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Surveillance assessment for veterinary biocidal products in Korea: A laboratory investigation

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Abstract

Veterinary biocides used in animal husbandry have the potential to cause human health concerns. Biocidal products for veterinary use, which contain pesticides approved in Korea, comprise 49 active ingredients within 234 products. Within 17 of these products there are 3 ingredients which are highly hazardous pesticides: coumaphos, dichlorvos and methomyl. In this study, the content of the active ingredients of 160 products sold domestically was investigated. Samples were collected for 119 biocidal products for veterinary use. These were analysed by high-performance liquid chromatography (HPLC) and gas chromatography (GC). Seventeen products were noncompliant (insufficient or excess quantity of active ingredients). The ingredients that were below the stated concentrations were amitraz, chlorpyrifos-methyl, cypermethrin, cyromazine, dichlorvos, fipronil, muscamone and trichlorfon. The ingredients that exceeded the stated concentrations were abamectin, fluvalinate and pyriproxyfen. The noncompliance rate in biocidal products for veterinary use was 9.19%. The results of this study show that three highly hazardous pesticides (coumaphos, dichlorvos and methomyl) and 10 active ingredients (abamectin, amitraz, chlorpyrifos-methyl, cypermethrin, cyromazine, fipronil, fluvalinate, muscamone, pyriproxyfen and trichlorfon) deviated from the stated concentrations. Thus, management plans should be established to ensure compliant veterinary drugs by post-distribution quality control, such as planning for regular inspection.

KEYWORDS
biocides, monitoring, pesticides, quality control

1 | INTRODUCTION

The scale of the domestic veterinary pharmaceutical market in South Korea has grown consistently, from 414.9 million USD in 2011 to 605.3 million USD in 2017. As of 2017, there are 305 companies manufacturing veterinary drugs, sanitary aids or devices, and 323 import companies. Sales of these products were 48 million USD in the year 2017, representing 7.8% of the domestic veterinary pharmaceutical market. Veterinary sanitary aids, based on the "Handling rules of veterinary medicinal products" (MAFRA (Ministry of Agriculture, Food, and Rural Affairs), 2017a) and "Guideline for scope and assignment of veterinary sanitary product" (APQA (Animal and Plant Quarantine Agency), 2015), include disinfectants, parasiticides, prophylactic anti-parasitic agents, repellents and insecticide baits. Sanitary aids exclude formulations administered directly to the animal, which are absorbed into the body. Recently, there have been cases that raised doubts regarding the efficacy of disinfectants, such as the avian influenza outbreak into South Korea during 2013–2016. Concerns have also been raised about the harmful effects of biocides, as in the global contamination of eggs with the insecticide fipronil (Stafford et al., 2018).

Therefore, it has been suggested that the harmful effects of veterinary biocides must be reviewed in humans and livestock. Moreover, quality control must be strengthened for approved products. In the case of disinfectants, this became a widespread societal issue following the national disasters caused by outbreaks of foot-and-mouth disease and avian influenza in the first half of 2016. The Animal and Plant Quarantine Agency (APQA) conducted a complete collection and inspection of the disinfectants used for control of foot-and-mouth disease and avian influenza. However, there remains a lack of research regarding quality control and monitoring for other veterinary sanitary products, especially insecticide-containing veterinary biocides. In accordance with current local guidelines (MAFRA (Ministry of Agriculture, Food, and Rural Affairs), 2017b), the APQA conducts annual collection and inspection of products, including approximately 1,650 veterinary drugs (Kang, et al., 2017). Quality control inspections are conducted for selected veterinary drugs that require focused control based on considerations regarding sales volume, unsuitable items and the scale of livestock treated with those drugs.

Thus, veterinary sanitary products, which are used in relatively small quantities, inevitably comprise a lower proportion of collections and inspections. The aim of this study was to ascertain the quality of these products in domestic circulation by the following procedures: monitoring methods of investigating approved ingredients and products, collecting products, and testing the ingredients and the concentrations of pesticides suspected of being harmful to livestock and humans. This study was undertaken to secure information useful for later establishment of policies for product quality control, such as designating specific items for control among pesticide-containing veterinary biocides.

2 | MATERIALS AND METHODS

2.1 | Survey of domestically approved veterinary biocide ingredients and products

An interrogation of a national database of product names for domestically approved pesticide-containing veterinary biocides was conducted by using the Veterinary Drug System operated by the Korean Animal Health Product Association (KAHPA).

2.2 | Collection and inspection of insecticide-containing veterinary biocides

One-hundred and sixty products that were in domestic circulation approved as insecticides-containing compound for veterinary use and veterinary sanitary products were purchased wholesale or obtained directly from the manufacturer in cases where purchase was difficult. Thirty-five active ingredients were tested among the 160 collected products. After recording data from the samples collected for each product, they were stored in a specimen storage room and used in tests.

2.3 | Chemicals and reagents

Reference standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), Korea Institute of Science and Technology (Seoul, South Korea), United States Pharmacopoeia (Rockville, MD, USA), and Wako (Osaka, Japan). All solvents used in chromatographic analysis were of HPLC grade and were purchased from Honeywell Burdick & Jackson (Ulsan, South Korea), J.T. Baker-Avantor (Center Valley, PA, USA), Sigma-Aldrich, and Daejung Chemicals & Metals Co. Ltd. (Siheung-si, South Korea). De-ionized water was purified by using a Milli-Q System (Millipore, Bedford, MA, USA). Syringe filters (0.2 μm PVDF and PTFE) were purchased from Whatman (Maidstone, United Kingdom) and Millipore (Waltham, MA, USA). Analytical columns used were XBridge™ C18 (4.6 mm × 150 mm, 5 μm, Waters Corporation, Milford, MA, USA), XTerra® RP18 (4.6 mm × 150 mm, 5 μm, Waters Corporation), Zorbax SB-C18 (2.1 mm × 50 mm, 1.8 μm, Agilent, Santa Clara, CA, USA) and HP-5 (30 mm × 0.25 mm, 0.25 μm, Agilent).

2.4 | Analytical devices

High-performance liquid chromatography (HPLC), gas chromatography (GC) and an automatic potentiometric titrator were used. For HPLC, a 1,200 series system (G1311A, Agilent) equipped with a UV-Detector and a 1,290 Infinity system (G4220A, Agilent) equipped with a UV-Detector were used. For GC, a 7890A system (G6440A, Agilent) equipped with an FID was used. The automatic potentiometric titrator used was a 794 Basic Titrino (Metrohm, Geneva, Switzerland).

2.5 | Analytical methods

The samples were tested using various certified methods such as the Korean Pharmacopoeia of Veterinary Medicinal Products

APQA (Animal and Plant Quarantine Agency), 2017, the Korean Pharmacopoeia (MFDS (Ministry of Food and Drug Safety), 2017) and other foreign test methods (BP (British Pharmacopoeia Commission), 2012; EP (European Pharmacopoeia Commission), 2012; JP (Society of Japanese Pharmacopoeia), 2017; USP (The United States Pharmacopoeial Convention), 2012), which are summarized in "Compendial Analysis Method for Veterinary Medicines, Animal and Plant Quarantine Agency (APQA)" (Table 1). HPLC, GC and titration methods were used for other chemical agents.

2.6 | Classification of risk for selection of insecticide-containing veterinary biocides requiring control

To compile a list of highly toxic insecticide-containing veterinary drugs requiring special control, the active substances in the collected biocide products were classified on the basis of insecticide hazard as defined by the World Health Organization (WHO) (WHO (World Health Organization), 2010). The classifications are shown in Table 2. The presence of coumaphos, dichlorvos and methomyl was designated as highly toxic substances and are included in "Ib" of Table 2.

TABLE 1 Developed method for analysis of veterinary biocidal products in Korea

Active ingredients	Method for analysis
Abamectin	Sample (in methanol)→Mp (80% methanol)→HPLC-UV (244 nm)
Amitraz	Sample (in acetonitrile)→Mp (70% acetonitrile)→HPLC-UV (294 nm)
Benzyl benzoate	Sample (in 70% acetonitril)→Mp (acetonitrile/DW/trifluoroacetic acid = 70/30/0.2) →HPLC-UV(250 nm)
Bifenthrin	Sample (in acetonitrile)→Mp (80% methanol)→HPLC-UV (254 nm)
Chlorpyrifos-methyl, Chlorfenapyr	Sample (in 60% acetonitrile)→Mp (60% acetonitrile)→HPLC-UV (220 nm)
Clothianidin	Sample (in 40% acetonitrile)→Mp (10 mM phosphoric acid/acetonitrile = 60/40)→HPLC-UV (244 nm)
Coumaphos, Propoxur	Sample (in acetonitril)→Mp (10 mM phosphoric acid/acetonitrile = 50/50)→HPLC-UV (265 nm)
Cymiazole	Sample (in MP)→Mp (0.05% phosphoric acid/acetonitrile = 80/20)→HPLC-UV (260 nm)
Cypermethrin, Chlorpyrifos	Sample (in acetonitrile) →Mp (75% acetonitrile)→HPLC-UV(220 nm)
Cyromazine	Sample (in methanol)→Mp (D.W/methanol/Triethylamine = 749/250/1)→ HPLC-UV (230 nm)
Deltamethrin	Sample (in acetonitrile)→Mp (0.05% phosphoric acid/acetonitrile = 80/ 20)→HPLC-UV (260 nm)
Dichlorvos	Sample (in methanol)→Mp (70% methanol)→HPLC-UV (220 nm)
Diflubenzuron	Sample(in acetonitrile)→Mp(DW/acetonitrile/trifluoroacetic acid = 50/40/10)→HPLC-UV(254 nm)
Dinotefuran	Sample(in methanol)→Mp(70% acetonitrile)→HPLC-UV(220 nm)
Muscamone	Sample(in chloroform)→GC-FID
Fenobucarb	Sample(in methanol)→Mp(65% methanol)→HPLC-UV(205 nm)
Fipronil, Methoprene	Sample(in acetonitrile/methanol/0.1% acetic acid glacial = 47/21/32)→ Mp(DW/acetonitrile, gradient)→HPLC-UV(284 nm)
Fluvalinate	Sample(in methanol)→Mp(75% acetonitrile)→HPLC-UV(254 nm)
Formic acid	Sample(in DW)→Mp(20 mM NaH2PO4 in 0.2% H_3PO_4/acetonitrile = 99/1)→HPLC-UV(210 nm)
Imidacloprid, Moxidectin	Sample(in acetonitrile)→Mp(10 mM phosphoric acid/acetonitrile, gradient)→HPLC-UV(245 nm)
Imidacloprid, Permethrin	Sample(in acetonitrile)→Mp(10 mM phosphoric acid/acetonitrile, gradient)→HPLC-UV(240 nm)
Phoxim	Sample(in tetrahydrofuran/acetonitrile = 10/90)→Mp(60% acetonitrile)→ HPLC-UV(280 nm)
Pyridaben	Sample(in acetonitrile)→Mp(10 mM phosphoric acid/acetonitrile = 20/80)→HPLC-UV(210 nm)
Pyriproxyfen	Sample(in methanol) →Mp(65% acetonitrile)→HPLC-UV(230 nm)
Spinosad	Sample(in methanol)→Mp(acetonitrile/methanol (50/50)→HPLC-UV (250 nm)
Tetrachlorvinphos	Sample(in MP)→Mp(70% methanol)→HPLC-UV(220 nm)
Tetramethrin	Sample(in acetonitrile)→GC-FID
Thiamethoxam	Sample(in acetonitrile)→Mp(10 mM phosphoric acid/acetonitrile, gradient)→HPLC-UV(254 nm)
Trichlorfon	Sample(in acetonitrile)→Mp(potassium phosphate buffer, pH3.0/ acetonitrile = 70/30)→HPLC-UV(210 nm)

Abbreviation: Mp, Mobile Phase.

3 | RESULTS

3.1 | Survey of pesticide-containing veterinary biocides

In this survey using the veterinary drug system operated by KAHPA, 234 approved pesticide-containing veterinary biocides currently available in Korea were identified. These products were produced by 54 manufacturers and contained one or more of 40 ingredients (Table 3). There were 6, 3 and 6 products containing the highly toxic substances coumaphos, dichlorvos and methomyl respectively.

3.2 | Analysis of the collected pesticide-containing veterinary biocides

In the analysis, 168 products were in the range 90%–120%, which satisfies the product quality control criteria. Seventeen products did not meet the criteria, and were either above or below the acceptable range (Figure 1). Products that could not meet the recommended criteria are listed in Table 4.

4 | DISCUSSION

In 2017, in some European and Asian countries, fipronil was detected in hen's eggs for human consumption, and as a result more than 7,000,000 eggs were destroyed (Stafford et al., 2018). Similarly, this was treated as a serious incident in Korea. Domestically sold eggs were tested for fipronil and destroyed if it was detected. Internal investigations revealed that the contamination was caused by the use of agricultural pesticides or excessive amounts of veterinary drugs to eliminate red mites from chickens. The present study was conducted to prevent a similar incident from occurring in the future. Biocide products which are currently in circulation tested to determine whether they had been made appropriately (i.e. to the specifications stated by the manufacturers). As shown in Table 3, 234 domestically approved veterinary biocide products, containing 49 specific ingredients, were identified. Of these, 185 products, containing 35 ingredients, were currently in distribution. Among the other products, most had been approved but were not being produced. There were no products being produced to order, so these were not able to be collected.

The APQA implements a post-production quality control system of collection and inspection of circulating veterinary drugs in order to prevent the production and distribution of low-quality drugs (MAFRA (Ministry of Agriculture, Food, and Rural Affairs), 2017b; Kang et al., 2015; Kang, et al., 2017). The products included in these inspections are mostly those with high volumes of sales or those that have consistently shown high rates of noncompliance. Because the biocides in this study have very low volumes of sales compared with drugs such as antibiotics, vaccines and nutritional supplements, they have rarely been selected for collection and inspection. Thus, they have remained a "blind spot" in post-production quality control systems.

Of 185 products, 17 exceeded or failed to meet the manufacturers' stated criteria (90%–120%) in the post-production quality control of veterinary drugs, thus, indicating a need for administrative action. Administrative action was taken for all 17 products, such as disposal of products of the relevant lots. In terms of the causes of unsuitability of these products, because none were close to their expiration dates, we suspected that changes in concentration were likely caused by problems during manufacture or failure to adhere to proper methods of storage during distribution.

TABLE 2 Hazard classification of pesticides, according to The World Health Organization (WHO) Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009

WHO Class		LD_{50} for the rat (mg/kg b.w.)		Collected pesticides
		Oral	Dermal	
I a	Extremely hazardous	<5	<50	
I b	Highly hazardous	5 – 50	50 – 200	Coumaphos, Dichlorvos, Methomyl
II	Moderately hazardous	50 – 2000	200 – 2000	Amitraz, Bifenthrin, Chlorphenapyr, Chlorpyrifos, Cypermethrin, Deltamethrin, Fenobucarb, Fipronil, Imidacloprid, Permethrin, Phoxim, Propoxur, Pyridaben, Pyrethrin, Trichlorfon
III	Slightly hazardous	<2000	<2000	Chlorpyrifos-methyl, Cyromazine, Diflubenzuron, Fluvalinate, Spinosad, Tetrachlorvinphos
U	Unlikely to present acute hazard	5,000 or higher		Methoprene, Pyriproxyfen, Tetramethrin

TABLE 3 The number of biocidal products for veterinary use and companies in Korea

Active ingredients (49)	Number of companies (54)	Number of products (234)
Abamectin	4	6
Allethrin + Synepirin	1	1
Alphamethrin	1	1
Amitraz	10	11
Azamethiphos	1	1
Bendiocarb + Dichlorvos	1	1
Benzyl benzoate	3	3
Bifenthrin	1	1
Bistrifluron	1	3
Carbaryl	1	1
Chlorpyrifos-methyl + Chlorfenapyr	1	1
Clothianidin	1	1
Coumaphos + Propoxur	1	1
Coumaphos	2	5
Cyfluthrin	1	2
Cymiazole	1	2
Cypermethrin	10	14
Cypermethrin + Chlorpyrifos	8	14
Cypermethrin + Chlorpyrifos +Methomyl	1	1
Cypermethrin + Dichlorvos	2	2
Cypermethrin + Piperonyl butoxide	1	1
Cypermethrin + Tetramethrin	4	5
Cyromazine	16	20
Deltamethrin	3	7
Deltamethrin + Cyromazine	1	1
Diazinon	1	1
Dichlorvos	3	3
Diflubenzuron	2	2
Difluron	1	1
Dinotefuran + Muscamone	2	2
Fenitrothion + Tetramethrin	1	2
Fenobucarb	2	5
Fipronil	9	13
Fipronil + Methoprene	1	2
Flumethrin	2	3
Fluvalinate	5	6
Imidacloprid	4	5
Imidacloprid + Flumethrin	1	2
Imidacloprid + Moxidectin	1	2
Imidacloprid + Permethrin	1	1
Lindane	1	1
Lindane + Benzyl benzoate	1	1
Lufenuron	1	3
Methomyl	5	5
Methomyl + Muscalure	1	1
Naled	2	2
Permethrin	13	15
Permethrin + Octachlorodipropylether	1	1
Permethrin + Pyrethrin	2	2
Permethrin + Pyrethrin +Piperonyl butoxide	1	1
Permethrin + Tetramethrin	1	1
Phosmet	3	4
Phoxim	1	2
Propoxur	2	4
Pyrethrin + Piperonyl butoxide	1	1
Pyridaben + Formic acid	1	1
Pyriproxyfen	2	2
Spinosad	1	1
Spinosad + Mibemycin oxim	1	1
Tetrachlorvinphos	8	8
Tetramethrin	1	1
Thiamethoxam	2	2
Trichlorfon	9	11
Trichlorfon + Benzyl benzoate	4	4

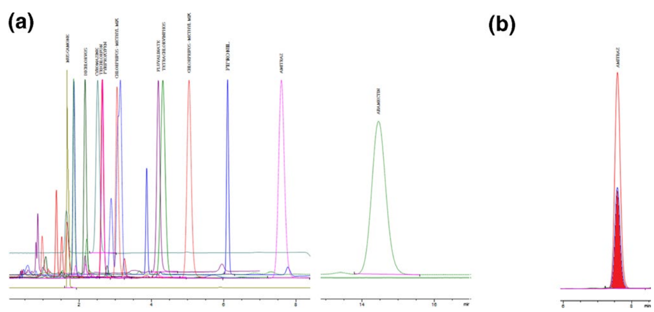

FIGURE 1 Representative chromatograms of noncompliant veterinary biocides. Superimposed chromatograms of (A) several biocide products with their respective standard compounds and (B) the Amitraz product with its standard

The rate of unsuitable products was 9.19% of all tested biocidal products for veterinary use. This is much higher than the average rate of unsuitable products in the regular annual collections and inspections of veterinary drugs for post-approval marketing surveillance assessment performed by the APQA (Kang, et al., 2017). In particular, products containing the highly toxic ingredients coumaphos, dichlorvos or methomyl, and products containing the 10

TABLE 4 Analytical results of biocidal products for veterinary use

Active ingredient	WHO Class	No.	Results
Abamectin		1	121.97%[b]
Amitraz	II	10	97.42%
			98.78%
			100.70%
			98.46%
			99.19%
			71.27%[a]
			39.93%[a]
			53.35%[a]
			109.53%
			107.64%
Benzyl benzoate		3	99.99%
			96.10%
			95.70%
Bifenthrin	II	5	105.41%
			99.13%
			93.79%
			103.57%
			99.14%
Bistrifluron		1	103.49%
Chlorfenapyr	II	1	101.70%
Chlorpyrifos	II	10	99.04%
			106.26%
			106.01%
			93.91%
			95.63%
			103.02%
			116.65%
			110.64%
			101.74%
			105.35%
Chlorpyrifos-methyl	III	1	84.27%[a]
Clothianidin		1	92.47%
Coumaphos	I b	1	102.55%
Cymiazole HCl		1	99.78%
Cypermethrin	II	14	97.04%
			104.12%
			107.05%
			85.13%[a]
			98.64%
			100.78%
			106.04%
			100.99%
			108.84%

(Continues)

TABLE 4 (Continued)

Active ingredient	WHO Class	No.	Results
			105.86%
			103.56%
			108.56%
			103.13%
			100.67%
Cyromazine	III	19	101.59%
			96.66%
			96.84%
			90.56%
			98.98%
			98.51%
			97.02%
			94.86%
			86.12%[a]
			88.20%[a]
			76.92%[a]
			83.99%[a]
			96.52%
			93.67%
			92.13%
			99.84%
			98.64%
			102.15%
			97.88%
Deltamethrin	II	1	108.99%
Dichlorvos	I b	2	85.50%[a]
			96.34%
Diflubenzuron	III	2	98.57%
			91.56%
Dinotefuran		5	107.92%
			101.45%
			98.07%
			101.11%
			114.02%
Fenobucarb	II	8	96.98%
			105.08%
			104.40%
			103.99%
			99.25%
			95.71%
			100.29%
			105.00%
Fipronil	II	9	101.57%
			93.96%
			96.75%

(Continues)

TABLE 4 (Continued)

Active ingredient	WHO Class	No.	Results
			96.06%
			95.36%
			99.09%
			98.67%
			95.54%
			83.31%[a]
Fluvalinate	III	2	145.25%[b]
			96.61%
Formic acid		2	94.43%
			97.30%
Imidacloprid	II	14	105.13%
			96.93%
			94.66%
			96.79%
			99.01%
			98.05%
			98.94%
			99.06%
			97.95%
			101.60%
			102.50%
			101.65%
			100.65%
			99.88%
			99.84%
Methoprene	U	5	99.84%
			94.27%
			98.69%
			96.34%
			94.80%
Moxidectin		8	100.80%
			100.11%
			101.35%
			104.90%
			101.99%
			103.25%
			103.87%
			102.92%
Muscamone		5	97.26%
			92.23%
			74.19%[a]
			70.38%[a]
			101.49%
Permethrin	II	7	101.57%
			103.97%

(Continues)

TABLE 4 (Continued)

Active ingredient	WHO Class	No.	Results
			104.24%
			100.73%
			101.11%
			102.43%
			104.85%
Phoxim	II	1	96.98%
Propoxur	II	1	102.55%
Pyridaben	II	2	99.76%
			101.60%
Pyriproxyfen	U	1	125.44%[b]
Spinosad	III	2	100.76%
			98.04%
Tetrachlorvinphos	III	2	95.98%
			89.53%
Tetramethrin	U	1	102.48%
Thiamethoxam		1	100.91%
Trichlorfon	II	10	94.79%
			99.48%
			99.20%
			99.59%
			100.67%
			95.18%
			88.74%[a]
			99.62%
			99.31%
			102.75%
Sum		185	

[a]Potency of biocides is unacceptably lower than their label claim.
[b]Potency of biocides is unacceptably higher than their label claim.

ingredients that were present in concentrations outside the acceptable range (i.e. abamectin, amitraz, chlorpyrifos-methyl, cypermethrin, cyromazine, fipronil, fluvalinate, muscamone, piperonyl butoxide, pyrethrin, pyriproxyfen and trichlorfon) should be prioritized for control.

4.1 | Conclusion

The noncompliant levels of these veterinary biocidal products indicate inappropriate manufacturing standards and demand strict monitoring control in Korea. These products should be selected as a group to be monitored called "veterinary biocides." A system of regular specific supervision of pharmaceutical affairs, or exploratory testing for residual substances in livestock, should be introduced to prevent the production and distribution of products that fail to meet acceptable levels of product quality.

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

AUTHOR CONTRIBUTION

JeongWoo Kang: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Writing-original draft; Writing-review & editing. **Md Akil Hossain:** Writing-original draft; Writing-review & editing. **Hae-chul Park:** Formal analysis; Investigation; Methodology; Validation. **Jae-Young Song:** Conceptualization; Supervision. **Yong-Sang Kim:** Resources. **Sung-won Park:** Supervision. **Kwang-jick Lee:** Conceptualization; Project administration.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data of this study are included in the manuscript.

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