REVIEW

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Determination of safe levels and toxic levels for feed hazardous materials in broiler chickens: a review

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Abstract

Feed safety is needed to produce and provide safe animal feeds for consumers, animals, and the environment. Although feed safety regulations have been set for each country, there is a lack of clear feed safety regulations for each livestock. Feed safety regulations are mainly focused on heavy metals, mycotoxins, and pesticides. Each country has different safe levels of hazardous materials in diets. Safe levels of hazardous materials in diets are mostly set for mixed diets of general livestock. Although there is a difference in the metabolism of toxic materials among animals, the safe level of feed is not specific for individual animals. Therefore, standardized animal testing methods and toxicity studies for each animal are needed to determine the correct safe and toxic levels of hazardous materials in diets. If this goal is achieved, it will be possible to improve livestock productivity, health, and product safety by establishing appropriate feed safety regulations. It will also provide an opportunity to secure consumer confidence in feed and livestock products. Therefore, it is necessary to establish a scientific feed safety evaluation system suitable for each country's environment. The chance of outbreaks of new hazardous materials is increasing. Thus, to set up appropriate toxic levels or safe levels in feed, various toxicity methods have been used to determine toxic levels of hazardous materials for humans and animals. Appropriate toxic testing methods should be developed and used to accurately set up and identify toxicity and safe levels in food and feed.

Keywords: Broiler chicken, Feed safety, Heavy metal, Mycotoxin, Pesticide, Toxicity

INTRODUCTION

Feed safety (i.e., safe feeds) is an essential prerequisite for producing and supplying animal products that are safe for consumers, animals, and the environment [1]. First, the demand for animal products is increasing due to the rapid increase of world population. Therefore, it is necessary to produce safe animal products for consumers because more animal products are required. Moreover, failure of controlling feed safety can lead consumers to have an increasing distrust of animal products, which will have a devastating effect on the livestock industry. Second, feed safety for animals needs to maintain animal welfare and health. Safe feeds can lead to appropriate animal production as well as animal health. Third,

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environmental pollution is an important issue because some feed materials are excreted to the environment such as soil and water. Therefore, safe feeds can minimize environmental pollution. Fourth, contamination of mycotoxins in feed can increase due to recent global warming. For this reason, safety management of mycotoxins in feed is important in hot and humid summers. Finally, the utilization of alternative feed resources is increasing because of rising feed ingredient prices. Therefore, it is necessary to secure safety of alternative feed resources. These problems are increasing the importance of safety management of feed due to changes in environmental conditions and the livestock industry. Therefore, all three factors relating to consumers, animals, and the environment are major issues in feed safety.

FEED SAFETY SYSTEMS IN VARIOUS COUNTRIES

Various countries have adopted their own feed safety regulations to produce safe animal feeds. Feed safety regulations are mainly focused on heavy metals, mycotoxins, and pesticides that frequently contaminate feed ingredients and become public concerns. Each country has different safe levels of hazardous materials in feed. Tables listed below show target hazardous materials and their safe levels for specific animals or general livestock from various countries such as Republic of Korea (Table 1), Japan (Table 2), Canada (Table 3), EU (Table 4), and US (Table 5).

TYPES OF FEED HAZARDS

Feed hazards such as heavy metals, pesticides, and melamine are toxic to animals and even humans after consuming contaminated animal products. Mycotoxins in feed have been extensively issued around the world.

Heavy metals

Heavy metals include arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium, zinc (Zn), manganese (Mn), cobalt (Co), and so on. Essential heavy metals, such as Zn, Mn, and Co are required for biological metabolism as a cofactor in enzymes for humans and animals [2]. On the other hand, some heavy metals such as As, Pb, Hg, and Cd are harmful to humans and animals when they consume high amounts of these heavy metals [3–5]. Moreover, WHO [6] has reported that As, Pb, Hg, and Cd are among the 10 hazardous chemicals of major public health concerns. These heavy metals are hazardous materials for animals due to the fact that they are not readily metabolized or excreted from the body [7,8]. Therefore, these heavy metals can lead to serious diseases and even death of humans and animals [6,9,10]. In general, As is easily found and distributed in rocks and soils. It is extensively used as pigments, glass, rodenticides, pesticides, and fungicides [11]. Water-soluble forms of As can be easily absorbed by the digestive system [12]. Thus, As is accumulated rapidly in tissues such as the liver, kidney, lung, and gastrointestinal tract. Until now, Pb is widely used in industrial materials such as pesticides, paint pigments, crystal glass production, and plumbing. However, Pb is one of the environmental pollutants that threaten humans and animals. In addition, Pb can cause biochemical problems, physiological problems, and behavioral dysfunctions in humans and animals [13]. When animals consume Pb-contaminated feeds, excretions of Pb are considerably slower than those of other heavy metals [7]. Moreover, Pb is usually deposited in the kidney, bone, and liver because of its slow excretion [7,12,14]. High concentrations of Pb in the bone are likely to be associated with the fact that Pb shows a great affinity for the bone by substituting calcium in the bone [7,15]. Thus, Pb might induce substantial adverse effects on performance and health. Also, Pb can accumulate in the organ of animals when

Table 1. Feed safety regulations of Republic of Korea[1)]

Hazardous materials	Feed type	Animal type	Safe level
Heavy metals			
Lead	Complete feed	Livestock	10 mg/kg
Fluorine	Complete feed	Chicken	250 mg/kg
Arsenic	Complete feed	Livestock	10 mg/kg
Mercury	Complete feed	Livestock	0.8 mg/kg
Cadmium	Complete feed	Livestock	2 mg/kg
Chromium	Complete feed	Fish	100 mg/kg
Mycotoxins			
Aflatoxin	Complete feed	Livestock	10 μg/kg
Ochratoxin A	Complete feed	Livestock	200 μg/kg
Pesticides			
Glyphosate	Complete feed	Livestock	5 mg/kg
Diazinon	Complete feed	Livestock	5 mg/kg
DDT	Complete feed	Livestock	0.5 mg/kg
Dimethoate	Complete feed	Livestock	1 mg/kg
Methomyl	Complete feed	Livestock	10 mg/kg
Fipronil	Complete feed	Livestock	0.02 mg/kg
Chlorpyrifos	Complete feed	Livestock	2.5 mg/kg
Fenthion	Complete feed	Livestock	1 mg/kg
Carbaryl	Complete feed	Livestock	5 mg/kg
Carbendazim	Complete feed	Livestock	20 mg/kg
Carbofuran	Complete feed	Livestock	0.2 mg/kg
Qintozene	Complete feed	Livestock	0.02 mg/kg
Terbufos	Complete feed	Livestock	0.3 mg/kg
Others[2)]			

[1)]Data from Ministry of Agriculture, Food and Rural Affairs [91].

[2)]There are 108 more pesticides.

DDT, dichloro-diphenyl-trichloroethane.

animals consume high amounts of Pb [4,16,17]. Commonly, Hg is a highly toxic material and is widely used for industrial materials such as fluorescent lamps, thermometers, electronics, and medicines. Recently, consumers have increased concerns on poisoning from Hg in agriculture. Excessive Hg exposure can induce disorders in renal function, hepatic functions, and nervous systems [18]. The major toxic effects of Hg often occur in the kidney because Hg causes necrosis of proximal tubular cells and demethylation [19,20]. In addition, Hg easily can enter the animal body via the gastrointestinal tract, respiratory system, and skin [21]. Thus, prolonged Hg ingestion or excessive exposure could induce detrimental effects on animal performance and health [3,22]. The Cd is produced through industrial activities, waste disposal, and fertilizer production. Humans and animals can be easily contaminated by Cd-contaminated food, feed, and soil. Excessive or chronic Cd exposure can induce histopathological damage, kidney and liver damage, and performance reduction in animals [9,23]. Thus, Cd might cause negative effects on animal performance and health when animals consume high concentrations of Cd in diets.

Pesticides

For many years, pesticides have been used for agricultural farming around the world. Up to now,

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Table 2. Feed safety regulations of Japan[1)]

Hazardous materials	Feed type	Animal type	Safe level
Heavy metals			
Arsenic	Complete feed	Livestock	2 mg/kg
Cadmium	Complete feed	Livestock	1 mg/kg
Lead	Complete feed	Livestock	3 mg/kg
Mercury	Complete feed	Livestock	0.4 mg/kg
Mycotoxins			
Aflatoxin B_1	Complete feed	Livestock	20 μg/kg
Deoxynivalenol	Complete feed	Livestock	1 mg/kg
		Older cattle	4 mg/kg
Zearalenone	Complete feed	Livestock	1 mg/kg
Pesticides			
Aldrin	Complete feed	Livestock	20 μg/kg
BHC	Complete feed	Livestock	5 μg/kg
DDT	Complete feed	Livestock	100 μg/kg
Endrin	Complete feed	Livestock	10 μg/kg
Fenvalerate	Complete feed	Poultry	500 μg/kg
		Pig	4 mg/kg
		Ruminant	8 mg/kg
Fipronil	Complete feed	Poultry	10 μg/kg
		Pig	20 μg/kg
Heptachlor	Complete feed	Livestock	20 μg/kg
Lindane	Complete feed	Livestock	50 μg/kg
Others			
Melamine	Complete feed	Livestock	2.5 mg/kg

[1)]Data from Food and Agricultural Materials Inspection Center [92,93].
BHC, benzene hexachloride; DDT, dichloro-diphenyl-trichloroethane.

pesticides are widely used in crop production, insect control, and forest protection to minimize infestations from insect pests in agriculture. In the animal industry, pesticides have been used to control insects. For instance, poultry red mite (*Dermanyssus gallinae*) is a blood-sucking pest that often causes problems for laying hens [24]. These mites show excessive multiplication and cause constant stress in laying hens, which can lead to decreased egg production and even death. In the case of broiler chickens, workers clean and disinfect the inside after completely emptying the broiler chicken house. Therefore, pesticides are needed to produce good quality crops, increase animal productivity, and improve animal health and welfare. However, excessive use of pesticides on animals can lead to contamination animal products such as meat, milk, and eggs by pesticides. Because pesticides are lipophilic, they can accumulate easily in fat depots of animals and their products [25]. Thus, broiler chickens are likely to be contaminated with pesticides through disinfection of the broiler chicken house, bedding materials, and contaminated feed [26,27]. For this reason, broiler chicken meat contaminated with pesticides can cause major problems in meat safety and human health. In addition, many countries face serious problems of eggs contaminated by pesticides such as fipronil [28]. Currently, many poultry scientists are making efforts to find ways of raising laying hens without using pesticides to control poultry mites.

Table 3. Feed safety regulations of Canada[1)]

Hazardous materials	Feed type	Animal type	Safe level
Heavy metals			
Aluminum	Complete feed	Livestock	3.2 mg/kg
Arsenic	Complete feed	Livestock	5.2 mg/kg
Cadmium	Complete feed	Livestock	0.05 mg/kg
Lead	Complete feed	Livestock	5 mg/kg
Mycotoxins			
Aflatoxin	Complete feed	Livestock	20 μg/kg
Deoxynivalenol	Complete feed	Cattle and poultry	5 mg/kg
		Pig, young calves, and lactating dairy animal	1 mg/kg
HT-2 toxin	Complete feed	Cattle and poultry	0.1 mg/kg
		Dairy animal	25 μg/kg
Diacetoxyscirpenol	Complete feed	Swine	2 mg/kg
		Poultry	1 mg/kg
T-2 toxin	Complete feed	Pig and poultry	1 mg/kg
Zearalenone	Complete feed	Gilt	1–3 mg/kg
		Cow	10 mg/kg
		Sheep and pigs	0.25–5 mg/kg
Ochratoxin A	Complete feed	Pig	0.2–2 mg/kg
		Poultry	2 mg/kg
Ergot	Complete feed	Ruminant	2–3 mg/kg
		Pig	4–6 mg/kg
		Chick	6–9 mg/kg

[1)]Data from Canadian Food Inspection Agency [94].

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi in human foods and animal feeds [29]. More than 200 types of mycotoxins have been identified. Aflatoxin (AF) and deoxynivalenol (DON) are well-known mycotoxins that frequently contaminate animal feeds [30]. Animal feeds contaminated by these mycotoxins can exert adverse effects on animal health, productivity, and mycotoxin residues in tissues [29]. Especially, mycotoxin residues in livestock tissues can cause problems for human health when humans consume contaminated livestock products [29]. Fungal growth and subsequent mycotoxin contamination are determined by various factors, including seasons, drought, location of grain cultivation, and harvesting time [31]. Mycotoxins contamination alone or in combination with pathogens has been associated with a variety of diseases, called "mycotoxicoses" [32]. The AF is produced by the genus *Aspergillus* [33]. Toxigenic *Aspergillus flavus* can produce AF B_1 and B_2 [34]. Toxigenic *Aspergillus parasiticus* can produce AF B_1, B_2, G_1, and G_2 [34]. Negative effects of AF on hepatic necrosis through free-radical production, lipid peroxidation, and inhibition of RNA and protein synthesis have been reported in various animals [35]. The DON is a secondary metabolite produced by *Fusarium graminearum* or *Fusarium culmorum* [36]. The DON mainly contaminates corn and wheat during growing and harvesting periods [37]. Although poultry is less sensitive to DON than other animals, feeding DON-contaminated corn to poultry could cause growth depression, health problems, and reproductive failures. The DON inhibits protein synthesis, and thus, cells and tissues with high protein synthesis are more damaged by DON contamination. These tissues include the small intestine, liver, bone marrow, lymph nodes,

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Table 4. Feed safety regulations of EU[1)]

Hazardous materials	Feed type	Animal type	Safe level
Heavy metals			
Arsenic	Complete feed	Livestock	2 mg/kg
Lead	Complete feed	Livestock	5 mg/kg
Fluorine	Complete feed	Ruminant	30–50 mg/kg
		Pig	100 mg/kg
		Poultry	350 mg/kg
Mercury	Complete feed	Dog and cat	0.4 mg/kg
		Others	0.1 mg/kg
Cadmium	Complete feed	Ruminant	1 mg/kg
Mycotoxins			
Aflatoxin B_1	Complete feed	Ruminant	50 μg/kg
		Pigs and poultry	20 μg/kg
Pesticides			
Aldrin	Complete feed	Livestock	10 μg/kg
Dieldrin	Complete feed	Livestock	0.2 mg/kg
Camphechlor	Complete feed	Livestock	0.1 mg/kg
Chlordane	Complete feed	Livestock	20 μg/kg
DDT	Complete feed	Livestock	20 μg/kg
Endosulfan	Complete feed	Livestock	0.1 mg/kg
Others			
Nitrites	Complete feed	Pet	15 mg/kg
Hydrocyanic acid	Complete feed	Chick	10 mg/kg
		Others	50 mg/kg
Free gossypol	Complete feed	Poultry	100 mg/kg
		Pig	60 mg/kg

[1)]Data from Official Journal of the European Communities [95].

spleen, thymus, and intestinal mucosa [36,38,39].

Melamine

Melamine is a 6-nitrogen-containing organic compound and contains 67% nitrogen. Melamine is widely used for various industry such as varnishes, paints, glues, and plastic packages. Therefore, melamine can be exposed to human food and animal feed. Previous research has reported the occurrence of kidney problem and deaths in infants and animals after eating foods and feeds contaminated by melamine [40–42]. In 2007, renal failure in dogs and cats fed melamine-contaminated pet food happened in US [40]. In 2008, a rapid increase in the number of renal failures in infants was also reported in China due to melamine fraudulently added to human foods and animal diets to increase their apparent Crude protein concentrations [42]. Therefore, melamine is known to be an extremely hazardous material worldwide. Melamine can be eliminated through urine in animals [43]. In addition, more than 90% of ingested melamine can be excreted within 24 h [43–45]. When humans and animals have prolonged melamine ingestion or excessive exposure, melamine might exert extremely negative effects on humans' health and animals' performance and health [40,46,47]. The reason for these symptoms is that melamine can be combined with cyanuric acid to form crystals in the kidney, resulting in renal failure in humans and animals [48]. In addition, high melamine intake can increase diarrhea, polyuria, and proteinuria in animals with

Table 5. Feed safety regulations of US[1)]

Hazardous materials	Feed type	Animal type	Safe level
Heavy metals			
Mercury	Complete feed	Aquatic animal	1 mg/kg
Mycotoxins			
Aflatoxin	Complete feed	Finishing pig	200 mg/kg
		Beef cattle, breeding pig, and mature poultry	100 mg/kg
		Immature animal	20 mg/kg
Fumonisin	Complete feed	Poultry	30 or 100 mg/kg
Deoxynivalenol	Grains	Chicken	10 mg/kg
Pesticides			
Aldrin	Complete feed	Livestock	30 μg/kg
Dieldrin	Complete feed	Livestock	30 μg/kg
BHC	Complete feed	Livestock	50 μg/kg
Chlordane	Complete feed	Livestock	100 μg/kg
Dicofol	Complete feed	Livestock	500 μg/kg
DDT	Complete feed	Livestock	500 μg/kg
Heptachlor	Complete feed	Livestock	30 μg/kg
Lindane	Complete feed	Livestock	100 μg/kg
Others			
Melamine	Complete feed	Livestock	2.5 mg/kg

[1)]Data from U.S. Food & Drug Administration [46,96–98].

renal problems [41].

NEGATIVE EFFECTS OF FEED HAZARDS

Growth performance

The body weight (BW), body weight gain (BWG), feed intake (FI), feed efficiency (FE, BWG/FI), and feed conversion ratio (FCR, FI/BWG) are sensitive and useful indicators of general animal health. In addition, mortality is one of the toxic indicators that can detect severe toxicity. Regarding heavy metals, Jahromi et al. [17] observed that 129-d-old male broiler chickens fed diets containing 200 mg/kg Pb had less BW, FI, and FE than those fed the control diet. Seven et al. [16] demonstrated that feeding diets containing 200 mg/kg Pb decreased BWG, FI, and FE of 42-d-old broiler chickens. However, Kim et al. [4] reported that BW, BWG, FI, FE, and mortality of 35-d-old broiler chickens were not influenced by increasing inclusion levels of Pb from 0 to 400 mg/kg in diets. In addition, Erdoğan et al. [49] reported no significant difference in FI and FCR of 42-d-old broiler chickens fed diets containing 200 mg/kg Pb. Kim et al. [3] reported that increasing inclusion levels of Hg from 0 to 500 mg/kg in diets decreased BW, BWG, FI, and mortality of 35-d-old broiler chickens. Parkhurst and Thaxton [22] observed that 35-d-old broiler cockerels fed drinking water containing greater than 250 mg/kg Hg had less BW and FI compared with those drinking water containing less than 250 mg/kg Hg. Feeding diets containing 2.5 μg/kg AF decreased BW of 21-d-old broiler chickens [50]. Kim et al. [51] observed that increasing inclusion levels of DON-contaminated distiller's dried grains with soluble (DDGS) in diets decreased BW, BWG, and FE of 28-d-old broiler chickens. Previous research has reported that feeding diets containing more than 10,000 mg/kg melamine decreased BW and FI of 21-d-old boiler chickens [52]. In addition,

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broiler chickens fed diets containing more than 25,000 mg/kg melamine had greater mortality than less than those fed diets containing less than 20,000 mg/kg melamine. Kim et al. [47] reported that feeding diets containing 10,000 mg/kg melamine decreased BW, BWG, and FI of 35-d-old male and female broiler chickens. Therefore, approximate toxic effects of hazardous materials can be determined based on changes in the growth performance of broiler chickens.

Hazardous material residue in tissues

The liver and kidney are known as the main organs with accumulation of hazardous materials [53,54]. In addition, these two organs are responsible for the metabolism and excretion of ingested hazardous materials [54]. The feather has also been widely used as an indicator for the assessment of hazardous materials [55–57]. Moreover, meat is one of the most important target organs for human foods. It can be a big problem because humans eat meat that might have been directly contaminated by hazardous materials. A previous study has reported that 42-day-old boiler chickens fed diets containing 200 mg/kg Pb show increased Pb residues in the liver and kidney [49]. Kim et al. [4] observed that increasing inclusion levels of Pb from 0 to 400 mg/kg in diets increased Pb residues in the liver, breast meat, and feather of 35-day-old broiler chickens. Kim et al. [3] reported that increasing inclusion levels of Hg from 0 to 500 mg/kg in diets increased Hg residues in the liver, breast meat, and feather of 35-day-old broiler chickens. Melamine residues in the kidney and breast are increased in broiler chickens fed diets containing greater than 10,000 mg/kg melamine than those fed a control diet [52]. Kim et al. [47] reported that feeding diets containing 10,000 mg/kg melamine increased melamine residues in the kidney and breast meat of 35-day-old male and female broiler chickens.

Blood measurements

The blood concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphate, total protein, albumin, and globulin have been widely used as indicators for liver functions [58,59]. The blood concentrations of creatinine and uric acid have been commonly applied as indicators of health status of the kidney [59,60]. Seven et al. [16] demonstrated that 42-day-old Ross broiler chickens fed diets containing 200 mg/kg Pb had greater the blood concentrations of AST than those fed a control diet. Kim et al. [4] reported that the serum concentrations of AST, creatinine, and uric acid were not influenced by increasing inclusion levels of Pb from 0 to 400 mg/kg in broiler diets. Ding et al. [61] reported that birds fed diets containing 100 mg/kg melamine and 33.3 mg/kg cyanuric acid had greater the serum concentrations of uric acid than those fed diets containing less than 100 mg/kg melamine and/or 33.3 mg/kg cyanuric acid. The serum concentrations of albumin, total protein, globulin, AST, and GGT were increased for 21-day-old broiler chickens fed diets containing 30,000 mg/kg melamine than for those fed diets containing less than 25,000 mg/kg melamine [52]. However, Kim et al. [47] observed that the plasma concentrations of ALT, AST, GGT, and total protein were not influenced by increasing inclusion levels of melamine from 0 to 10,000 mg/kg in broiler diets.

Antioxidant capacity

Reactive oxygen species are produced when tissue's oxidant and antioxidant balance is disrupted [62]. To protect against oxidative stress, all living organisms have evolved with antioxidant systems of both enzymatic and non-enzymatic mechanisms [63]. Major antioxidant enzymes are superoxide dismutase, catalase (CAT), and glutathione peroxidase (GPx), whereas glutathione concentration (GSH) is a non-enzymatic antioxidant [64]. Seven et al. [16] reported that birds fed diets containing 200 mg/kg Pb had greater CAT activities in the liver and kidney compared

with those fed a control diet. In addition, feeding diets containing 200 mg/kg Pb increased GSH activities in the plasma and heart of broiler chickens compared with feeding the control diet. The GPx activities in the liver were increased in 42-day-old broiler chickens fed diets containing 10 mg/kg Pb compared with those fed a basal diet [65].

METHODS FOR TOXICITY TESTING

Acute toxicity testing includes various protocols for LD_{50} testing, eye irritancy testing, skin irritancy testing, and other organ testing [66]. Short-term toxicity studies with rodents and non-rodents [67,68] are commonly performed for 14 or 28 days. The OECD guideline [69] recommends acute toxicity testing with a stepwise toxicity testing method using three animals of only one sex (male or female). Results of acute toxicity testing include measurements (e.g., BW) and daily detailed observations (e.g., pathology). Lipnick et al. [70] reported that females were commonly more sensitive to a conventional LD_{50} test than males. However, males can be more sensitive if we have information on higher sensitivity of males to structural and toxicological properties of hazardous materials. Therefore, it is desirable to use only one sex for toxicity testing. Acute toxicity testing is usually the initial step of testing toxic effects of hazardous materials. Its main objective is to provide information on potentially toxic effects of hazardous materials during their short-term exposure [71]. The first survey can be performed to predict the extent of toxicity range. Acute toxicity is achieved by administering one or a few doses of hazardous materials to animals. In addition, at least 2 to 4 times repeated-steps might be required to determine the acute toxicity of hazardous materials [69,71].

Sub-chronic toxicity testing is normally conducted for 90 days [72,73]. This test is required to study adverse effects of repeated exposure over a relatively long term of an experimental animal. Although target compounds are not toxic based on acute toxic tests, some compounds might be toxic after a long-term exposure at a low dose due to a potential increase in accumulation, changes in enzyme levels, and disruption of physiologic and biochemical homeostasis. The OECD [74–76] has reported detailed methods for sub-chronic toxicity testing. Sub-chronic oral, dermal, or inhalation toxicity may be determined based on initial information on toxicity identified in acute toxicity tests. At least 20 animals (10 males and 10 females) should be allotted to each group. At least three graded concentrations of hazardous materials should be applied. Hazardous materials are administered to animals via feeding, drinking water, or gavage. Results of sub-chronic toxicity testing include general measurements (e.g., BW, BWG, FI, and water intake) and daily and detailed observations (e.g., ophthalmological examination, haematology, clinical biochemistry, urinalysis, pathology, and histopathology).

Chronic toxicity testing is performed by administering hazardous materials for more than 12 months [77]. Chronic toxicity testing checks mutagenic, teratogenic, and carcinogenic effects [78]. Chronic toxicity testing offers prediction with regard to long-term toxic effects of hazardous materials in animals, and it may be concluded to the human safe level of the hazardous materials. The OECD [79] has reported detailed methods of chronic toxicity testing. Chronic toxicity testing can be performed using oral, dermal, and inhalation routes. In general, chronic toxicity testing is performed through oral administration. Hazardous materials are commonly administrated to animals via feeds, drinking water, or gavage. In particular, nutritional imbalance should not occur. Hazardous materials are administrated to experimental animals for approximately 12 months. The experimental period can change to be shorter (e.g., 6 or 9 months) or longer (e.g., 18 or 24 months). The experimental period can be adjusted based on requirements of particular regulatory regimes or for specific biological mechanisms. Results of chronic toxicity testing include general measurements

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(e.g., weighing at least once a week, FI and water intake, and FE) and daily detailed observations (e.g., ophthalmological examination, haematology, clinical biochemistry, urinalysis, and pathology).

Toxicity testing methods in U.S. Food & Drug Administration (FDA)

FDA's guidelines for toxicity studies are shown in Table 6. Short-term toxicity studies with rodents and non-rodents [67,68] are commonly carried out for 14 or 28 days. Results of the toxicity study could provide initial information for further sub-chronic or chronic toxicity studies. Hazardous materials are administered to animals via feed, drinking water, or gavage. Subchronic toxicity studies with rodents and non-rodents [72,73] are usually carried out for 90 days. Results of subchronic toxicity studies can also be used for predicting target doses of hazardous materials for future chronic toxicity studies. Chronic toxicity studies with rodents [77] should be conducted for at least 12 months. Results of chronic toxic study can be used to identify the toxicity of a food ingredient when the ingredient is fed to humans or other animals for a long period of time. Both control and treatment groups should have at least 20 rodents per sex per treatment group. In all studies, the same number, sex, species, and strains should be used. Excessive mortality due to poor animal management should be avoided. If there is a very high mortality, the study is required to be repeated. Experimental animals should be weighed at least once a week. FI and water intake should be recorded and measured weekly during the whole toxicity study. Clinical problems including opthalmological examination, hematology, clinical chemistry, urinalyses, neurotoxicity testing, and immunotoxicity can be examined.

Toxicity testing methods in Organization for Economic Cooperation and Development (OECD)

OECD guidelines for toxicity testing of hazardous materials (chemicals) have a set of globally established specifications (Table 7). OECD guidelines refer to acute toxicity testing including procedures for acute oral toxicity [80], acute dermal toxicity: fixed dose procedure [81], acute inhalation toxicity [82], acute dermal irritation/corrosion [83], acute eye irritation/corrosion [84], acute oral toxicity – fixed dose procedure [85], acute oral toxicity – acute toxic class method [69], and acute oral toxicity: up-and-down procedure [86]. Detailed methods are presented in Table 7.

Table 6. U.S. Food & Drug Administration (FDA)'s guideline for the toxicity studies[1)]

Name	Species	Number	Sex	Exposure
Acute toxicity studies				
Short-term toxicity studies with rodents	Rodents (usually rats and mice)	10 rodents per sex per group	Both	14 or 28 days
Short-term toxicity studies with non-rodents	Non-rodents (usually dogs)	2 or 4 non-rodents per sex per group	Both	14 or 28 days
Sub-chronic toxicity studies				
Subchronic toxicity studies with rodents	Rodents (usually rats and mice)	20 rodents per sex per group	Both	90 days
Subchronic toxicity studies with non-rodents	Non-rodents (usually dogs)	4 non-rodents per sex per group	Both	90 days
Chronic toxicity studies				
Chronic toxicity studies with rodents	Rodents (usually rats and mice)	10 or 20 rodents per sex per group	Both	12 months
One-year toxicity studies with non-rodents	Non-rodents (usually dogs)	4 non-rodents per sex per group	Both	12 months

[1)]Data from U.S. Food & Drug Administration [67,68,72,73,77,99].

Table 7. Organization for Economic Co-operation and Development (OECD)'s guideline for the testing of chemicals[1)]

No.	Name	Species	Number	Sex	Exposure
Acute toxic studies					
401	Acute oral toxicity	Mammalian	5 males and 5 females	Both	< 24 hours
402	Acute dermal toxicity: fixed dose procedure	Adult rat or healthy young adult animals	Sequential manner with 2 animals	Female	< 24 hours
403	Acute inhalation toxicity	Healthy young adult animals	5 males and 5 females	One or both	4 - 6 hours
404	Acute dermal irritation/corrosion	Albino rabbit or healthy young adult rabbits	-	-	4 hours
405	Acute eye irritation/corrosion	Albino rabbit or healthy young adult rabbits	-	-	8 – 24 hours
420	Acute oral toxicity – fixed dose procedure	Rodent species or healthy young animals	5 animals	Female	< 24 hours
423	Acute oral toxicity – acute toxic class method	Rodent species or healthy young animals	6 animals (3 animals per step)	Female	< 24 hours
425	Acute oral toxicity: up-and-down procedure	Rodent species or healthy young adult animals	5 animals	Female	< 24 hours
Sub-chronic toxic studies					
407	Repeated dose 28-day oral toxicity study in rodents	Rat or other rodents	5 females and 5 males	Both	28 days
408	Repeated dose 90-day oral toxicity study in rodents	Rat or other rodents	10 females and 10 males	Both	90 days
409	Repeated dose 90-day oral toxicity study in non-rodents	Dogs, pigs, or mini-pigs	4 females and 4 males	Both	90 days
410	Repeated dose dermal toxicity: 21/28-day study	Adult rat, rabbit, or guinea pigs	5 females and 5 males	Both	21/28 days
411	Subchronic dermal toxicity: 90-day study	Adult rat, rabbit, or pigs	10 females and 10 males	Both	90 days
413	Subchronic inhalation toxicity: 90-day study	Healthy young adult rodents	10 females and 10 males	Both	90 days
Chronic toxic studies					
452	Chronic toxicity studies	Rodents	20 animals per sex per group	Both	12 months

[1)]Data from Organisation for Economic Co-operation and Development [69,74–76,79–86,100–102].

Toxicity testing methods in European Food Safety Authority (EFSA)

EFSA [87] has reported technical guidelines regarding tolerance and efficacy studies in target animals. The experimental design should be set based on hazardous materials and animal species. In addition, the experiment should be carried out considering health status and husbandry conditions of animals. The number of animals and replicates should be set within the permissible range of statistical analysis. The experimental design of a tolerance study should have at least three dietary groups: (1) a control group that should not contain hazardous materials, (2) a treatment group 1 that contains hazardous materials at the maximum concentrations in diets and/or water, and (3) treatment group 2 that contains 10-fold higher concentrations in diets and/or water as compared to treatment group 1. If 100-fold higher concentrations are tolerable, haematology or routine blood chemistry is not mandatory. If hazardous materials are tolerable only at a lower concentration, the study should be designed with a margin of safe levels for hazardous materials. The EFSA [87] has also set the experimental period for a tolerance study. The proposed period of the tolerance study is presented in Table 8. EFSA's technical guidance also includes bioavailability and bioequivalence testing methods for digestion studies, balance studies, and palatability studies.

Method for determining safe levels and toxic levels of feed hazards

Linear regression analysis is used to calculate the intercept and slope. Based on linear regression analysis (Fig. 1), anybody can estimate safe levels of hazardous materials by regressing dietary

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Table 8. European Food Safety Authority (EFSA)'s durations of the tolerance study[1)]

Animal	Approximate study period			Study duration	
	Start	Age	BW	Efficacy	Tolerance
Pigs					
Piglets (suckling)	Birth	21–42 days	6–11 kg	14 days	14 days
Piglets (weaned)	21–42 days	120 days	35 kg	42 days	42 days
Piglets (both)	Birth	120 days	35 kg	42 days	42 days
Pigs for fattening	≤ 35 kg	120–250 days	80–150 kg	> 70 days[1)]	42 days
Sows for reproduction	First[2)]	-	-	2 cycles	1 cycle
Poultry					
Chickens for fattening	Hatch	35 days	1.6–2.4 kg	35 days	35 days
Chickens reared for laying	Hatch	~ 16 weeks	-	112 days	35 days
Laying hens	16–21 weeks	~ 13 months	-	168 days	56 days
Turkeys for fattening	Hatch	~ 14 weeks	~ 7–10 kg	84 days	42 days
Turkeys for breeding	30 weeks	~ 60 weeks	-	6 months	56 days
Turkey reared for breeding	Hatch	30 weeks	~ 15 kg	Entire	42 days
Bovines					
Calves for rearing	Birth	4 months	145 kg	56 days	42 days
Calves for fattening	Birth	6 months	180 kg	> 84 days	28 days
Cattle for fattening	Full[3)]	10–36 months	350–700 kg	168 days	42 days
Dairy cows	-	-	-	84 days	56 days
Cows for reproduction	First[2)]	-	-	2 cycles	1 cycle

[1)]Until slaughter weight (Data from FEEDAP [87]).

[2)]First insemination.

[3)]Full development.

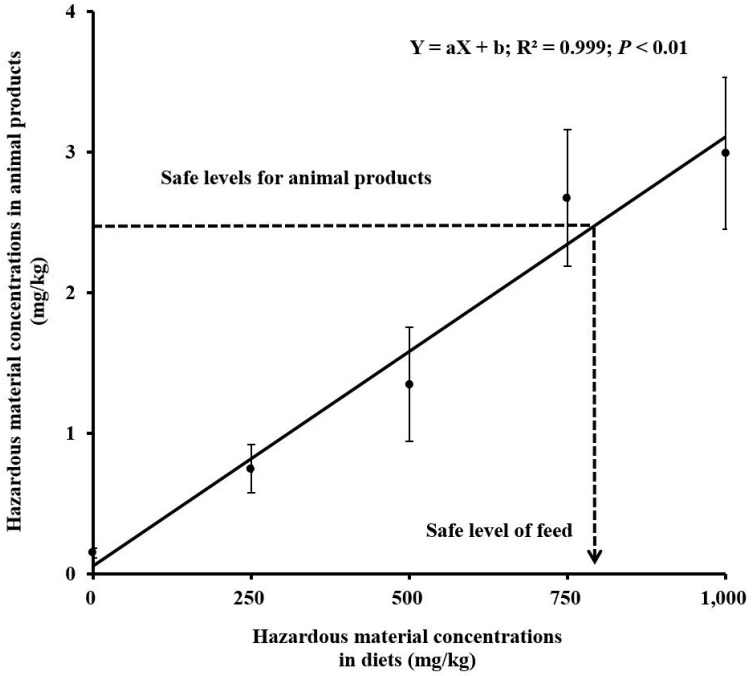

Fig. 1. Linear regression analyses to set the safe level of the hazardous materials in diets. The equation of linear regression analysis was Y = aX + b, where X is hazardous material concentrations and Y is hazardous material concentrations in animal products.

concentrations of hazardous materials to concentrations (i.e., residue) of animal products such as meats, milk, and eggs. For example, Kim et al. [47] have carried out a regression analysis and indicated a linear relationship between dietary melamine concentrations (x variables) in broiler diets and melamine concentrations in breast meat (y variables). Melamine was added to the basal diet at a concentration of 0, 250, 500, 750, or 1,000 mg/kg melamine. Results indicated that the linear regression was Y = 0.003X + 0.0583 (R^2 = 0.968; p < 0.01). Based on this equation, safe level of melamine concentrations in broiler diet was estimated to be 814 mg/kg when safe concentrations of melamine in the breast meat were set to be 2.5 mg/kg according to the international safe limit [46,88]. Therefore, a linear regression analysis can identify the safe level of livestock products based on hazardous material residues in animal products and the value (i.e., safe limits) set for food safety regulations.

A one-slope broken-line analysis [89,90] can estimate toxic levels of hazardous materials based on animal performance (Fig. 2). The first line remains in the plateau state and then shows breakpoint. At the breakpoint, the second line indicates a decreasing slope. This breakpoint provides the toxic level of a diet or water. In our previous experiment, for example, seven broiler diets were formulated to provide increasing melamine concentrations of 0, 250, 500, 750, 1,000, 5,000, or 10,000 mg/kg in diets. Results indicated that birds fed diets containing 10,000 mg/kg melamine had less BWG compared with those fed other diets. This result showed a toxic level of only 10,000 mg/kg melamine in diets for the productivity of broiler chickens. However, this result did not provide an accurate toxic level in diets based on animal performance because the toxic level was solely determined by how we set treatment levels. Thus, we predicted the toxic level of melamine in broiler diets based on BWG using a nonlinear regression (NLIN) procedure of SAS (SAS Institute., Cary, NC, USA). The one-slope broken-line analysis was as follows: Y = L + U × (X –

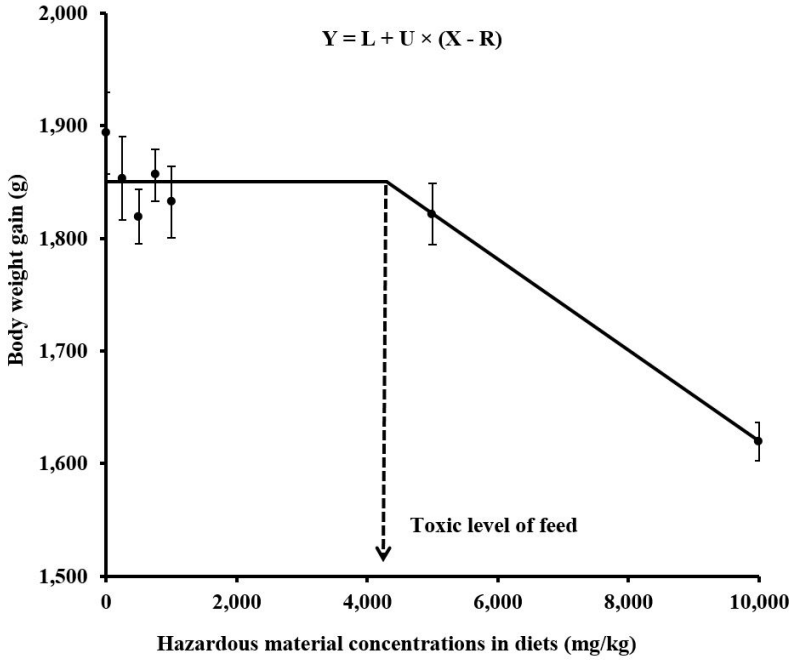

Fig. 2. The one-slope broken-line analysis of body weight gain (BWG) at different concentrations of hazardous materials in diets. The equation of one-slope broken-line analysis was Y = L + U × (X – R), where L is the maximum value of BWG (asymptote), U is the slope, X is hazardous material concentration in diets, and R is the toxic level of hazardous materials in diets (breakpoint x value).

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R), where L was the maximum value of BWG (asymptote), U was the slope, X was melamine concentration in diets, and R was the toxic level of melamine in diets (breakpoint x value). Results indicated that the equation was Y = 1,851 − 0.0404 × (X − 4,292). Based on this equation, the toxic level of melamine in broiler diets was predicted to be 4,292 mg/kg. Therefore, the one-slope broken-line analysis could be used to obtain accurate toxic levels of hazardous materials.

In conclusion, feed safety is an essential requirement for protecting humans, animals, and the environment. Institutions of various countries are responsible for protecting and promoting public health through animal feed safety regulations. There are many hazardous materials that threaten the safety of feed in the world. Hazardous materials that negate animal and human health include heavy metals, pesticides, mycotoxins, and others. The chance of outbreaks of new hazardous materials is increasing. To set up appropriate toxic levels (or safe levels), various toxicity methods can be used to determine toxic levels of hazardous materials for humans and animals. Appropriate toxic testing methods should be developed and used to accurately set up and identify toxicity and safe levels of hazardous materials in food and feed.

REFERENCES

1. den Hartog J. Feed for food: HACCP in the animal feed industry. Food Control. 2003;14:95-9. https://doi.org/10.1016/S0956-7135(02)00111-1
2. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: a review. Afr J Food Sci. 2010;4:200-22.
3. Kim JH, Lee HK, Park GH, Choi HS, Ji SY, Kil DY. Determination of the toxic level of dietary mercury and prediction of mercury intake and tissue mercury concentrations in broiler chickens using feather mercury concentrations. J Appl Poult Res. 2019;28:1240-7. https://doi.org/10.3382/japr/pfz090
4. Kim JH, Park GH, Han GP, Choi HS, Ji SY, Kil DY. Prediction of lead intake and tissue lead concentrations in broiler chickens using feather lead concentrations. Biol Trace Elem Res. 2020;193:517-23. https://doi.org/10.1007/s12011-019-01726-2
5. Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. Front Pharmacol. 2021;12:643972. https://doi.org/10.3389/fphar.2021.643972
6. WHO [World Health Organization]. Chemicals of major public health concern [Internet]. 2020 [cited 2023 Jan 6]. https://www.who.int/teams/environment-climate-change-and-health/chemical-safety-and-health/health-impacts/chemicals
7. Humphreys DJ. Effects of exposure to excessive quantities of lead on animals. Br Vet J. 1991;147:18-30. https://doi.org/10.1016/0007-1935(91)90063-S
8. Wu X, Cobbina SJ, Mao G, Xu H, Zhang Z, Yang L. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ Sci Pollut Res. 2016;23:8244-59. https://doi.org/10.1007/s11356-016-6333-x
9. Scheuhammer AM. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. Environ Pollut. 1987;46:263-95. https://doi.org/10.1016/0269-7491(87)90173-4
10. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003;68:167-82. https://doi.org/10.1093/bmb/ldg032
11. Gupta DK, Tiwari S, Razafindrabe BHN, Chatterjee S. Arsenic contamination from historical aspects to the present. In: Gupta D, Chatterjee S, editors. Arsenic contamination in the environment. Cham: Springer; 2017. p.1-12.
12. Govind P, Madhuri S. Heavy metals causing toxicity in animals and fishes. Res J Anim Vet

Fish Sci. 2014;2:17-23.
13. Kumar A, Singh N, Pandey R, Gupta VK, Sharma B. Biochemical and molecular targets of heavy metals and their actions. In: Rai M, Ingle A, Medici S, editors. Biomedical applications of metals. Cham: Springer; 2018. p. 297-319.
14. Choi H, Ji SY, Jo H, Song M, Kim BG. Excessive dietary lead reduces growth performance and increases lead accumulation in pigs. Anim Biosci. 2021;34:102-8. https://doi.org/10.5713/ajas.20.0220
15. DeMichele SJ. Nutrition of lead. Comp Biochem Physiol A Physiol. 1984;78:401-8. https://doi.org/10.1016/0300-9629(84)90567-X
16. Seven İ, Aksu T, Tatlı Seven P. The effects of propolis and vitamin C supplemented feed on performance, nutrient utilization and carcass characteristics in broilers exposed to lead. Livest Sci. 2012;148:10-5. https://doi.org/10.1016/j.livsci.2012.05.001
17. Jahromi MF, Liang JB, Ebrahimi R, Soleimani AF, Rezaeizadeh A, Abdullah N, et al. Protective potential of Lactobacillus species in lead toxicity model in broiler chickens. Animal. 2017;11:755–61. https://doi.org/10.1017/S175173111600224X
18. EFSA [European Food Safety Authority] Panel on Contaminants in the Food Chain (CONTAM). Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA panel on contaminants in the food chain. EFSA J. 2012;10:2985. https://doi.org/10.2903/j.efsa.2012.2985
19. Ware RA, Burkholder PM, Chang LW. Ultrastructural changes in renal proximal tubules after chronic organic and inorganic mercury intoxication. Environ Res. 1975;10:121-40. https://doi.org/10.1016/0013-9351(75)90078-X
20. Wolfe MF, Schwarzbach S, Sulaiman RA. Effects of mercury on wildlife: a comprehensive review. Environ Toxicol Chem. 1998;17:146-60. https://doi.org/10.1002/etc.5620170203
21. Friberg L, Vostal JJ. Mercury in the environment: an epidemiological and toxicological appraisal. Cleveland, OH: CRC Press; 1972.
22. Parkhurst CR, Thaxton P. Toxicity of mercury to young chickens: 1. effect on growth and mortality. Poult Sci. 1973;52:273-6. https://doi.org/10.3382/ps.0520273
23. Korénekova B, Skalická M, Nad P, Šály J, Korének M. Effects of cadmium and zinc on the quality of quail's eggs. Biol Trace Elem Res. 2007;116:103-9. https://doi.org/10.1007/BF02685923
24. Chirico J, Eriksson H, Fossum O, Jansson D. The poultry red mite, Dermanyssus gallinae, a potential vector of Erysipelothrix rhusiopathiae causing erysipelas in hens. Med Vet Entomol. 2003;17:232-4. https://doi.org/10.1046/j.1365-2915.2003.00428.x
25. Kahunyo JM, Maitai CK, Frøslie A. Organochlorine pesticide residues in chicken fat: a survey. Poult Sci. 1986;65:1084-9. https://doi.org/10.3382/ps.0651084
26. Kan CA. Prevention and control of contaminants of industrial processes and pesticides in the poultry production chain. Worlds Poult Sci J. 2002;58:159-67. https://doi.org/10.1079/WPS20020015
27. MacLachlan DJ . Physiologically based pharmacokinetic (PBPK) model for residues of lipophilic pesticides in poultry. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2010;27:302-14. https://doi.org/10.1080/19440040903296683
28. EFSA [European Food Safety Authority], Reich H, Triacchini GA. Occurrence of residues of fipronil and other acaricides in chicken eggs and poultry muscle/fat. EFSA J. 2018;16:5164. https://doi.org/10.2903/j.efsa.2018.5164
29. da Rocha MEB, Freire FCO, Maia FEF, Guedes MIF, Rondina D. Mycotoxins and their effects on human and animal health. Food Control. 2014;36:159-65. https://doi.org/10.1016/

https://doi.org/10.5187/jast.2023.e26

j.foodcont.2013.08.021
30. Ji C, Fan Y, Zhao L. Review on biological degradation of mycotoxins. Anim Nutr. 2016;2:127-33. https://doi.org/10.1016/j.aninu.2016.07.003
31. Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. Food Res Int. 2014;62:11-9. https://doi.org/10.1016/j.foodres.2014.02.023
32. Raju MVLN, Devegowda G. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). Br Poult Sci. 2000;41:640-50. https://doi.org/10.1080/713654986
33. Blunden G, Roch OG, Rogers DJ, Coker RD, Bradburn N, John AE. Mycotoxins in food. Med Lab Sci. 1991;48:271-82.
34. Cotty PJ. Influence of field application of an atoxigenic strain of Aspergillus flavus on the populations of A. flavus infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology. 1994;84:1270-7. https://doi.org/10.1094/Phyto-84-1270
35. McLean M, Dutton MF. Cellular interactions and metabolism of aflatoxin: an update. Pharmacol Ther. 1995;65:163-92. https://doi.org/10.1016/0163-7258(94)00054-7
36. Recharla N, Park S, Kim M, Kim B, Jeong JY. Protective effects of biological feed additives on gut microbiota and the health of pigs exposed to deoxynivalenol: a review. J Anim Sci Technol. 2022;64:640-53. https://doi.org/10.5187/jast.2022.e40
37. Cote LM, Beasley VR, Bratich PM, Swanson SP, Shivaprasad HL, Buck WB. Sex-related reduced weight gains in growing swine fed diets containing deoxynivalenol. J Anim Sci. 1985;61:942-50. https://doi.org/10.2527/jas1985.614942x
38. Feinberg B, McLaughlin CS. Biochemical mechanism of action of trichothecene mycotoxins. In: Beasley VR, editor. Trichothecene mycotoxicosis: pathophysiologic effects. Boca Raton, FL: CRC Press; 1989. p. 27-35.
39. Devegowda G, Murthy TNK. Mycotoxins: their effects in poultry and some practical solutions. In: Duarte DE, editor. The mycotoxin blue book. Nottingham: Nottingham University Press; 2005. p. 25-56.
40. Burns K. Researchers examine contaminants in food, deaths of pets. J Am Vet Med Assoc. 2007;231:1636-8. https://doi.org/10.2460/javma.231.11.1632
41. Dobson RLM, Motlagh S, Quijano M, Thomas Cambron R, Baker TR, Pullen AM, et al. Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicol Sci. 2008;106:251-62. https://doi.org/10.1093/toxsci/kfn160
42. WHO [World Health Organization]. Melamine-contaminated powdered infant formula in China – update 2 [Internet]. 2008 [cited 2023 Jan 6]. https://www.who.int/emergencies/disease-outbreak-news/item/2008_09_29a-en
43. Dorne JL, Doerge DR, Vandenbroeck M, Fink-Gremmels J, Mennes W, Knutsen HK, et al. Recent advances in the risk assessment of melamine and cyanuric acid in animal feed. Toxicol Appl Pharmacol. 2013;270:218-29. https://doi.org/10.1016/j.taap.2012.01.012
44. Mast RW, Jeffcoat AR, Sadler BM, Kraska RC, Friedman MA. Metabolism, disposition and excretion of [14C]melamine in male Fischer 344 rats. Food Chem Toxicol. 1983;21:807-10. https://doi.org/10.1016/0278-6915(83)90216-8
45. Hau AK, Kwan TH, Li PK. Melamine toxicity and the kidney. J Am Soc Nephrol. 2009;20:245-50. https://doi.org/10.1681/ASN.2008101065
46. FDA [U.S. Food and Drug Administration]. Interim safety and risk assessment of melamine

and its analogues in food for humans; availability [Internet]. 2008 [cited 2023 Jan 6]. https://www.federalregister.gov/documents/2008/11/13/E8-26869/interim-safety-and-risk-assessment-of-melamine-and-its-analogues-in-food-for-humans-availability
47. Kim JH, Choi HS, Goo D, Park GH, Han GP, Delos Reyes JB, et al. Effect of dietary melamine concentrations on growth performance, excreta characteristics, plasma measurements, and melamine residue in the tissue of male and female broiler chickens. Poult Sci. 2019;98:3204-11. https://doi.org/10.3382/ps/pez050
48. Brown CA, Jeong KS, Poppenga RH, Puschner B, Miller DM, Ellis AE, et al. Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. J Vet Diagn Invest. 2007;19:525-31. https://doi.org/10.1177/104063870701900510
49. Erdoğan Z, Erdoğan S, Aksu T, Baytok E. The effects of dietary lead exposure and ascorbic acid on performance, lipid peroxidation status and biochemical parameters of broilers. Turk J Vet Anim Sci. 2005;29:1053-9.
50. Campbell ML Jr, May JD, Huff WE, Doerr JA. Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. Poult Sci. 1983;62:2138-44. https://doi.org/10.3382/ps.0622138
51. Kim JH, Park GH, Han GP, Kil DY. Effect of feeding corn distillers dried grains with solubles naturally contaminated with deoxynivalenol on growth performance, meat quality, intestinal permeability, and utilization of energy and nutrients in broiler chickens. Poult Sci. 2021;100:101215. https://doi.org/10.1016/j.psj.2021.101215
52. Brand LM, Murarolli RA, Gelven RE, Ledoux DR, Landers BR, Bermudez AJ, et al. Effects of melamine in young broiler chicks. Poult Sci. 2012;91:2022-9. https://doi.org/10.3382/ps.2011-02044
53. Yılmaz F, Özdemir N, Demirak A, Tuna AL. Heavy metal levels in two fish species Leuciscus cephalus and Lepomis gibbosus. Food Chem. 2007;100:830-5. https://doi.org/10.1016/j.foodchem.2005.09.020
54. Zhang L, Mu X, Fu J, Zhou Z. In vitro cytotoxicity assay with selected chemicals using human cells to predict target-organ toxicity of liver and kidney. Toxicol In Vitro. 2007;21:734-40. https://doi.org/10.1016/j.tiv.2007.01.013
55. Goede AA, De Bruin M. The use of bird feathers for indicating heavy metal pollution. Environ Monit Assess. 1986;7:249-56. https://doi.org/10.1007/BF00418017
56. Pilastro A, Congiu L, Tallandini L, Turchetto M. The use of bird feathers for the monitoring of cadmium pollution. Arch Environ Contam Toxicol. 1993;24:355-8. https://doi.org/10.1007/BF01128733
57. Kim JH, Kil DY. Comparison of toxic effects of dietary organic or inorganic selenium and prediction of selenium intake and tissue selenium concentrations in broiler chickens using feather selenium concentrations. Poult Sci. 2020;99:6462-73. https://doi.org/10.1016/j.psj.2020.08.061
58. Lu T, Harper AF, Zhao J, Corl BA, LeRoith T, Dalloul RA. Effects of a dietary antioxidant blend and vitamin E on fatty acid profile, liver function, and inflammatory response in broiler chickens fed a diet high in oxidants. Poult Sci. 2014;93:1658-66. https://doi.org/10.3382/ps.2013-03827
59. Huang Q, Gao X, Liu P, Lin H, Liu W, Liu G, et al. The relationship between liver-kidney impairment and viral load after nephropathogenic infectious bronchitis virus infection in embryonic chickens. Poult Sci. 2017;96:1589-97. https://doi.org/10.3382/ps/pew455
60. Erdogan Z, Erdogan S, Celik S, Unlu A. Effects of ascorbic acid on cadmium-induced oxidative stress and performance of broilers. Biol Trace Elem Res. 2005;104:19-31. https://

https://doi.org/10.5187/jast.2023.e26

doi.org/10.1385/BTER:104:1:019
61. Ding XM, Zhang KY, Wang L, Bai SP. Toxicity of melamine and cyanuric acid in broilers and residues in tissues. Hum Exp Toxicol. 2012;31:174-84. https://doi.org/10.1177/0960327111422405
62. Ahamed M, Siddiqui MKJ. Environmental lead toxicity and nutritional factors. Clin Nutr. 2007;26:400-8. https://doi.org/10.1016/j.clnu.2007.03.010
63. Ohtsuka A, Kojima H, Ohtani T, Hayashi K. Vitamin E reduces glucocorticoid-induced oxidative stress in rat skeletal muscle. J Nutr Sci Vitaminol. 1998;44:779-86. https://doi.org/10.3177/jnsv.44.779
64. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys. 1986;2:501-14. https://doi.org/10.1016/0003-9861(86)90305-X
65. Cheng YF, Chen YP, Wen C, Wang WB, Wang AQ, Zhou YM. Evaluation of dietary palygorskite supplementation on growth performance, mineral accumulations, antioxidant capacities, and meat quality of broilers fed lead-contaminated diet. Biol Trace Elem Res. 2018;181:314-22. https://doi.org/10.1007/s12011-017-1047-6
66. Bourdeau P, Somers E, Richardson GM, Hickman JR. Short-term toxicity tests for non-genotoxic effects. Chichester: John Wiley & Sons; 1990.
67. FDA [U.S. Food and Drug Administration]. Toxicological principles for the safety assessment of food ingredients. Redbook 2000: Chapter IV.C.3.a., Short-Term Toxicity Studies with Rodents [Internet]. 2003 [cited 2023 Jan 6]. https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm078339.htm
68. FDA [U.S. Food and Drug Administration]. Toxicological principles for the safety assessment of food ingredients. Redbook 2000. Chapter IV.C.3.b., Short-Term Toxicity Studies with Non-Rodents [Internet]. 2003 [cited 2023 Jan 6]. https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm078342.htm
69. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 423: acute oral toxicity - acute toxic class method [Internet]. 2002 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264071001-en.pdf?expires=1526609490&id=id&accname=guest&checksum=2E3838F1CAFF47AD7482296CE27FEF25
70. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, et al. Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. Food Chem Toxicol. 1995;33:223-31. https://doi.org/10.1016/0278-6915(94)00136-C
71. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275-87. https://doi.org/10.1007/BF01234480
72. FDA [U.S. Food and Drug Administration]. Toxicological principles for the safety assessment of food ingredients. Redbook 2000. Chapter IV.C.4.a., Subchronic toxicity studies with Rodents [Internet]. 2003 [cited 2023 Jan 1]. https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm078345.htm
73. FDA [U.S. Food and Drug Administration]. Toxicological principles for the safety assessment of food ingredients. Redbook 2000. Chapter IV.C.4.b., Subchronic toxicity studies with non-Rodents [Internet]. 2003 [cited 2023 Jan 6]. https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm078346.

htm
74. OEC [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 410: repeated dose dermal toxicity: 21/28-day study. 1981 [cited 2023 Jan 6]. https://read.oecd-ilibrary.org/environment/test-no-410-repeated-dose-dermal-toxicity-21-28-day-study_9789264070745-en#page1
75. OEC [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 408: repeated dose 90-day oral toxicity study in rodents [Internet]. 1998 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070707-en.pdf?expires=15266 25012&id=id&accname=guest&checksum=06CA51DD579D5DC8BFA5F8952577F944
76. OECD [Organization for Economic Co-operation and Development. OECD guidelines for the testing of chemicals, Section 4: Test no. 413: subchronic inhalation toxicity: 90-day study [Internet]. 2017 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070806-en.pdf?expires=1 526610423&id= id&accname=guest&checksum=B22324CCB6506D45DC524CC73E4FB775
77. FDA [U.S. Food and Drug Administration]. Toxicological principles for the safety assessment of food ingredients. Redbook 2000. Chapter IV.C.5.a., Chronic toxicity studies with rodents [Internet]. 2007 [cited 2023 Jan 6]. https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm078349.htm
78. Orcu HH. Fungicides and their effects on animals. In: Carisse O, editor. Fungicides. Rijeka: InTech; 2010. p. 349-62.
79. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 452: chronic toxicity studies [Internet]. 2009 [cited 2023 Jan 6]. https://read.oecd-ilibrary.org/environment/test-no-452-chronic-toxicity-studies_9789264071209-en#page1
80. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 401: acute oral toxicity. 1987 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/environment/test-no-401-acute-oral-toxicity_9789264040113-en
81. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 402: acute dermal toxicity: fixed dose procedure [Internet]. 2017 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070585-en.pdf?expir es=15265 65285&id=id&accname=guest& checksum=4BEF2130B7EC3CEF9C2DFE9EF3C9FF3B
82. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 403: acute inhalation toxicity [Internet]. 2009 [cited 2023 Jan 6]. https://read.oecd-ilibrary.org/environment/test-no-403-acute-inhalation-toxicity_9789264070608-en#page1
83. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 404: acute dermal irritation/corrosion [Internet]. 2002 [cited 2023 Jan 6]. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg404.pdf
84. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 405: acute eye irritation/corrosion [Internet]. 2021 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264185333-en.pdf?expires=1 526608649& id=id&accname=guest&checksum=1BA63D264B9744B0785

https://doi.org/10.5187/jast.2023.e26

DB6B518E426CD
85. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 420: acute oral toxicity – fixed dose procedure [Internet]. 2002 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070943-en.pdf?expires =1526609048&id =id&accname=guest&checksum=833D28C593713ECA6002DB33A718F3EC
86. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 425: acute oral toxicity: up-and-down procedure [Internet]. 2008 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/environment/test-no-425-acute-oral-toxicity-up-and-down-procedure_9789264071049-en
87. FEEDAP [EFSA Panel Additives and Products or Substances used in Animal Feed]. Technical guidance: tolerance and efficacy studies in target animals. EFSA J. 2011;9:2175. https://doi.org/10.2903/j.efsa.2011.2175
88. WHO [World Health Organization]. UN strengthens regulations on melamine, seafood, melons, dried figs and labelling: consumers to benefit from new food safety standards. 2012 [cited 2023 Jan 6]. https://www.who.int/news/item/04-07-2012-un-strengthens-regulations-on-melamine-seafood-melons-dried-figs-and-labelling
89. Robbins KR, Saxton AM, Southern LL. Estimation of nutrient requirements using broken-line regression analysis. J Anim Sci. 2006;84:E155-65. https://doi.org/10.2527/2006.8413_supplE155x
90. Alhotan RA, Vedenov DV, Pesti GM. Estimation of the maximum safe level of feed ingredients by spline or broken-line nonlinear regression models. Poult Sci. 2017;96:904-13. https://doi.org/10.3382/ps/pew317
91. MAFRA [Ministry of Agriculture, Food and Rural Affairs]. Acceptance standard for hazardous materials in feed [Internet]. 2017 [cited 2023 Jan 6]. https://www.law.go.kr/%ED%96%89%EC%A0%95%EA%B7%9C%EC%B9%99/%EC%82%AC%EB%A3%8C%20%EB%93%B1%EC%9D%98%20%EA%B8%B0%EC%A4%80%20%EB%B0%8F%20%EA%B7%9C%EA%B2%A9
92. FAMIC [Food and Agricultural Materials Inspection Center]. Regulatory frameworks to ensure feeds safety in Japan. Regulation value of pesticides (ministerial ordinance) [Internet]. 2015 [cited 2023 Jan 6]. http://www.famic.go.jp/ffis/feed/r_safety/r_feeds_safety22.html#mycotoxins
93. FAMIC [Food and Agricultural Materials Inspection Center]. Regulatory frameworks to ensure feeds safety in Japan. Regulation value of pesticides, heavy metals and mycotoxins (administrative guideline) [Internet]. 2017 [cited 2023 Jan 6]. http://www.famic.go.jp/ffis/feed/r_safety/r_feeds_safety22.html#mycotoxins
94. CFIA [Canadian Food Inspection Agency]. RG-8 regulatory guidance: contaminants in feed (formerly RG-1, chapter 7). Section 4: metal contaminants [Internet]. 2017 [cited 2023 Jan 6]. https://inspection.canada.ca/animal-health/livestock-feeds/regulatory-guidance/rg-8/eng/1347383943203/1347384015909?chap=0
95. OJEC [Official Journal of the European Communities]. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed [Internet]. 2002 [cited 2023 Jan 6]. http://eur-lex.europa.eu/resource.html?uri=cellar:aca28b8c-bf9d-444f-b470-268f71df28fb.0004.02/DOC_1&format=PDF
96. FDA [U.S. Food and Drug Administration]. Guidance for industry: action levels for poisonous or deleterious substances in human food and animal feed [Internet]. 2000 [cited 2023 Jan 6]. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatory

Information/ucm077969.htm
97. FDA [U.S. Food and Drug Administration]. Guidance for industry: fumonisin levels in human foods and animal feeds [Internet]. 2001 [cited 2023 Jan 6]. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm109231.htm
98. FDA [U.S. Food and Drug Administration]. Guidance for industry and FDA: advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed [Internet]. 2010 [cited 2023 Jan 6]. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm120184.htm
99. FDA [U.S. Food and Drug Administration]. Toxicological principles for the safety assessment of food ingredients. Redbook 2000. Chapter IV.C.5.b., One-year toxicity studies with non-rodents [Internet]. 2003 [cited 2023 Jan 6]. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm078348.htm
100. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 411: subchronic dermal toxicity: 90-day study [Internet]. 1981 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070769-en.pdf?expires=152 6610083&i d=id&accname=guest&checksum=7AA1122C0B7A5838AE314CFFE00E512A
101. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 409: repeated dose 90-day oral toxicity study in non-rodents [Internet]. 1998 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070721-en.pdf?expires =1526626052 &id=id&accname=guest&checksum=235426B40465F9AE9C8314D9E593F1D4
102. OECD [Organization for Economic Co-operation and Development]. OECD guidelines for the testing of chemicals, Section 4: Test no. 407: repeated dose 28-day oral toxicity study in rodents [Internet]. 2008 [cited 2023 Jan 6]. https://www.oecd-ilibrary.org/docserver/9789264070684-en.pdf?expires=152662578 5&id=id&accname=guest&checksum=9FB9FA64A75B5C12884B9428A4A022AF

https://doi.org/10.5187/jast.2023.e26