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Research Article

A Recent Trend of Residual Pesticides in Korean Feed

Jin Young Jeong, Minseok Kim, Youl-Chang Baek, Jaeyong Song, Seul Lee, Ki Hyun Kim, Sang Yun Ji, Hyun-Jeong Lee, Young Kyun Oh and Sung Dae Lee*
Animal Nutrition & Physiology Team, National Institute of Animal Science, Wanju 55365, Korea.

ABSTRACT

Pesticide application in agriculture provides significant benefits such as protection from disease, prevention of harmful insects, and increased crop yields. However, accurate toxicological tests and risk assessments are necessary because of many related adverse effects associated with pesticide use. In this review, we discuss and analyze residual pesticides contained in livestock feed in Korea. A pesticide residue tolerance standard for livestock feed has not been precisely established; so, risk assessments are required to ensure safety. Standards and approaches for animal criteria and appropriate methods for evaluating residual pesticides are discussed and analyzed based on technology related to animal product safety in Korea. The safety of livestock feed containing pesticides is assessed to establish maximum residue limits relative to pesticides. Analysis of residual pesticides in milk, muscle, brain, and fat was performed with a livestock residue test and safety evaluation of the detected pesticide was performed. Efficacy of organic solvent extraction and clean-up of feed was verified, and suitability of the instrument was examined to establish if they are effective, rapid, and safe. This review discussed extensively how pesticide residue tolerance in livestock feed and hazard evaluation may be applied in future studies.

(**Key words** : Feed, Risk Assessment, Pesticides, Maximum Residue Limit)

Ⅰ. INTRODUCTION

Agricultural pesticide application is necessary to prevent disease and reduce pests to increase agricultural production yield and quality. Pesticides are potential contributing factors to the increase in agricultural production. Nevertheless, Pesticides have been used for their low cost and effectiveness; however, they are known to have hazard effects. Pesticide residue in feedstuff is very important for the health and welfare of animals and humans. Agricultural commodities with constant exposure to pesticides have been shown particular interest, and public health has been considered because of residual pesticides in animal feed. Pesticide assessment has become important before spraying onto feedstuff and to assess the potential effect it might have on livestock. The majority of pesticide residue tests have been performed on blood, egg, fat, and meat using various analytical methodologies. Appropriate methods for detecting residual pesticides have been improved and introduced to regulate pesticide usage. However, the continuous use of pesticides has challenged feed safety worldwide. The standard for the registration of pesticides has been amended to include alternative approaches, and to reduce experimental animal and maximum residue limits (MRLs) according to the Rural Development Administration of Korea. The MRL is hard to determine accurately via feed intake because exposure to pesticide MRLs is different depending on the type of feed (Mekonen et al., 2014). For feed safety in livestock, the positive list system (PLS) is proposed at a uniform level (less than 0.01 mg/kg) of non-detection and manages each pesticide under acceptable daily intake (ADI), except for pesticides with residual standards. Therefore, the PLS requires strict controls to prevent undesirable abuse of pesticides. The types of livestock feedstuff supplied include pasture, hay and silage, corn, rice straw, cereal grains, wheat, soybeans, canola, and cottonseed. Therefore, to undertake a risk assessment of residual pesticides it is necessary to determine an accurate estimation of the hazard.

MRLs are the legally calculated pesticide residue levels in food and feedstuffs determined by the Codex Alimentarius Commission (CAC) and other authorities for pesticide residues in agricultural commodities (Ehling and Reddy, 2015). Eggs, meat, and milk have a MRL of 0.01 mg/kg based on the limit

* Corresponding author : Sung Dae Lee, Animal Nutrition & Physiology Team, National Institute of Animal Science, Wanju 55365, Korea.
Tel: +82-63-238-7487, Fax: +82-63-238-7497, E-mail: jeong73@korea.kr

of determination (CAC, 2016). Risk assessments examining pesticide exposure are required to establish pesticide MRLs in foods and feedstuffs according to the Ministry of Food and Drug Safety of Korea. In the present review, the PLS, major contaminants, MRLs, applications, analytical methods, and risk assessments are introduced and analyzed, and future perspectives discussed.

1. Positive list system (PLS) in food and feedstuffs

A PLS prohibits the use of unregistered pesticides than registration of pesticides or MRL of 0.01 mg/kg. Thus, pesticide limits are strengthened to prevent undesirable abuse of pesticides for food and feed safety. Non-registered pesticides in Korea have residual pesticide limits set at 0.01 ppm, as do registered pesticides with import tolerance (IT). Pesticide MRLs have been established for the use of pesticides in agricultural crops via risk assessments by the National Law Information Center in Korea (Table 1). When the PLS is implemented, it will be applied to all agricultural products from January 2019; however, it did begin in December 2016 for tropical fruits and nuts. Yet, pesticide residue tolerance limits in Korea have not been established to date, with only the establishment of residual pesticide limits for some foods

Table 1. Pesticide maximum residue limits in livestock feed

Pesticides	Single ingredient								Formula feed
	Cereals						Beans	Roughages	
	Wheat	Barley	Rye	Corn	Oats	Sorghum			
2,4-Dichlorophenoxyacetic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	400	0.5
Aldicarb	0.05	0.05	0.02	0.5	0.2	0.5	-	1	0.4
Aldrin/Dieldrin	-	-	-	-	-	-	0.05	0.03	0.03
Bifenthrin	0.5	0.5	0.5	15	0.5	0.5	0.7	15	0.5
Carbaryl	5	5	5	5	5	5	5	250	5
Chlorpyrifos-methyl	10	6	7	7	10	10	-	6	6
Cypermethrin	10	10	10	10	10	10	-	30	10
Carbendazim	20	20	20	20	20	20	20	20	20
Carbofuran	0.2	0.2	0.2	0.2	0.2	0.2	-	13	0.2
Chlorpyrifos	5	0.5	10	0.5	2	0.75	-	13	2.5
Dichlorvos	2	2	2	2	2	2	-	10	2
Disulfoton	5	3	5	3	0.05	-	-	10	4
Fenitrothion	6	6	6	6	6	6	-	10	6
Fenthion	-	-	-	5	-	-	-	5	1
Flusilazole	5	5	5	5	5	5	5	5	5
Kresoxim-methyl	5	5	5	5	5	5	-	5	5
Methiocarb	0.05	0.05	-	-	-	-	0.5	0.05	0.05
Paraquat	0.05	0.05	10	0.1	0.5	0.5	-	5	1
Diazinon	5	5	5	5	5	5	-	10	5
Dichloro-diphenyl-trichloroethane	0.5	0.5	0.5	0.5	0.5	0.5	-	0.5	0.5
Diflubenzuron	1.5	1.5	1.5	1.5	1.5	1.5	40	3	6
Dimethoate	1	0.04	0.2	1	0.2	0.2	-	2	1
Ethylenedibromide	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5
Glyphosate	5	5	0.2	5	5	5	-	500	5
Methomyl	5	10	10	10	10	10	0.2	20	10
Methoprene	5	5	5	5	5	5	5	0.01	5
Permethrin	10	10	10	10	10	10	50	55	10
Phenthoate	1	1	1	1	1	1	-	10	1
Prochloraz	40	40	40	40	40	40	-	40	40
Propiconazole	2	2	2	-	-	-	5	18	2
Quintozene	0.03	0.01	-	0.01	-	-	0.05	0.03	0.02
Terbufos	0.2	0.2	0.2	0.2	0.2	0.3	-	1	0.3

and feed. Pesticides that have a MRL established only for corn are also detected in rice straw. Overall, the purpose of the PLS is to provide a system for food or feed safety management within the range of ADI by non-registered pesticides using uniform limits or MRLs.

2. Major contaminations in agricultural commodities

Agricultural pesticides, heavy metals, mycotoxins, persistent organic pollutants, radioactivity, and veterinary drugs are major contaminations according to the National Law Information Center in Korea (Table 2). In particular, residual pesticides have negative effects on humans and livestock. Pesticides can remain in agricultural commodities and livestock products. The undesirable abuse of pesticides might create a potentially hazardous influence for livestock and human. Pesticides might also cause adverse effects on non-target products.

Residual pesticides commonly remain in food, feed, soil, drinking water, and air from the use of pesticides and includes degradation, reaction and conversion products, and metabolites (Dhaliwal, 2006). Secondary metabolites such as mycotoxins are produced from species of filamentous fungi on the seeds of agricultural commodities. Mycotoxin contamination of agricultural commodities is a serious concern for livestock and public health. Mycotoxins that are subject to the law include aflatoxins, fumonisins, ochratoxins, cyclopiazonic acid, deoxynivalenol, patulin, and zearalenone produced by species of *Aspergillus*, *Fusarium*, and *Penicillium*, with aflatoxins and fumonisins posing the greatest risk to animals and humans worldwide (Reddy et al., 2009). The frequency, magnitude, and cause of mycotoxin contamination of agricultural commodities must be discussed further in the future. Heavy metal concentrations in agricultural commodities have been analyzed including potentially hazardous substances such as Cd, Cr, Cu, Hg, Pb, Zn, Sb, Co, and Ni. Heavy metal content in feedstuff and food must be established to provide new insight into contamination levels and monitoring to remove potential risks to human health. Radioactivity released from nuclear accidents has also been exposed to food and feed. The environmental hazard to humans and animals depends on the types of radionuclides and quantity of radioactivity [e.g., radioiodine (I-131) and radiocaesium (Cs-134 and Cs-137)]. Veterinary drugs or derivative products provide significant benefits to improve the weight gain, enhance feed efficiency, and prevent hereditary and infectious diseases in livestock. However, if animals for meat production had critical risk based on veterinary drugs, then the animal products after slaughter is a major concern for human health. Therefore, veterinary drug residue possesses a potential hazard of feed and food contamination.

3. Pesticides application lists in feed

Pesticides are of concern to agricultural crops and risk to human health in Korea. Pesticides that are applied to agricultural commodities and generally used pesticides are listed in Table 3. Pesticides are applied to improve crops yields in livestock agriculture via various mechanisms. Consumers and producers are exposed to pesticides, either directly from human exposure or indirectly via the consumption of livestock products. In 2017, almost 2,972 types of pesticides were

Table 2. The classification of hazardous substances in feedstuff

Substances	Contaminants
Pesticide	Group I : Aldrin+Dieldrin, Heptachlor, Procloraz, Propiconazole, etc. Group II : Aldicarb, Carbaryl, Cabendazim, Carbofuran, etc. 2,4-D (Dichlorophenoxyacetic acid), Paraquat, Glyphosate(Glyphosate+Sulfosate), Ethylenedibromide (EDB)
Heavy metals	Cadmium, Lead, Mercury, Inorganic tin, Chrome, Antimony, Arsenic, Selenium, Barium, Aluminium, Boron, Cobalt, Copper, Manganese, Nickel, Strontium, Tin, Organic tin, Zinc
Mycotoxins	Aflatoxin, Ochratoxin A (Management) Deoxynivalenol, Zearalenone, Fumonisins (Recommendation)
Radioactivity	Cesium, Iodine
Veterinary drugs	Salinomycin, Monensin sodium, Lasalocid sodium, Narasin, Maduramycin ammonium, Semduramicin, Clopidol, Fenbendazole, Diclazuril

approved in accordance with the legal framework in Korea. Food and feed production must improve livestock product yields, with the use of pesticides in agriculture increasing crop production (Aktar et al., 2009). The abuse of pesticides can result in environmental problems owing to their ability to enter crops and livestock as well as creating human health problems. Poisoning by pesticides is a global health problem. The World Health Organization reported that, worldwide, pesticide poisoning results in 250,000 deaths every year. Pesticide poisoning can range from nausea, allergies, and headaches to neurological disorders, cancer, and reproductive malfunction (Farina et al., 2017). Pesticide residue limits are necessary to predict the concentrations of pesticide use and pesticide application. Each agricultural crop has significantly different MRLs. Residual pesticides found in humans come from consuming livestock products (80%) more than from the air (10%) or drinking water (10%). Therefore, human health is threatened by consuming livestock products containing residual pesticides even when the pesticides are registered for toxicity testing.

4. Pesticide maximum residue limits (MRLs)

Pesticides are generally used to improve crop yield and efficiency in agriculture. The use of pesticides to improve feed production leads to an uncontrolled release of undesired substances into the feedstuff. Therefore, despite the benefits associated with pesticide use in agriculture, there is a trend of

Table 3. Comparison of pesticides among the Codex Alimentarius Commission (CAC), Japan, and Korea

Pesticides	Korea	CAC	Japan	Pesticides	Korea	CAC	Japan
2,4-D	O	O	O	Chlorpyrifos-methyl	O		O
Abamectin		O		Clethodim	O	O	
Acephate	O	O	O	Clofentezine		O	
Acetamiprid		O		Clothianidin	O	O	
Acetochlor		O		Cyanazine	O		O
Alachlor	O		O	Cyantraniliprole		O	
Aldicarb	O	O	O	Cycloxydim	O	O	
Aldrin	O		O	Cyflumetofen		O	
Aminocyclopyrachlor		O		Cyfluthrin	O	O	O
Aminopyralid	O	O		Cyhalothrin	O	O	O
Atrazine	O		O	Cypermethrin	O		
Azinphos-Methyl	O	O		Cypermethrins		O	
Azoxystrobin	O	O		Cyproconazole	O	O	
Benomyl			O	Cyprodinil	O	O	
Bensultap			O	DDT	O		O
Bentazone	O	O	O	Deltamethrin	O		O
BHC	O		O	Diazinon	O	O	O
Bifenazate		O		Dicamba	O	O	O
Bifenthrin	O	O		Dichlobenil		O	
Bitertanol	O	O		Dichlorvos	O		O
Boscalid	O	O		Dieldrin			O
Bromoxynil			O	Difenoconazole	O	O	
Buprofezin		O		Diflubenzuron	O	O	
Captan	O		O	Dimethenamid-P	O	O	
Carbaryl	O	O	O	Dimethoate	O	O	O
Carbendazim	O	O	O	Dinotefuran		O	
Carbofuran	O	O	O	Diquat	O	O	O
Carbosulfan		O		Disulfoton	O	O	
Cartap	O		O	Dithiocarbamates	O	O	
Chlorantraniliprole	O	O		Edifenphos	O		

pesticide challenges based on risk assessments for livestock and human health.

MRLs have been determined for agricultural crops and feedstuffs by establishing pesticide tolerance levels and continuous monitoring. The exceedance levels of registered pesticide must assess the MRLs to avoid the use of hazardous agricultural commodities. Non-registered or banned pesticides cannot be used in food and feedstuff production at any concentration. Pesticide MRLs are specified by the CAC. Pesticides are used in agriculture to control insects and diseases with the goal of improving crop quantity or quality. However, pesticides can affect human health from agricultural operations (Fry et al., 2016). The potentially hazardous substances remain in agricultural commodities even though there is an interval between the spraying of the pesticides and the pre-harvest time. Therefore, residual pesticides must be controlled for to prevent any hazard or abuse of pesticides in livestock and humans. Some countries have registered and established MRLs of their own in certain food and feedstuffs. Therefore, a standard for MRLs has been established because trade disputes between countries can occur. The European level and the CAC of the Food and Agriculture Organization have set MRLs (CAC, 2012). Residual levels in agricultural commodities have been measured in accordance with these MRLs (Jallow et al., 2017). In addition, the dietary consumption of residual pesticides is determined by the analysis of food (Kim et al., 2016). For the risk assessment of residual pesticides, an appropriate approach is required to determine the residual pesticides below the MRL and to discover the identity in food and feedstuffs. Multi-residue methods or single residue methods can be applied, e.g., sample collection, homogenization with appropriate solvent, sample separation, purification, and clean-up, followed by chromatography. Multi-residue methods are commonly utilized to monitor or screen for pesticides. Single residue methods are tested on each pesticide in each feedstuff to determine the half-life, dissipation patterns, pre-harvest interval, and pre-harvest residue limits (Jang et al., 2014; Grimalt and Dehouck, 2016)

5. Suitable analytical methodologies

Utilizing the appropriate analytical method for the sample containing pesticides is important to determine the MRLs. A sample weight of 10–50 g was approved to reduce sampling error, ensure analytical sample homogeneity, and increase the detection levels and quantity by the Ministry of Food and Drug Safety, Republic of Korea. The approaches via accurate sample preparation is not necessary 10-50 g as suggested sample amount (Farha et al., 2015).

5.1. Sample preparation

Accurate sample preparation techniques are important to ensure the accuracy and homogeneity of the experimental sample (Grimalt and Dehouck, 2016). An appropriate sample amount for pesticide residue analysis is 1–5 kg of the agricultural crop. This sample is then homogenized by cryogenic grinding. Finally, after pulverization, a small amount (10–20 g) is used for extraction and assessment (Farha et al., 2015).

5.2. Sample extraction

Sample extraction must consider the physico-chemical characteristics and polarity of the pesticides. Improvements in extraction processes and analytical methods have enhanced the analytical accuracy and precision and reduced the complexity of sample treatments (Grimalt and Dehouck, 2016). The analysis of organochlorine pesticides in foodstuffs has been developed using acetonitrile and petroleum ether (Mills et al., 1963).

An extraction technique using acetone was developed to analyze pesticides that have greater polarity than organochlorine, and then dichloromethane and petroleum is used to clean-up with florisil. The acetone extraction method was developed in 1983 by the Dutch Food and Consumer Products Safety Authority. The analysis method using ethyl acetate was established in 1989 by the Swedish National Food Administration and involved clean-up using gel permeation chromatography (Grimalt and Dehouck, 2016). Acetone has higher polarity than ethyl acetate and dichloromethane. The polar pesticides with anhydrous sodium sulfate are introduced to the water phase. A new method with acetonitrile extraction was developed with a clean-up that used dispersive solid phase extraction analysis (Anastassiades et al., 2003). The sample extract method using acetonitrile is more effective because it has fewer analytical steps and less solvent

is required (Grimalt and Dehouck, 2016). Currently, pesticides are analyzed with acetonitrile solvent because of its higher extraction ability. Acetonitrile has a higher efficiency of extraction in agricultural commodities that have a high moisture content (Lee et al., 1991). A clean chromatogram can be separated by $MgSO_4$ because of a decrease in the aqueous phase and heat generation (Rizzetti et al., 2016). Therefore, many factors such as sample amount, solvent volume, and high recovery rate must be considered to determine the optimal conditions for pesticide analysis. The best extraction methods have been established for the identification of pesticides in crops including homogeneity, dispersing extraction, solid-phase micro-extraction, and microwave-assisted extraction (Berrada et al., 2004; Ueno et al., 2004; Fenoll et al., 2007; Singh et al., 2007). Table 4 lists the various extraction solvents applied during the analysis of agricultural crops.

5.3. Instrumentation

The analytical equipment that are used to quantify and identify crops include gas chromatography (GC) with electron capture detector, GC-mass spectrometry (GC-MS), GC-electrolytic conductivity detector, high resolution gas chromatograph-low resolution mass spectrometer/high resolution mass spectrometer, high resolution gas chromatograph with electron capture detector, and liquid chromatography with ultraviolet absorbance detection. The analysis can identify and quantify low level analytes and unknown components by gas chromatography (Farina et al., 2017) or liquid chromatography (Farha et al., 2015). Furthermore, liquid chromatography coupled with ultraviolet detection has been used under optimal conditions (Du et al., 2014; Farha et al., 2015) derived from liquid chromatography or with tandem mass spectrometry (Kim et al., 2016). GC with micro-electron capture detection (μECD), nitrogen phosphorous detector (NPD) (Al Mahmud et al., 2013; Farajzadeh et al., 2015), GC-MS, GS–ion trap mass spectrometry (ITMS; Abdelhameed et al., 2014) and GC-MS/MS (Vidal et al., 2002) have been developed for pesticide monitoring because of the high selectivity and separation and identification of MS. However, GC-MS/MS and ITMS are very expensive to use. The coupling of GC with MS/MS has been introduced to replace the traditional GC detectors; however, traditional GC detectors can still be used although they are less sensitive. An advanced approach based on GC or LC coupled with a MS detector should be developed for optimal conditions. To date, numerous studies for determining residual pesticides have been published using GC or LC coupled with a MS detector and a novel measuring method.

6. Pesticide residue testing of livestock products

An alternative approach that uses animal requirements to assess the potential risk of feedstuff containing residual pesticide has been developed because long-term dietary testing is difficult in humans. However, extrapolation or interpolation from animal results is difficult. The negative effects of feedstuffs containing hazardous substances such as pesticides might affect animals also. Further study is required to improve the optimal conditions of pesticide MRLs using experimental animals. Roughage and feedstuffs are a variety of assessment types because the cultivation area and methods are different. The scope and process for pesticides registration varies in a country-specific manner. Therefore, in Korea, an optimal model is required to propose pesticide residue limits based on

Table 4. Polarity index in commonly used solvents

Solvent	Solvent polarity index (P)
Hexane	0.1
Toluene	2.4
Chloroform	2.7
Diethyl ether	2.8
Ethyl acetate	4.4
Acetone	5.1
Acetonitrile	5.8

the local situation. The MRL for the PLS should be proposed based on residual pesticides by livestock products tolerance testing.

7. Risk assessment of dietary intake in feed-based pesticides

Finally, the risk assessment of long-term dietary intake of pesticides is estimated by theoretical maximum daily intake and ADI. Short-term dietary intake of pesticides is calculated between national estimated short-term intake and acute reference dose (ARfD). If the long-term and the short-term dietary intake exposure do not exceed the ADI and ARfD, then the residual pesticides in livestock products are safe for human health. Pesticide MRLs reflect the approved use of a pesticide in food and feedstuffs. However, risk assessment systems for pesticide registration are performed by chronic and acute risks in livestock products. Exposure to pesticides has been calculated by measuring meat, liver, blood, and urine samples from the USA and Korea. Observations showed generally higher concentrations of pesticide metabolites and residue levels in young adults than in children or older adults because young adults have a higher food intake and more exposure to livestock products. Therefore, pesticide residues in livestock products as food are the main source of exposure for humans. A study was undertaken to calculate the human health risk based on the estimated short-term food intake (Liu et al., 2013). Dietary intake to exposed organophosphate pesticides was measured as ARfD. However, long-term health risk was estimated as the estimated daily intake (EDI) and ADI. Therefore, accurate determination of residual pesticides and toxicity levels is important in food and feedstuffs, and scientific inferences have been discussed and published. Both the traditional approaches and alternative methods must allow for pesticide toxicity, economic considerations, and health and welfare in human and livestock. Because pesticides are extensively utilized and there is an associated legal consideration, risk assessments of hazardous substances are very important. In Korea, the good laboratory practice system related to hazard testing of pesticides is managed by the National Institute of Animal Science. Pesticide testing should be performed in accordance with the standards and methods of registration of pesticides by the Rural Development Administration. The risk to food safety is caused by pesticides as well as from pesticide-resistant pathogens (Wolejko et al., 2016). Exposure to pesticides must not exceed the ADI level from the consumption of livestock products. Thirty-one types of pesticides were found to have higher levels than the MRLs based on risk assessments using the EDI and ADI (Park et al., 2016).

8. Conclusions and future perspectives

This review discussed the general considerations for food and feedstuff safety in association with pesticide contamination. Pesticide application has contributed to improving agricultural produce yields and quality via the prevention of pests and diseases worldwide. However, human and livestock health problems can be caused by residual pesticide concentrations and the type of feedstuffs. Feedstuff is important for livestock nutrition via direct or mixed-use supply. Therefore, the assessment of residual pesticide levels is important for establishing the appropriate processing technologies. The PLS containing pesticide MRLs for feed safety should be carefully applied in future studies

Ⅱ. CONFLICT OF INTEREST

The authors declare no conflict of interest

Ⅲ. ACKNOWLEDGEMENTS

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