Journal of Nutrition & Food Sciences

Aytenfsu et al., J Nutr Food Sci 2016, 6:4
http://dx.doi.org/10.4172/2155-9600.1000524

Review Article Open Access

Review on Chemical Residues in Milk and Their Public Health Concern in Ethiopia

Samuel Aytenfsu[1], **Gezehegne Mamo**[2] **and Bedaso Kebede**[1*]

[1]*Veterinary Drug and Animal Feed Administration and Control Authority, Ministry of Livestock and Fisheries, Addis Ababa, Ethiopia*

[2]*College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia*

[*]**Corresponding author:** Bedaso Kebede, Veterinary Drug and Animal Feed Administration and Control Authority, Ministry of Livestock and Fisheries, Addis Ababa, Ethiopia, Tel: 251913136824; E-mail: **Kebede.bedaso@yahoo.com**

Received date: May 12, 2016; **Accepted date:** June 16, 2016; **Published date:** June 23, 2016

Abstract

Chemical residues which could contaminants cow milk are chlorinated pesticides, organophosphates, herbicides, fungicides, antihelminthetic drugs, antibiotic, hormones, detergents and disinfectants, nitrites, poly chlorinated, poly brominated biphenyles, dioxins, mycotoxins, heavy metals and somatotropin hormone. Antibiotics such as beta-lactams, tetracycline, amino glycosides (e.g. streptomycin, neomycin and gentamycin), macrolides (e.g. erythromycin) and sulfonamides are the source of residues. Because of the public health significance, milk and milk products contaminated with antibiotics beyond a given residue levels, considered unfit for human consumption. Problems associated with antibiotic residues in milk include the risk of allergic reactions and occurrence of antibiotic-resistant bacteria, teratogenicity risk to the foetus, hypoplasia in developing teeth, aplasia of bone marrow, chronic insidious intake lead to elevated cancer risk and disruption of body's reproductive, immune, endocrine and nervous system. Organochlorine pesticides are substances containing contaminants classes: The dichlorodiphenylethanes, the chlorinated cyclodienes (aldrin, dieldrin, heptachlor, etc.), and the hexachlorocyclohexanes (lindane). The most common effects of the wide spread of organochlorine compounds in the environment are birth defects, neurological effects, behavioral effects, reproductive effects and cancer. The occurrence of chemical residues in the milk of lactating cows is a matter of public health concern, since dairy products are widely consumed by infants, children and many adults throughout the world. Governments have responsibility for making regulations to protect consumers against harm arising from chemical in food. The regulation of illegal residues in foods is a cooperative effort of Food Safety and Inspection Services, Feed and Drug Administration and Environmental Protection Agency. However, in Ethiopia not yet attention given regarding to set standards, control measures and monitoring program of chemical residues in animal originated foodstuff.

Keywords: Antibiotics; Chemical; Chromatography; Residues; Pesticides; Public health

Abbreviations

ADIs: Aceptable Daily Intakes; AFB1: Aflatoxin B1; AFM1: Aflatoxin M1; CAC: Codex Alimentarius Commission; CCPs: Critical Control Points; CDC: Center for Disease Control and Prevention; DDD: Dichloro Diphenyl Dichloroethane; DDT: Dichlorodiphenyltrichloroethane; ELISA: Enzyme-Linked Immunosorbent Assay; EU: European Commission; FAO: Food and Agriculture Organization; FDA: Food and Drug Authorities; FSIS: Food Safety Inspection Services; HACCP: Hazard Analysis Critical Control Points; HCB: Hexa Chloro Benzene; HCH: Hexa Chloro Cyclohexane; HPLC: High Performance Liquid Chromatography; IARC: Agency for Research on Cancer; IGF: Insulin-like Growth Factor; MRLs: Maximum Residues Limits; MU: One Mouse Unit; NOAEL: No Observed Adverse Effect Levels; OIE: Organization for Animal Health; PBBs: Poly Brominated Biphenyles; ppm: Parts Per Million; USA: United States of America; WHO: World Health Organization; WTO: World Trade Organization

Introduction

Milk production inextricably linked to the environment. Animals may become exposed to chemical substances during their production cycle which have been identified to date could come from drugs aimed at treating diseases or application of chemicals for the control of weeds, insects, fungi and rodents has enabled agricultural productivity and intensity to increase. These chemicals are environmental contaminants linked to atmospheric pollution, feed, soil and/or water. Uncontrolled processes interfering with the food delivering ecological system or with its natural compounds lead to residues in milk and result risk to human and environmental health [1]. Chemical residues which have been or are found in milk are chlorinated pesticides, organophosphates, herbicides, fungicides, antihelminthetic drugs, antibiotic, detergents and disinfectants, nitrites, polychlorinated, poly brominated biphenyles, dioxins, mycotoxins, heavy metals and somatotropin hormone. Any of these compounds may persist at collection, preparation processes of dairy products and they considered as residues [2-4].

The use of antibiotics therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries. Unauthorized antibiotic use may result in residues of these substances in milk and tissues [5]. Antibiotic residues are small amounts of drugs or their active metabolites, which remain in milk after treating the cows [6]. Because of the public health significance, milk and milk products contaminated with antibiotics beyond a given residue levels, is considered unfit for human consumption [7]. Problems associated with antibiotic residues in milk include the risk of allergic reactions,

Citation: Aytenfsu S, Mamo G, Kebede B (2016) Review on Chemical Residues in Milk and Their Public Health Concern in Ethiopia . J Nutr Food Sci 6: 524. doi:10.4172/2155-9600.1000524

aplasia of the bone marrow, carcinogen [8] and increased resistance of pathogens towards antibiotics [6].

Most of the foods produced for human consumption grown using organochlorine pesticides. Organochlorine pesticides are substances containing chemically combined chlorine and carbon. Dairy herds mainly exposed to these contaminating residues through their feed. After the residues metabolized they are stored in fat reserves from which they enter the circulation and partially eliminated in the milk resulting contamination [9]. The most common effects of organochlorine compounds in milk are birth defects, neurological effects and behavioral effects, reproductive effects and cancer [10,11].

In Ethiopia, few research reports indicate the existence of chemical residues contamination in milk and meat such as Oxytetracycline and Penicillin G antibiotic residue in cow milk [12]. Tetracycline residue levels in slaughtered beef cattle and organochlorine pesticide residues in human and cow's milk were some of the studies which highlight the significance of the problem of chemical residues in Ethiopia [13,14].

In order to safeguard human health the World Health Organization (WHO) and the Food Agriculture Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods [15]. A regulatory limit for antibiotic residues and other chemical residues imposed on the dairy industry in many countries [16,17]. However, Ethiopia has not yet adapted international standards or established specifications for chemical residue limits in the milk and other consumable animal products. The Ethiopian livestock industry has not established any control programs to ensure the safety of the milk. The chemical residue limits, which apply to both the parent chemicals and its metabolites, need to have strong legislations have to be enforced at all levels in Ethiopia livestock industry in order to protect the health of the consumers.

Therefore the objectives of this review are:

- To review the type, source and detection methods of chemical residues in animal products mainly milk.
- To review the public health importance of chemical residues in milk.
- To forward potential recommendation for prevention and control of the risk of chemical residue contamination in milk.

Sources of Chemical Contaminants of Milk

Most of chemical contaminants in milk and dairy products are antimicrobials (antibiotics and sulfonamides), anthelminthitic drugs, pesticides, detergents and disinfectants, nitrites, polychlorinated, dioxins, mycotoxins, heavy metals and somatotropin hormone [18].

Veterinary drugs

Much of the veterinary treatment of dairy cattle involves intramammary infusion of antibiotics to control mastitis. Some drugs apply to control endoparasites, ectoparasites and several illnesses and to increase milk production [2,19]. The most commonly used antimicrobials in dairy cattle can group into five major classes. These include the beta-lactams (e.g. penicillins and cephalosporins), tetracyclines (e.g. Oxytetracycline, tetracycline and chlortetracycline), amino glycosides (e.g. streptomycine, neomycin and gentamycin), macrolides (e.g. erythromycin) and sulfanomides (e.g. sulfamethazines) [8,20]. These drugs administered to animals by many routes such as intravenous, intramuscular injection routes, orally in the food and water, topically on the skin and by intra mammary and intrauterine infusions. Theoretically, all of these routes may lead to residues appearing in milk and dairy products [3,8]. Whenever any route with an antibiotic treats a lactating cow measurable levels of the antibiotic are usually detectable in the milk for a few days after the last treatment. There are Maximum Residues Limits (MRLs) for some drugs in milk according EU regulations that have shown in Table 1.

No.	Drugs	Concentration μg/kg
1	Benzylpenicillin	4
2	Tetracycline	100-200
3	Oxytetracycline	100
4	Chlortetracycline	100
5	Trimethoprim	50
6	Ceftiofur	100
7	Striptomycine	200-1000
8	Oxfendazole	10
9	Sulphonamiduse	100

Table 1: MRLs of some antibiotic drugs in milk according to EU regulations.

Antihelminthic drugs, which used to remove internal parasites such as flukes, tapeworms (cestodes) and nematodes (round worms) are important in animal production systems. The oxyclosanide, closantel and rafoxanide are active against *Fasciola hepatica* (liver fluke). Albendazole is a widely used benzimidazole antihelminthic that has shown efficacy against the important species of round worms, tapeworms and flukes from animals and humans. Following administration to cattle and sheep, albendazole is readily absorbed from the gut and rapidly transform to various metabolites, the major metabolites being albendazole sulfoxide, albendazole sulfone and albendazole 2-amino sulfone. These metabolites can account for all residues found in milk and dairy products at any time point that are both bioavailable and of toxicological significance [21]. Residues of benzimidazole compounds can occur in milk and dairy products and it is necessary to observe withdrawal periods for milk after therapy [22].

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; however, ivermectin has a teratogenic effect in laboratory animals like rats, rabbits and mice [23].

Bovine growth hormone

Bovine Growth Hormone or Bovine Somatotropin (BST), which administered to animals, is a genetically engineered protein hormone either identical or similar to the natural bovine growth hormone produced by pituitary gland. Its primary function is to increase milk production of dairy lactating cattle. Therefore, BST is a protein hormone that increases milk production in cows between 10 and 15% [24]. An increase in milk yield typically occurs within 5 days after beginning of treatment. When BST treated cows were consuming sufficient quantities of nutrients to meet the energy needs for additional milk synthesis, body lipid mobilization did not increase, but lipid synthesis were instead reduced. Bovine Growth Hormone increases activity and/or longevity of mammary secretary cells, probably via Insulin-like Growth Factor (IGF)-I produced by the liver and/or the mammary gland [25]. IGF-I is a portion of the effects of BST on lactation in dairy cows. The **raw milk** and pasteurized milk could have been levels of IGF-I of 5.6 and 8.2 ng/mL, respectively. Therefore, IGF-I is not destroyed by the pasteurization process but the heating of milk for the preparation of infant formula denaturizes IGF-I and significantly, reduction (35-48%) levels of IGF-I compared to **raw milk** [18]. Bovine Growth Hormone is probably stimulating immunological responses of animals and hence increasing the milk cell count [25]. It can cause to increase incidence of clinical mastitis in cows [18] and sub clinical mastitis in ewes. This prompted concern that increased use of antibiotics to treat the mastitis might lead to increased residues of such drugs in milk. Therefore, the risk to human health comes not from Bovine Growth Hormone residues but from the possibility that residues from the antibiotics used to treat the udder infections could end up in the contaminated milk supply [26].

Pesticides and insecticides

Contamination of feeds arises in the field or store where treatment with pesticides occurs. Chlorinated pesticides and related compounds such as Dichloro diphenyl trichloroethane (DDT), Dichloro diphenyl dichloroethane (DDD), Polychlorinated biphenyls (PCBs), chlorinated cyclodienes (aldrin, dieldrin, heptachlor, etc.), the hexachlorocyclohexanes (lindane) and Dioxins can enter milk and dairy products when the cow consumes contaminated feed. The chlorinated hydrocarbons are extremely durable, persistent, endocrine-disrupting activities, bioaccumulating and widely distributed toxic compounds that find their way into the food chain usually through use in controlling environmental or animal pests [27].

The occurrence of persistent organochlorine compounds in the environment is changing relatively slowly over a span of years; similar time trends are characteristic of contents in fish, meat, eggs, and dairy products, which are the foods with the greatest contributions to the intake of organochlorine compounds [28].

As much as 20% of an ingested chlorinated hydrocarbon excretes in milk. Chlorinated hydrocarbons adhere to milk fat and butter contains a much higher proportion of these insecticides [3]. Residues of some chlorinated compounds in milk and dairy products show in Table 2. DDT can accumulate in fatty tissues and can transfer into milk and dairy products. Organochlorine pesticides such as DDT and hexachlorocyclohexane (HCH) have banned in china since 1983. Residues of such compounds may persist in the environment and cause contamination through the food chain). Presented organochlorine pesticide residues (owing to their use in sanitary actions) indicating a human exposure through milk and dairy products [18].

Mycotoxins

Some of the moulds produce various toxic metabolites under appropriate temperature and moisture conditions. These metabolites, which may be hazardous for human health, called mycotoxins [29]. Aflatoxin M1 (AFM1) found in the milk of animals that fed with Aflatoxin B1 (AFB1) containing feed [30]. It can produce by moulds of feeds that have been harvested damp, have not adequately dried, or are improperly stored. These substances through the feedstuff can infect dairy cattle. The content of AFM1 in milk is entirely dependent on the presence of the precursor AFB1 in the ration of dairy cattle and it can numerically express as feed to milk ratio. The forming of AFM1, metabolite of AFB1, occurs in liver and it secrets into milk in the mammary gland of dairy cows [31]. Many researchers reported that there was a linear relationship between the amount of AFM1 in milk and AFB1 in feed consumed by the animals. On the other hand, AFM1 levels in milk show a **seasonal variation** and the toxin amount have differences in the products, which produced from the toxin containing milk. It reported that after uncovering of negative effects of aflatoxins on public health, various international foundations related with this subject paid attention and in 1993, International Agency for Research on Cancer (IARC) of WHO included AFB1 as primary and AFM1 as secondary groups of carcinogenic compounds [31,32]. Many countries have carried out various control and inspection programs on this subject concerning about public health for many years. According to the results obtained, maximum aflatoxin levels were determined for food and feed by considering each country's conditions and finally regulations were established [33].

Pesticides	Types of dairy products	Mean level (mg/kg of fat)
HCB	Raw milk	0.016
	Milk powder	0.0656
	Ras cheese	0.0037
	Damietta cheese	0.0045
	Spanish pasteurized milk	0.007
Aldrin-dieldrin	Cheese	0.2 ng/g
	milk	0.0074-0.0271
	Milk powder	0.0038
Dieldrin	Ras cheese	0.0039-0.0068
	Damietta cheese	0.0025
HCH	milk	0.094
	Spanish pasteurized milk	0.015
	Milk powder	0.0149
	Ras cheese	0.00865
	Damietta cheese	0.0115
	Butter	0.093
DDT	milk	0.159
	Butter	0.049
	Milk powder	0.0546

HCB: Hexachlorobenzene; HCH: Hexachlorocyclohexane; DDT: Dichlorodiphenyltrichloroethane

Table 2: Mean level of pesticide content of milk and dairy products [18].

Heat treatments like pasteurization were not effective in the reduction of formation of AFM1 [33]. It has relatively increased in cheese samples because of its affinity to casein fraction in milk and due to the water solubility of this toxin lower level of AFM1 found in cream and butter than that of bulk-tank milk. All of these findings indicated that AFM1 with different levels could be available in dairy products made from contaminated milk. Consequently, this subject is a serious problem for the public health since all the age groups including infants and children consume these products worldwide. For this reason, milk and dairy products have to be inspecting continuously for AFM1 contamination at least twice a year. Beside this, it is important to have low levels of AFM1 in the feeds of dairy animals and in order to achieve this purpose, feeds of dairy cows should be kept away from contamination as much as possible [34].

Nitrates and nitrites

Nitrates and nitrites are chemicals used in fertilizers, in rodenticides (to kill rodents), and as food preservatives [35]. Nitrates and nitrites come in various forms, but when dried are typically a white or crystalline powder. Nitrate (NO^{3-}) and nitrite (NO^{2-}) are also naturally occurring compounds that are a metabolic product of microbial digestion of wastes containing nitrogen, for example, animal feces or nitrogen-based fertilizers. The use of nitrate fertilizer on crops can result in higher concentrations in some crop residue of animal feed [36].

Heavy metals

Heavy metals enter the human and animal body mainly by the routes of inhalation and ingestion. With increasing environmental pollution, a heavy metal exposure assessment study is necessary [37,38]. Heavy metals produce toxic effects by replacing essential metal ions existing in the chelates present in body. The intake via ingestion depends upon food habits. Lead (Pb) and cadmium (Cd) are toxic for human and children are more sensitive to these metals than adults are. The metals, namely copper (Cu) and zinc (Zn), are essential micronutrients and have a variety of biochemical functions in all living organisms. While Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element-to-element. Milk is the fundamental food for infants and the daily intake of the heavy metals Pb, Cd, Cu and Zn can be determine by different age groups of infants through different milks and baby foods. Heavy metals can enter to milk and dairy products and affect the health of people who have consumed contaminate milk and dairy products [39].

Toxic Agents in an Organism

Living things continuously exposed to external chemical 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 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) exposed to chemicals 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, dermal (e.g. external antiparasitic agents in ruminants), parenteral (e.g. antibiotic treatment in large animals) and even inhalation (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). Chemicals 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 [1].

Absorption

Many toxic agents enter with foodstuff and 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 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-ionized), which is determined by surrounding pH. Weak acids will become more easily absorbed in the stomach while the rest will happen in bases of the intestines. Hydrophilic substances having a small molecular weight 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. It should be born in mind that certain factors may sometimes alter chemicals 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 minimizing its potential adverse effect [40].

Distribution

Once it has been absorbed, a toxic agent 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 specialized transport; however, certain toxicants do not cross the membranes and distributed via the blood flow. Some accumulated in determined parts of the organism as result of their binding to proteins or their high solubility in fat. When a toxicant becomes stored, then equilibrium reached with the free fraction, which is in the plasma. Thus, when the chemical metabolized or excreted, then substance released from the storage site, thereby meaning that the chemicals half-life could become very long. Albumin is the main plasmatic protein transporting chemicals. 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 exploited for recognizing exposure, whether in humans or animals [41].

Many organic compounds are very stable and lipophilic, accumulated in the environment, rapidly absorbed and concentrated in body fat. The toxicants accumulated in fat because they dissolved in it. A substance stored in fat is not toxic for the carrier, but there is rapid lipid mobilization, for example, poisoning may occur during long periods of fasting. Human beings [1] could consume animal fat, a potential reserve of liposoluble toxicants.

Metabolism

The objective of chemicals metabolism is to increase characteristics regarding an increase in substances hydro solubility so that they can more easily excreted. This process occurs in two phases; hydrolysis, reduction and oxidation reactions 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 chemicals constituted in their substrate. Conjugation reactions occur during phase 2, mainly with glucuronic acid, glutathione conjugates and sulphates; such reactions 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 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 [40].

Excretion

Toxicants eliminated from the body by various routes, the kidneys being the most important organ for excreting chemicals since it is the main elimination route. The biliary route involving the faeces is the other elimination route for toxic substances, which 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 concept of withdrawal time established to avoid the accumulation of drug residues in animals. It defined as being the time required after a drug administered to an animal to ensure that drug residues in marketable products (meat, milk, eggs, viscera or other edible products) are below a determined maximum residue limit (MRL) (Table 3) [41].

Antibiotics	Food stuff	Concentration above MRL	References
Oxytetracycline	Meat	71.3	Hunde and Molla [13]
Oxytetracycline	Milk	73.91 (8.5%)	Abebew [12]
Penicillin G	Milk	17.39 (8.5%)	Abebew [12]

Table 3: Studies of antibiotics concentration above MRL in Ethiopia.

Analytical Methodologies for Chemical Residues

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 programmers 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. The prior treatment, which a sample has received, is very important for ensuring that methods reach desired detection levels and an acceptable level of exactitude and precision. Enabling the factors responsible for analyte loss to be controlled during such procedure [1]; this would include the presence of functional reactive groups which can interfere with such determination [42].

There is an immense variety of methods for identifying, confirming and quantifying analytes, which used individually or coupled to each other in a suitable way. These methods grouped into bioassays, microbiology assays, immunochemical assays and physical-chemical assays [43].

Bioassays

Biological methods for determining toxic residues in foodstuffs can use both *in vivo* and *in vitro* [43]. The mouse bioassay is the most used one and even accepted by regulating entities. A toxin extract intraperitoneally injected into mice having around 20 g body weights in the mouse bioassay and their survival monitored from 24-48 hours. One mouse unit (MU) 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 [1].

Microbiological assays

The microbiological methods used for detecting antimicrobial residues in foodstuffs based on inhibiting microbial growth, microbial receptor activity and enzymatic reactions and applied to all types of matrices, usually milk, meat, eggs and honey.

Microbial inhibition methods 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 thermophiles* [1]. The analysed milk sample applied on the agar surface either directly or with a paper disc called disc assay plate methods. In the course of incubation, the diffusion of the sample into the medium takes place (the agar diffusion principle), and if the sample contains inhibitor agents, reduction or total inhibition occurs of the tested microorganism growth. Depending on the method used, the presence of inhibitor agents in the tested sample indicated by the formation of a clear zone of inhibition around the disc (disc assay plate methods) or a change in the medium colour [8,44]. Microbial growth inhibition methods (wide spectral rapid tests) vary in the type of the testing organism, indicator, incubation period and temperature, spectrum and detection levels of the agents analysed. A series of these methods use as the testing microorganism *Bacillus stearothermophilus var. calidolactis*: Blue Star/6/7 (Enterotox Lab., Germany), Charm Blue Yellow Test (Charm Sciences Inc., USA), Delvo test SP-NT (Gist-brocades BV, The Netherlands), Copan milk test (Copan Italia, Italy), Eclipse 50 (Zeu-Inmunotec SL, Spain). *Bacillus stearothermophilus* is an outstanding testing microorganism for its properties from which the most important, according to Katz and Siewierski [45], are the ability of rapid growth at higher temperatures (64°C) and a high sensitivity to the β-lactam antibiotics [44].

Microbial receptor activity is an alternative method for establishing the β-lactam group antibiotics (active forms of the β-lactam structure) is the use of the receptor proteins. β-Lactam specific receptor proteins or penicillin-binding proteins (PBP) were successfully used in some methods and commercially produced tests (Biacore analysis, Penzym test, Beta Star test, SNAP test, Charm Safe Level test and Delvo-X-Press test and others) for establishing the β-lactam antibiotics residues [8,46]. PBP is found frequently in bacterial cell walls. Penicillin sensitive bacteria have various penicillin binding proteins which, judging on their molecular weights; divided into two groups: Proteins with low or high molecular weights [47]. These proteins are further divided in subgroups by the amino acid sequence. Various PBPs have different functions. They include transpeptidase, transglycosylase, and carboxypeptidase activities [47].

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 very reduced prior cleaning treatment. These assays 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, radioimmunoassay, the fluorescent immunoassay and the chemiluminescence immunoassay [1].

Modern immune analytical methods accomplish an increase in the sensitivity by labeling one of the reagents–the antigen or the antibody. The label can be a radioisotope, enzyme, fluorescence or chemical scintillation agent [8,48,49]. Non-isotopic immunoassays such as ELISA, fluorescence polarization immunoassay, particle-concentration immunoassay, particle-concentration fluorescence immunoassay, and monoclonal-based immunoassays, in all likelihood, play an increasingly important role in antibiotics screening immunoassay determinations [48]. The most frequently used immunochemical method for rapid diagnostics of veterinary drug residues is enzyme immunoanalysis. As an enzyme label, horseradish peroxidase, alkaline phosphatase, glucose oxidase, pyruvate dehydrogenase and recombinant β-galactosidase are used. These enzymes catalyse the reactions that cause the substrates degradation and form coloured products that can read spectrophotometrically or visually [44].

For pesticide determination, new assays and sensors for cheaper and faster on-site analysis being developed. Enzymatic sensors, based on the inhibition of a selected enzyme, are the most extended biosensors used for the determination of these compounds [50]. Biosensors based on enzyme inhibition although sensitive, are not selective and cannot used for quantification of either an individual or a class of pesticides. Nevertheless, introduction of recombinant enzyme for biosensor applications can solve the problem. The organophosphorus hydrolase (OPH) is able to hydrolyze a number of OP pesticides such as paraoxon and parathion and chemical warfare agents such as sarin and soman. Hydrolysis of these OP pesticides generates p-nitrophenol, which is an electroactive and chromophoric product. Thus, OPH could be combined with an optical transducer to measure the absorbance of p-nitrophenol or with an amperometric transducer to monitor the oxidation or reduction current of this product [51]. In a different approach, biosensors based on immunological assays have been developed with limit of detection of 0.1 $\mu g.L^{-1}$ and application of the River Analyzer immunosensor in the determination of pesticides such as atrazine, simazine, isoproturon, 2,4-D, alachlor and paraquat in natural waters. Recently, a label-free direct piezoelectric immunosensor built on a flow-through cell used for the determination of 2,4-D in water with a limit of detection around 0.2 $\mu g.L^{-1}$. However, there are very limited applications of biosensor detection of pesticide compounds in meat system [52].

A great number of specific sensors for bacterial toxins and mycotoxins developed for food and environmental control [53]. Thus, an integrated optical sensor reported for the analysis of aflatoxin B in corn. A light-addressable potentiometric immunosensor based on the commercial device for the analysis of saxitoxin and ricin been described. An impedance-based immunosensor has been prepared by using an ultrathin platinum film with an immobilized layer of antibodies against the staphylococcal enterotoxin B. Various evanescent wave immunosensors been reported to be capable of detecting botulin with very low limits of detection. A rapid and sensitive immunosensor for the detection of the Clostridium botulinum toxin A been developed. This fiber optic-based biosensor utilizes the evanescent wave of a tapered optical fiber where antibodies antitoxin A been covalently immobilized at the distal end. The toxin detected by means of a rhodamine label, within a minute at concentrations as low as 5 ng/mL [54].

Physico-chemical assays

Physical-chemical methods 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 developed for achieving this objective. Separation methods based on the principles of chromatography and generally coupled to high sensitivity and selectivity detection techniques leading to quantifying an analyte with a high level of precision and exactitude and its unequivocal identification at very low concentration levels [1]. The chromatographic methods used for determining analytes in complex matrices would be gas chromatography, high performance liquid chromatography (HPLC), ionic chromatography, size exclusion chromatography, supercritical fluid chromatography, affinity chromatography. 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, inductively-coupled plasma atomic emission spectrometry, nuclear magnetic resonance, mass spectrometry and mass spectrometry in tandem [55].

A number of analytical techniques such as colorimetric method, Thin Layer Chromatography, High Performance-Thin Layer Chromatography is used for mycotocins, Gas Liquid Chromatography, Gas Chromatography-Mass Spectrometry, High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry-Mass Photometry etc., are established for detection and quantification of pesticide residues in animal tissues [56]. However, colorimetric and Thin Layer Chromatography methods are limited only for qualitative determination due to its low sensitivity. Gas Liquid Chromatography or Gas Chromatography-Mass Spectrometry though normally used for determination of non-volatile compounds (organochlorine and organophosphorus pesticides), but also effectively utilized for determination of thermo-labile compounds such as synthetic pyrethroid and N-methyl carbamate pesticides [53].

Public Health Concerns

General public health importance of chemicals

The presence of chemical contaminants in milk are very important for consumers and it can be a matter of public health concern as well as many of unknown diseases in human because of milk and dairy products are widely consumed by humans throughout the world [2].

An important concern of veterinary toxicology is the possible transmission of harmful substances from milk and dairy products to human population. This concerns primarily antibiotics in use as feed additives. They include tetracycline, nitrofuranes, penicillin, streptomycin and sulfonamides [18]. There may be biologically active metabolites of antimicrobial in milk and dairy products that could result anaphylaxis and allergic shock in sensitized individuals such as penicillin. Penicillin is not inactivated by pasteurization or drying and levels as low as 0.03 $IU.mL^{-1}$ [57]. Overuse of antimicrobial in agriculture production cause to toxicity in human and animals. They can cause some disruptions like aplasia of the bone marrow (e.g. chloramphenicol) [8] and carcinogen (e.g. oxytetracycline and furazolidone), 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 [58]. In addition, nitrofuranes can react with nitrite to yield (carcinogenic) nitrosamines [8], tetracycline can generate bacterial resistance; oxytetracycline particularly induces antibiotic resistance in coli form microorganisms present in the human intestine. The emergence of resistant bacteria within animals and the transfer of antibiotic resistance genes(R-factor) from non-pathogenic bacteria to other bacteria or human pathogens that will lead to widespread resistance [8,20,57].

Scientist at Center for Disease Control and Prevention (CDC) began tracking a new type of *Salmonella* Newport 9+, which is resistant to nine antibiotics including ceftriaxone, one of the few drugs that kill most bacteria and the drug of choice for children when *Salmonella* enter the blood stream. The appearance of resistance pathogenic organisms such as *Salmonella spp.*, *E. coli*, *Campylobacter spp.* and Methicillin Resistant *Staphylococcus aureus* has been given more concern with regard to drug residues [59,60].

Despite their beneficial use, embryotoxicity, teratogenicity and other adverse effects in a variety of animal species caused by benzimidazoles or their metabolites [61]. Thus, considerable attention been focused on the threat to human health stemming from consumed food of animal origin such as milk, cheese and butter. The European Union [62] has established maximum Residue Limits (MRLs) in certain products of animal origin, including meat and milk, but not for other dairy products. The need for more intensive residue controls becomes stronger considering several studies, which indicate, that benzimidazoles not degraded after microwave and oven baking, storage at -18°C for three to eight months and after cooking [63].

Among all residues, pesticides receiving most interest worldwide in recent years. The United Nations has estimated that about 2 million poisoning and 10,000 deaths occur each year from pesticides, with about three-fourth of this occurring in developing countries. The acute and malicious consumption involving higher dose results in death whereas, chronic insidious intake lead to elevated cancer risk and disruption of body's reproductive, immune, endocrine and nervous system [64].

Pesticides, e.g. Hexachlorocyclohexane (HCHs), can cause damages on central nervous system, reproductive and endocrine. Organochlorine pesticides characterize by their high lipophilicity and long elimination half-lives, which have neurotoxin, birth defects, behavioral effects, reproductive effects, cancer, disruption of the normal homeostasis of the endocrine functions [10]. An epidemiological study in Belgium found that women with at least 0.5 ppb levels of organochlorine pesticides, DDT and/or hexachlorobenzene have at least a 5 times higher incidence of breast cancer than those with lower levels [11].

Mycotoxins can also present in milk and dairy products and can create public health problems in humans. Aflatoxin M1 in milk is a carcinogenic metabolite of aflatoxin B1. Aflatoxin M1 in milk and dairy products led to increase the risk of liver cancer. Excretion of AFM1 into milk causes hepatocellular carcinoma in rat and rain trout. Due to the potent carcinogenicity of AFM1, most countries regulate both AFB1 in dairy cattle feed and the AFM1 in milk. The tolerance level for AFM1 in milk varies among countries from 0.05 μg/kg in Europe to 0.5 μg/kg in the United States [65]. The tolerance level for AFM1 in milk and dairy products in Iran is also 0.05-0.5 μg/kg [66].

Exposure to higher levels of nitrates or nitrites has been associated with increased incidence of cancer in adults, and possible increased incidence of brain tumors, leukemia, nasopharyngeal (nose and throat) tumors and "blue baby syndrome" (methemoglobinemia) in children in some studies [67]. Likely exposure pathways for children include ingesting contaminated drinking water, milk and most commonly of concern for private wells and foods containing preservatives (Reinik, 2005).

The health implications from heavy metals lead to kidney damage, cardiovascular diseases, induction of hypertension, growth inhibition, interference in haemoglobine synthesis, irreversible changes in brain and nerve cells and also some of these residues are known to be carcinogenic in nature. The pulmonary and nervous systems and skins are the main target organs of arsenic contamination. Cadmium associated with kidney damage and lead considered to has been associated with learning deficits in children. Copper and zinc are essential micronutrients but in higher amount may influence metallic taste to the product resulting unacceptability of the product [38].

Status of chemical residues in Ethiopia

In Ethiopia, few studies have indicated the existence of chemical residue in milk and other animal products. In Central Ethiopia the prevalence of oxytetracycline and penicillin, G Residues in milk and meat showed that higher than maximum residue limits [12]. Determination of pesticide residue in South-West Ethiopia (Asendabo, Serbo and Jimma) the mean estimated daily intake of DDT by infants from mother's milk and cow milk samples showed that 62.17 μg/kg body weight, which is about three times higher than the acceptable daily intake set by WHO/FAO for total DDT, 20 μg/kg of body weight. This alarmingly high daily intake value is which cause for concern, since children are highly susceptible to effects from such environmental contaminants [14]. Other study on cow milk in Addis Ababa indicated the prevalence of toxic metals such as Cd, Pb, Fe and Zn are amongst the elements that have caused most concern in terms of adverse effects to human health (Table 4) [68,69].

Prevention and Control of Chemical Contaminants

Governments have responsibility for making regulations to protect consumers against harm arising from chemical in food. The regulation of illegal residues in foods is a cooperative effort of FSIS (Food Safety and Inspection Services), FDA (Feed and Drug Administration) and EPA (Environmental Protection Agency). Veterinary residues regulated through Maximum Residues Levels (MRLs) which permitted in milk and dairy products on sale. Milk withheld from sale for a specific period after veterinary therapy (usually 72-96 h) to ensure that no residues persist. Even with the possibility of a recognized withholding period for the milk, few drugs authorized for use in lactating cows [57]. Therefore, if the milk with holding times applied, there should be no detectable residues in milk. The amounts decrease rapidly. For example, the amount of amoxicillin found in milk 36 h after an intra mammary application of 200 mg was 1 $mg.dL^{-1}$ [2]. Besides, Food and Agriculture Organization (FAO) and World Health Organization (WHO) have established of MRLs and ADI of some veterinary residues in milk for consumer protection (Table 5).

Parameter	Concentration in Addis Ababa by Admasu et al. [69]	Concentration in Addis Ababa by Dawd et al. [68]
Cd	0.18	0.101
Fe	1.27	1.27
Pb	2.63	0.998
Zn	5.33	4.9

Table 4: Heavy metal concentrations of cow's milk in study of Addis Ababa [68].

Types	Chemicals	ADI	Recommended MRLs(mg/L)
Antimicrobiol	Cftiofure	0-50	100
	Sulfonamide	0-50	25
	Streptomycin	0-50	200
	Oxytetracycline	0-3	100
	Gentamicin	0-4	200
	Benxylpencillin	30	4
Insecticides	Cypthrinflu	0-20	40
	Cypemethrin	0-50	50
Anthehelminthic	Thialbendazole	0-100	100
	Albendazole	0-50	100
Production aid	Bovine somatotropine	Not specified	Not specified

Table 5: Residues of some veterinary drugs in cow's milk for human consumption [18].

ADI (Acceptable Daily Intake): ADI for a food is the health endpoint and represents an estimate of the amount of a chemical residue that ingested daily over the lifetime of an individual in the general population without appreciable risk. MRLs: Maximum residue limits.

Regulation

The regulation of residues handled by each country throughout the world has a tendency towards uniform approach. However, much greater enforcement should be done with the passage of sanitary and Phytosanitary measures. The relevant aspects of the Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs and pest control chemicals in Food processing Animals [70] applied to minimize or prevent the introduction of chemical residues in milk or milk products. The international organizations such as Codex Alimentarius Commission (CAC) have also set their standards using the term Maximum Residue Limits (MRLs) and Acceptable Daily Intake (ADI) levels of various pesticide residues in milk and milk products.

In Ethiopia, pesticide and veterinary drug use and registration are regulated under ministry of agriculture; however, it lacks standards for control and monitoring of chemical residues as well as economic, social and environmental risk and inherent toxicity to mammals, wildlife and plants.

Risk assessment of residues

International Organization such as Codex Alimentarius Commission have taken initiation of harmonization of chemical residues in food through establishment of statutory limitations viz., Maximum Residue Limits (MRLs), Acceptable Daily Intake (ADI) levels, acute reference dose, No Observed Adverse Effect Levels (NOAEL) etc. However all these statutory limitations are important principles for risk assessment of residues in foods including milk. For statutory limits of residues, the terms tolerances or Maximum Residue Limits (MRLs) frequently used by regulatory agencies. Nevertheless, both the term tolerances and MRLs are synonymous; former used in United States, while later used in Canada and the European Union. The term MRL may be defined as the maximum concentration of marker residue (e.g. parent compound, metabolites etc.), expressed in parts per million (ppm) or parts per billion (ppb) on fresh weight basis, that is legally permitted or recognized as acceptable in or on food. The MRLs established based on package of toxicology and residue data and these referred to as safety file and the residue file. The toxicology data are used not only to characterize the biological properties of the molecules, but also to identify a suitable No Observed Effect Levels (NOELs), which in turn is used to calculate an Acceptable Daily Intake (ADI), the quantity, which if consumed over a human lifetime will have no adverse effect on consumer health. The identification of NOELs, the calculation of ADI values and the establishment of MRLs is a complex scientific process involving toxicology, pharmacology, microbiology, residue kinetics and analytical chemistry but the MRL is established at a magnitude which ensures that the ADI will not be exceeded by the consumer when eating food of animal origin [71].

Total quality management and quality assurance program

Responsibility for chemical contaminants control in milk and dairy products lies with all participitants in the production process, from farmers through to consumers. Consumer and governmental concern over the potential for the introduction of chemical contaminants on the farm into the food supply is an important issue affecting policy for the livestock industry. One goal of total quality management is to prevent the occurrence of chemical contaminants, especially antibiotic residues in raw milk Shipped from the farm. An effective approach to meet this goal is the implementation of HACCP procedures, which are part of the Milk and Dairy Beef Quality Assurance Program for antibiotic avoidance [72]. The Milk and Dairy Beef Quality Assurance Program conceived as a HACCP program. The objectives of the HACCP program are to identify hazards associated with a process and to determine the acceptable limits in production process. Also, it determines the Critical Control Points (CCP) that prevent those hazards, to develop a management strategy that addresses the identified CCP, to monitor the success of the program and to make any necessary changes to management procedures in response to outcome of the monitoring. Evaluation of early implementations of the Milk and Dairy Beef Quality Assurance Program by representatives of milk receivers and veterinarians identified deficiencies in the Milk and Dairy Beef Quality Assurance Program as a HACCP program [72-75].

Conclusions and Recommendations

Chemical contaminants in milk and dairy products are results of environmental contamination, poor veterinary service and misuse of antibiotics, uncontrolled use of pesticides, insecticide and biological toxins ingested by animals. These contaminants as residues in the milk have significant public health importance, since dairy products are widely consumed by infants, children and many adults throughout the world. Chemical contamination not completely prevented, or eliminated from milk and dairy products. However, the implementation of food safety and a regulatory laws in dairy and plant farms is required to reduce chemical residues in milk and dairy products. Therefore, the following recommendations forwarded:

- Strict national legislation passed on livestock sector to avoid unnecessary use of chemicals.
- Avoid using antibiotics in the veterinary field without a veterinarian's prescription and educate dairy owners on drug withdrawal period of treated animals.
- National chemical residues control and monitoring program should be design to set standards on the use of chemicals (antimicrobials, insecticides, pesticides, etc.).
- Training to farmers and personnel about good manufacturing practices and monitoring are useful to reduce chemical contaminants in milk and dairy products.
- Further research on the effect of chemical residues to human and alternative control system should be encouraged.

Authors Contribution

Authors equally contributed.

Conflict of Interest

Authors declare that no conflict of interest.

References

1. María CL, Mary T (2012) Chemical Residues in Animal Food Products: An Issue of Public Health, Public Health Methodology, Environmental and Systems Issues. National University of Colombia, Colombia 51: 641-648.
2. Jensen RG (1995) Handbook of Milk Composition. Academic Press Inc, UK.
3. Hubbert WT, Hagstad HV, Spangler E, Hinton MH, Hughes KL (1996) Food Safety and Quality Assurance (Foods of Animal Origin). (2nd edn.), Iowa State University Press, Ames.
4. Abdel WA, Dawood RM, Abd E, Maha A, Helal S, et al. (2004) Detection of organochlorine pesticide residues in samples of cow milk collected from Sohag and Qena governorates. Ass Univ Bull Environ Res 7: 105-116.
5. Ivona K, Mate D (2002) Evaluation of the Sensitivity of Individual test Organisms to Residual Concentrations of selected types of drugs. Slov Vet Res 9: 78-82.
6. CAC (1998) Codex Alimentarius Commission, Reports of the sessions of the codex committees on residues of veterinary drugs in Foods. Washington DC, USA.
7. Hillerton JE, Halley BI, Neaves P, Rose MD (1999) Detection of Antimicrobial Substances in Individual Cow and Quarter milk samples using Delvotest microbial inhibitor tests. J Dairy Sci 82: 704-711.
8. Mitchell JM, Griffiths MW, McEwen SA, McNab WB, Yee AJ (1998) Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. J Food Prot 61: 742-756.
9. Martínez MP, Angulo R, Pozo R, Jodral M (1997) Organochlorine pesticides in pasteurized milk and associated health risks. See comment in PubMed Commons below Food Chem Toxicol 35: 621-624.
10. Windham B (2002) The health effects of pesticides, Pesticid.
11. Tilman S (2003) Belgium, occupational and environmental medicine. Belgium 60: 348-351.
12. Abebew DS (2008) Detection and Determination of Oxytetracycline and Penicillin G Antibiotic Residue Levels In Bovine Bulk Milk from Debrezeit and Nazareth Dairy Farms. Proceedings of the 1st International Technology, Education and Environment Conference.
13. Hunde AB, Molla BZ (2012) Tetracycline Residue Levels in Slaughtered Beef Cattle from Three Slaughterhouses in Central Ethiopia. Global Veterin 6: 546-554.
14. Gebremichael S, Birhanu T, Tessema DA (2013) Analysis of Organochlorine Pesticide Residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South-Western Ethiopia. Chem Publ 5: 1652-1657.
15. FAO/WHO (1995) Residues of some veterinary drugs in animals and foods. Monographs prepared by the forty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives. FAO Food Nutr Pap 41: 1-112.
16. FDA USA (1996) Evaluation and Use of Milk. Antimicrobial drug screening tests report. Centre for food safety and applied nutrition. Feed and Drug Control Authorities Prime Connection, Rockville, USA.
17. Folly M, Machado S (2001) Antibiotic Residues Determination, using microbial inhibition, protein binding and immunoassays methods, in pasteurized milk commercialized in the northern region of Rio de Janeiro State, Brazil. Cienc Rural 31: 95-98.
18. Jahed KGR (2007) Chemical Contaminants in Milk and Public Health Concerns: A Review. Intern J Dairy Scie 2: 104-115.
19. Korsrud GO, Boison JO, Nouws JF, MacNeil JD (1998) Bacterial inhibition tests used to screen for antimicrobial veterinary drug residues in slaughtered animals. J AOAC Int 81: 21-24.
20. Sundlof SF, Kaneene JB, Miller RA (1995) National survey on veterinarian-initiated drug use in lactating dairy cows. J Am Vet Med Assoc 207: 347-352.
21. Schenck FJ, Callery PS (1998) Chromatographic methods of analysis of antibiotics in milk. J Chromatogr A 812: 99-109.
22. Fletouris DJ, Botsoglou NA, Psomas IE, Mantis AI (1997) Determination of the Marker Residue of Albendazole in Cheese by Ion-pair liquid Chromatography and Fluorescence Detection. J Dairy Sci 80: 2695-2700.
23. Moreno L, Alvarez L, Ceballos L, Sánchez BS, Lanusse C (2008) Pattern of ivermectin and doramectin (cattle) residues in muscular tissue from various anatomical locations. Food Additives and Contaminants: Part A 25: 406-412.
24. Hoagland TA, Zinn SA, Tripp MW, Schreiber D, Cowan WA (1997) Effect of Bovine Somatotropin (BST) on milk production in angus and hereford cows. J Anim Sci 75: 131-131.
25. Hilliard Y, Colleau JJ, Disenhaus C, Lerondelle C, Mouchet C, et al. (1998) Recombinant growth hormone: Potential interest and risks of its use for bovine milk production-A Review. J Anim Prod 11: 15-32.
26. Cohick WS (1998) Role of the insulin-like growth factors and their binding proteins in lactation. J Dairy Sci 81: 1769-1777.
27. Mukerjee D (1998) Health risk of endocrine-disrupting ortho-substituted PCBs emitted from incinerators. Environ Eng Sci 15: 157-169.

28. Robertson G, Lebwitz M, Needham L, O'Rourke MK, Rogan S, et al. (2002) Distribution of residential organochlorine pesticide residues along Arisona/Mexico border. Proceeding: USA, Indoor Air 63: 6-68.
29. Sharma RP, Salunkhe DK (1991) Mycotoxins and Phytoalexins. CRC Press, London.
30. Wood GE (1991) Aflatoxin M1. In: Mycotoxins and Phytoalexins, Sharma RP, Salunkhe DK (edn.). CRC, London, pp: 145-163.
31. Cathey CG, Huang ZG, Sarr AB, Clement BA, Phillips TD (1994) Development and evaluation of a minicolumn assay for the detection of aflatoxin M1 in milk. J Dairy Sci 77: 1223-1231.
32. Dragacci S, Gleizes E, Fremy JM, Candlish AA (1995) Use of immunoaffinity chromatography as a purification step for the determination of aflatoxin M1 in cheeses. Food Addit Contam 12: 59-65.
33. Jahed KGR (2006) Aflatoxin M1 Related to Consumption of Contaminated Milk and Dairy Products. J Iran Vet Council, Iran.
34. Bakirci I (2001) Aflatoxin: A study on the occurrence of aflatoxin M1 in milk and milk products produced in van province of Turkey. Food Contr 12: 47-51.
35. WHO (2006) International Program on Chemical Safety, Environmental Health Criteria 5: Nitrates, Nitrites and N-Nitroso Compounds, Geneva, Switzerland.
36. Reinik M, Tamme T, Roasto M, Juhkam K, Jurtsenko S, et al. (2005) Nitrites, nitrates and N-nitrosoamines in Estonian cured meat products: intake by Estonian children and adolescents. Food Addit Contam 22: 1098-1105.
37. Ikeda M, Zhang ZW, Moon CS, Imai Y, Watanabe T, et al. (1996) Background exposure of general population to cadmium and lead in Tainan city, Taiwan. Arch Environ Contam Toxicol 30: 121-126.
38. Raghunath R, Tripathi RM, Khandekar RN, Nambi KS (1997) Retention times of Pb, Cd, Cu and Zn in children's blood. Sci Total Environ 207: 133-139.
39. Licata P, Trombetta D, Cristani M, Giofrè F, Martino D, et al. (2004) Levels of "toxic" and "essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy. Environ Int 30: 1-6.
40. Lehman-McKeeman LD (2008) Absorption, distribution and excretion of toxicants, In: Casarett and Doul's Toxicology. The basic science of poisons (7th edn.), Curtis Klaassen, New York, USA, pp: 1-1309.
41. Panter KE, James LF (1990) Natural plant toxicants in milk: a review. J Anim Sci 68: 892-904.
42. LoBrutto R, Patel T (2007) Method Validation, In: HPLC for Pharmaceutical Scientists, Wiley Interscience, New Jersey, USA, pp: 455-502.
43. FAO (2004) Marine biotoxins, FAO Food and Nutrition Paper 80, Rome, Italy.
44. Botsoglou NA, Fletouris DJ (2001) Drug Residues in Foods, Pharmacology, Food Safety, and Analysis. Marcel Dekker, New York.
45. Katz SE, Siewierski M (1995) Bacillus stearothermophilus disc assay: a review. J of AOAC International 78: 1408-1415.
46. Gustavsson E, Sternesjo A (2004) Biosensor Analysis of β-lactams in Milk: Comparison with Microbiological, Immunological, and Receptor-based Screening Methods. J AOAC International 87: 614-620.
47. Massova I, Mobashery S (1998) Kinship and diversification of bacterial penicillin-binding proteins and beta-lactamases. Antimicrob Agents Chemother 42: 1-17.
48. Roeder AM, Roeder M (2000) Antibiotics in foods of animal origin. In: Francis F. Encyclopedia of Food Science and Technology. (2nd edn.), John Wiley and Sons Inc., New York 1: 54-63.
49. Stepaniak L, Sorhaug T, Jedrychowski L (2003) Immunochemical. In: Roginski H.: Encyclopedia of Dairy Sciences. Elsevier Science Ltd., London 1: 62-66.
50. Choi JW, Kim YK, Lee IH, Min J, Lee WH (2001) Optical Organophosphorus Biosensor Consisting of Acetylcholinesterase/ Viologen Hetero Langmuir-Blodgett film. Biosensors Bioelectronics 16: 937-943.
51. Mulchandani A, Chen W, Mulchandani P, Wang J, Rogers KP (2001) Biosensors for direct determination of organophosphate pesticides. Biosens Bioelectron 16: 225-230.
52. Mallat E, Barzen C, Abuknesha R, Gauglitz G, Barcelo D (2001) Fast determination of paraquat residues in water by an optical immunosensor and validation using capillary electrophoresis-ultraviolet detection. J Anal Chim Acta 427: 165-171.
53. Delehanty JB, Ligler FS (2002) A microarray immunoassay for simultaneous detection of proteins and bacteria. Anal Chem 74: 5681-5687.
54. Kumar P, Colston JT, Chambers JP, Rael ED, Valdes JJ (1994) Detection of botulinum toxin using an evanescent wave immunosensor. Biosens Bioelectron 9: 57-63.
55. Mastovska K (2011) Multiresidue analysis of antibiotics in food of animal origin using liquid chromatography-mass spectrometry. Methods Mol Biol 747: 267-307.
56. Pecorelli I, Bibi R, Fioroni L, Galarini R (2004) Validation of a confirmatory method for the determination of sulphonamides in muscle according to the European Union regulation 2002/657/EC. J Chromatogr A 1032: 23-29.
57. Bjorland J, Odegaard SA, Waage S, Yndestad M, Hormazabal V (1998) Concentrations of benzyl penicillin in milk of dairy cows after intra mammary administration. Norsk Veterinaer Tidsskrift 10: 5-11.
58. Medina M, González D, Ramírez A (2008) Detection of antimicrobial residues in animal tissues and tetracyclines in bones. Revista de Salud Animal 30: 110-115.
59. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, et al. (1998) Emergence of multidrug-resistant Salmonella enterica serotype typhimurium DT104 infections in the United States. N Engl J Med 338: 1333-1338.
60. Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, et al. (2000) Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N Engl J Med 342: 1242-1249.
61. Tsiboukis D, Sazakli E, Jelastopulu E, Leotsinidis M (2013) Anthelmintics residues in raw milk. Assessing intake by a children population. Polish J of Vet Scie 16: 85-91.
62. EU (2010) European Union Commission Regulation No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
63. Tsiboukis D, Sazakli E, Gortzi O, Hadjichristodoulou C, Matara C, et al. (2010) Assessing quality of raw milk in southern Greece in the aspect of certain benzimidazole residues. Food Addit Contam Part B Surveill 3: 73-79.
64. Horrigan L, Robert SL, Walker P (2002) How Sustainable Agriculture can Address the Environmental and Human Health Harms of Industrial Agriculture. Environ Health Perspect 110: 445-456.
65. WHO (2002) Evaluation of certain mycotoxins in food. Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organ Tech Rep Ser 906: 1-62.
66. Jahed KGR (2005) A Model for Application of Hazard Analysis Critical Control Point (HACCP) system in production of Iranian white cheese stored in salt brine. Proceedings of the 8th National Congress on Environmental Health, Tehran, Iran, pp: 333.
67. Volkmer BG (2005) Influence of nitrate levels in drinking water on urological malignancies: a community-based cohort study. BJU Int 95: 972-976.
68. Dawd AG, Gezmu TB, Haki GD (2012) Essential and Toxic Metals in cow's whole milk from selected sub-cities in Addis Ababa, Addis Ababa University, Center for Food Science and Nutrition, Ethiopia. Inter J of Food Science 1: 12-19.
69. Admasu E, Chandravanshi BS, Wondimu T, Akalu G (2005) Levels of some nutrients and non-essential metals in cow's whole milk delivered to Addis Ababa, Ethiopia. Msc Thesis, School of Graduate Studies, Addis Ababa University, Addis Ababa, Ethiopia.

70. CAC (2009) Codex Alimentarius Commission, Code Of Hygienic Practice For Milk and Milk Products. Milk and Milk Product (2nd edn.), CAC/GL.71-2009.

71. Hartzell GE (1996) Overview of combustion toxicology. Toxicology 115: 7-23.

72. Sischo WM, Kiernan NE, Burns CM, Byler LI (1997) Implementing a quality assurance program using a risk assessment too on dairy operations. J Dairy Sci 80: 777-787.

73. Europian Commission (1997) Regulation Laying Down A Community Procedure for the Establishment of Maximum Residue Limits of Veterinary Medical Products in Foodstuffs of Animal Origin. Offic J 224: 1-8.

74. Tennant DR (1997) Food Chemical Risk Analysis. Blakie Academic and Professional. (1st edn.), Chapman and Hall, UK, pp: 381-398.

75. Tripathi RM, Raghunath R, Sastry VN, Krishnamoorthy TM (1999) Daily intake of heavy metals by infants through milk and milk products. Sci Total Environ 227: 229-235.