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Original Article

Environmental Risk Assessment for Veterinary Antibiotics and Hormone in Malaysian Agricultural Soil

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Abstract

Background: Repeated applications of animal manure as fertilizer are normal agricultural practices in Malaysia that may release veterinary antibiotics to environment from treated animals.

Methods: Environmental risk assessment (ERA) on 5 commonly used antibiotics and one hormone in Malaysian broiler farm was calculated using the ratio of measured environmental concentration (MEC) and predicted no effect concentration (PNEC) in the environment. PNEC was derived from the available acute and chronic toxicity data in the open peer-reviewed literature. Risk quotients (RQ) were then calculated for 5 antibiotics (erythromycin, norfloxacin, sulfadiazine, trimethoprim, and tylosin) and one hormone (progesterone).

Results: RQ for tylosin has exceeded 1, indicating that this compound has high risk of acute toxicity in Malaysian agricultural soil while trimethoprim and tylosin possessed medium risk of chronic toxicity. The rest of the compounds showed low risk or no risk in acute or chronic ecological toxicity.

Conclusion: The release of tylosin, trimethoprim, norfloxacin and progesterone from broiler manure to agricultural soil may potentially harm the environment.

Keywords: Veterinary antibiotic, Environmental risk assessment, Risk quotient, Malaysia

Introduction

Livestock producers often misused veterinary pharmaceuticals as supplement in animal feed to increase weight gain and prevent disease among their livestock. These chemicals are introduced increasingly without realising the consequences for the environment, direct and indirect effects for human health.

Antibiotics are compounds that present naturally in the environment. However, the frequent use of antibiotics either by human to treat diseases or as animal feed supplements has raised concerns about the increased adaptation of antibiotics resistant bacteria (1) and bioaccumulation of veterinary pharmaceuticals in plants (2-7).

Among the endocrine disrupting compounds (EDCs), steroid hormones are of particular concern because of their capacity to induce strong endocrine responses. Steroid hormones can exist

as either natural or synthetic substances, mimicking the effects of endogenous estrogens as EDCs through binding to specific receptors common to non-target organisms (invertebrates, fish, reptiles, birds and mammals) (8).

Therefore, the investigation of pharmaceutical pollution in broiler manure has special importance because they constitute a major source of hazardous unmetabolized veterinary pharmaceutical to the environment via fertilization of agricultural soil. An environmental risk assessment is conducted based on the study reported by Ho et. al (9) in order to assess the potential risk of 6 compounds (erythromycin, norfloxacin, sulfadiazine, trimethoprim, tylosin and progesterone) in agricultural soil by evaluating the ratio between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC).

Materials and Methods

Soil samples were collected from 10 respective agricultural fields in Selangor, Negeri Sembilan and Melaka in Malaysia according to Ho et. al (9). The target antibiotics and hormone in soil samples were ultrasonic extracted using the optimized extraction buffer (MeOH: ACN: 0.1M EDTA: McIlvaine buffer (pH 4), 30:20:25:25). The extracts were then cleaned up using Oasis HLB 3cc/60mg cartridge and analyzed in liquid chromatography tandem mass spectrometry (LC-MS/MS).

The risk of quotient (RQ) is a useful tool to characterize potential ecological risk of many contaminants in the environment (10-12). RQ was calculated as the ratio between maxiumum measured environmental concentration (MEC) and predicted no effect concentration (PNEC) (Eq. 1). Maximum MEC was obtained from the occurrence data whereas PNEC was estimated by dividing the lowest values of acute EC_{50} or LC_{50} or the chronic no observed effect concentration (NOEC) with a default assessment factor (AF). All values below the method quantification limit (MQL) were set to 0.

Up until now, there are limited toxicological data of antibiotics and hormones in terrestrial organisms have been reported. Therefore, $PNEC_{soil}$ values were estimated from $PNEC_{water}$ values by applying the equilibrium partition approach as suggested by Martin et al. (13) (Eq. 2).

$$\text{Risk Quotient} = \frac{MEC_{soil}}{PNEC_{soil}} \qquad \text{Eq. (1)}$$

$$PNEC_{soil} = PNEC_{water} \times K_d \qquad \text{Eq. (2)}$$

$$PNEC_{acute} = \frac{EC_{50} or LC_{50}}{1000} \qquad \text{Eq. (3)}$$

$$PNEC_{chronic} = \frac{NOEC}{AF} \qquad \text{Eq. (4)}$$

According to the Technical Guidance Document on Risk Assessment of the European Commission 2003, when only short-term/acute toxicity data EC_{50}/LC_{50} are available, the calculation of $PNEC_{water}$ is obtained from EC50/LC50 divided by an assessment factor (AF) of 1000. The availability of long-term/chronic NOEC values for one, two or three trophic levels are depending on assessment factor (AF) of 100, 50 and 10 respectively (European Commission, 2003). For risk characterization, a commonly used risk-ranking criterion was applied. When the RQ equals or exceeds 1, an ecological "high risk" is suspected. "Low risk" is suspected when $0.01 < RQ < 0.1$, whereas, $0.1 < RQ < 1$ indicates "medium risk" (11, 14)

Results

Risk quotients (RQ) and the corresponding levels of potential ecological risks to the terrestrial environment were calculated as shown in Table 1 and Table 2 respectively. Acute and chronic environmental risks of each compound were calculated based on the availability of PNEC and K_d data from the literature.

The acute environmental risks of 5 antibiotics are shown in Table 1. The results showed that tylosin possessed high acute ecological risk after one-month application of the broiler manure to agricultural soil, while trimethoprim is estimated to have medium acute risk in the environment. Norfloxacin has shown low acute ecological risk while erythromycin and sulfadiazine are estimated to

have no acute ecological risk in the terrestrial environment.

The chronic environmental risks of 3 antibiotics and one hormone are summarized in Table 2. Two antibiotics, trimethoprim, and tylosin were estimated to have medium chronic risk in terrestrial environment. The results showed that norfloxacin and progesterone possessed low chronic risk in the terrestrial environment after manure amendment.

Table 1: Estimated risk quotients and corresponding levels of potential acute ecological risks to the terrestrial environment

	Lowest EC_{50} or LC_{50} (ng/L)	AF	$PNEC_{water}$ (ng/L)	K_d (L/kg)	$PNEC_{soil}$	MEC[a] (ng/kg)	RQ (MEC/PNEC)	ERA
Erythromycin	3.1×10^{7}[b]	1000	3.1×10^{4}	164.76	5.0×10^{6}	0	0	No
Norfloxacin	7.0×10^{7}[b]	1000	7.0×10^{4}	7943	5.5×10^{8}	9.6×10^{4}	1.7×10^{-4}	Low
Sulfadiazine	2.2×10^{6}[c]	1000	2.2×10^{3}	2.8	6.2×10^{3}	0	0	No
Trimethoprim	1.6×10^{7}[b]	1000	1.6×10^{4}	25.7	4.1×10^{5}	6.0×10^{4}	0.15	Medium
Tylosin	1.4×10^{6}[b]	1000	1.3×10^{3}	128	1.8×10^{5}	6.8×10^{5}	3.84	High

[a](9), [b](15), [c](11)

Table 2: Estimated risk quotients and corresponding levels of potential chronic ecological risks to the terrestrial environment.

	Lowest NOEC (ng/L)	AF	$PNEC_{water}$ (ng/L)	K_d (L/kg)	$PNEC_{soil}$	MEC[a] (ng/kg)	RQ (MEC/PNEC)	ERA
Norfloxacin	$1.7E\times10^{5}$[b]	100	1.7×10^{3}	7943	1.3×10^{7}	9.6×10^{4}	7.1×10^{-3}	Low
Trimethoprim	1.0×10^{6}[b]	100	1.0×10^{4}	25.7	2.6×10^{5}	6.0×10^{4}	0.23	Medium
Tylosin	3.0×10^{5}[b]	50	6.0×10^{3}	128	7.7×10^{5}	6.8×10^{5}	0.88	Medium
Progesterone	1.0×10^{5}[d]	50	2.0×10^{3}	204	4.1×10^{5}	2.4×10^{4}	0.059	Low

[a](9), [b](15), [d](16)

Discussion

The results of the ERA performed in this study indicate that the release of antibiotic and hormone residues after soil amendment is posing certain ecological risks to soil environment. In this study, only the maximum MEC were considered for the calculation of RQ in the worst-case scenario. However, there are some limitations to assess the environmental risk of all target compounds reported by Ho et al. (9); because we are not able to collect all the toxicity data and the solid-water partition coefficient values in the literature. Moreover, the toxicity of the mixture of compounds was not considered. The potential environmental risk of each compound was characterized base on three risk levels: 0.01-0.1, low risk; 0.1-1, medium risk; and >1, high risk.

Previous studies are focussed in aquatic toxicity of pharmaceuticals; ecotoxicity of pharmaceuticals in soil environment was not well documented. However, RQ of trimethoprim in soil was previously reported by Martin et al. (13), the results of the study showed that trimethoprim has no risk in digested sludge amended soil and compost amended soil. Other compounds, such as eryth-

romycin, tylosin and norfloxacin showed low risk, moderate risk and low risk respectively in aquatic environment (15).

Conclusion

Veterinary antibiotics and hormone were detected in the broiler manure and the manure was subsequently applied onto agricultural soil as fertilizer. The MEC of these compounds in manure amended agricultural soil revealed that the application of broiler manure to agricultural soil may potentially pollute the environment and exerts considerable ecological risk in the soil environment. The results of this study showed that tylosin has high acute risk in Malaysian agricultural soil while trimethoprim and tylosin exerted medium chronic risk in the soil environment. The environmental risk of pharmaceuticals in agricultural soil is very much depending on the farm management and the sorption affinity of the compounds. The MEC in manure and soil is highly depending on the amount of antibiotics administered to the animals by the farmers. Therefore, it is advised to treat or compost the animal manure before applying on agricultural soil to reduce the ecological risk.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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