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Pharmaceuticals in the Environment and Management Practices in Abroad

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Pharmaceuticals in the Environment and Management Practices in Abroad

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1. Concerns and Primary Usage of Antibiotics

Antibiotics were originally designed to inhibit the activity of microorganism, viruses, and eucaryotic cells as therapeutic purpose for humans (Thiele-Bruhn, 2003). For animals, antibiotics are used not only to treat and prevent the illness therapeutically but also to promote growth as a non-therapeutic purpose (Tolls, 2001). However, antibiotics released to the environment are recently regarded as micropollutants and there is an increased concern to introduced antibiotics in environment due to the possibility of production of resistant bacteria in the ecosystem. These resistant bacteria can transfer the resistance genes to other bacteria and eventually render certain antibiotics useless (Ohlsen et al., 2003).

Previous studies have attempted to identify antibiotic resistance genes (ARG) in several environmental compartments (Chee Sanford et al., 2001) and these researchers have found tetracycline resistance genes in lagoons and groundwater located in two swine production facilities. They concluded in this study that groundwater or soil could be significant reservoirs for distribution of ARG. In addition, ARG has been found in farmland treated with pig manure slurry (Sengelov et al., 2003), wastewater, surface water, and drinking water biofilms (Schwartz et al., 2003), and sewers receiving hospital effluent (Guardabassi et al., 1998). This results indicate that ARG can be produced anywhere in the environment.

There are two aspects that antibiotics have a high potential to be released into environment and caused the adverse effects. The first factor is enormous usage amount of antibiotics for human and animals. According to a recent report (Mellon et al., 2001), the estimated total annual consumption of antibiotics was 35 million pounds in USA. Among the total usage amount, 3 million pounds that is 9% of total consumption were used for human and 2 million pounds occupied 6% of total consumption was used for animals as therapeutic purpose. However, most of the antibiotics were used in the absence of disease

for non-therapeutic purpose mainly as growth promoters for animals and usage amount was estimated to 25 million pounds that is 70% of total consumption.

The second aspect is pharmacokinetic mechanism of antibiotics. Antibiotics are intended to act efficiently at low doses and excreted completely from the body after administration. Consequently, antibiotics can be released up to 50% as a parent compounds or metabolites normally conjugated with glucose or other polar compounds (Thiele-Bruhn, 2003;Ternes, 2001).

As a result of two aspects described above, antibiotics can be introduced in environment and produce ARG into the ecosystem, potentially threatening human health.

2. Extraction of Antibiotics in Aqueous and Sediment

Antibiotics are mainly comprised of a non-polar backbone with functional groups causing high polarity. Certain antibiotics have more than two acid dissociation constants (pKa) and can be dissociated or protonated depending on pH of media. In addition, physicochemical properties of antibiotics exposed in strong acidic or basic condition can be altered and limited the proper analysis of antibiotics. Thus, general procedure to analyze the residual of antibiotics in different environmental compartments is difficult to establish and various analytical techniques have been documented in previous studies depending on different target antibiotics and sample matrices.

In general, solid phase extraction (SPE) technique is the most popular method to extract residual of antibiotics in aqueous matrix. Main mechanism of SPE method is to increase the retention efficiency of antibiotics in SPE cartridge or disk and at the same time, to remove any impurities present in the sample. To obtain optimized condition for SPE method, selection of proper cartridges or disk, pre-conditioning of cartridge prior to load the sample, and choosing appropriate organic solvent to extract residuals in the cartridge need to be considered. Among various SPE cartridges, HLB (Hydrophilic Lipophilic Balanced) cartridge has been used commonly to extract various antibiotics in aqueous matrix due to acceptance in wide range of pH and no silanols that can cause irreversible binding with certain compounds in the cartridge (Lindsey et al., 2001;Zhu et al., 2001;Kolpin et al., 2002;Loffler and Ternes, 2003a;b;Barreiro and Lores, 2003).

Pre conditioning of cartridge prior to loading the sample can make suitable condition in the cartridge to enhance the recovery efficiency of target antibiotics. Common procedure is to use adequate amount of organic solvents (i.e. methanol or acetonitrile etc.) to remove any organic residuals in cartridge followed by removing any impurities in the cartridge with deionized (DI) water. In certain cases, acidic or basic condition is preferred to retain antibiotics and applied in the cartridge (Lindsey et al., 2001;Kolpin et al., 2002;Barreiro and Lores, 2003;Miao and Koenig, 2002;McArdell et al., 2003;Wiegel et al., 2004).

To extract retained target antibiotics in the cartridge, various organic solvents are commonly used. Extracted target antibiotics are normally concentrated in adequate

temperature to lower the limit of quantification (LOQ) making enable to measure sub microgram of concentration of antibiotics in environment.

Developing robust and reliable analytical method for solid matrix is more challenging due to complexity of matrix and still lack of information is available. Using traditional extraction method, Soxhlet extraction, has been diminished because this method can produce significant amount of hazardous organic solvent and also require more time and labor (Koester et al., 2003). Instead of this traditional method, demanding of accelerated solvent extraction (ASE) referred to pressurized liquid extraction (PLE), adequate buffer solution extraction, and liquid liquid extraction (LLE) has been increased. New developed extraction techniques are considered to reduce solvent consumption, sample-handling time, and to increase the extraction efficiency. PLE method uses elevated pressure and temperature at short time under static condition to allow appropriate contact time with extraction solvent and sample for extraction (Ferrer and Furlong, 2002). Application of PLE method to extract environmental solid samples has been documented in previous studies (Ferrer and Furlong, 2002;Golet et al., 2002a;Christian et al., 2003;Jacobsen et al., 2004;Halling-Sorensen et al., 2005).

Buffer solution extraction method is an alternative method to extract target antibiotics in solid matrix. Since very polar and non-polar extractants might cause the incomplete extraction in solid matrix, weakly acidic or basic buffer solution is frequently used (Thiele-Bruhn, 2003). Certain organic solvent can be combined with buffer solution depending on physicochemical properties of target antibiotics. Hamscher et.al. (Hamscher et al., 2002) used citrate buffer solution (pH 4.7) to extract three tetracyclines from soil and same method was adapted to compare different class of antibiotics in sandy soil (Hamscher et al., 2005). Haller et.al. (Haller et al., 2002) used basic condition (pH 9.0) of buffer solution to extract sulfonamides and trimethoprim in manure. Buffer solution combining with organic solvent was adapted to extract various classes of antibiotics in animal feed (Caballero and Lapasio, 2002), manure (Kolz et al., 2005a;Kolz et al., 2005b;Liguoro et al., 2003), and soil (Blackwell et al., 2004a;Aga et al., 2005).

Although LLE method can produce certain amount of organic solvent, this method is still used to extract antibiotics from solid matrix. Schlusener et.al. (Schlusener et al., 2003) used ethyl acetate to extract eight antibiotics from liquid manure and two ionophore antibiotics was extracted from animal feed with methanol (Bertini et al., 2003).

3. Separation and Detection of Antibiotics

A common technique to separate and detect antibiotics in extracted environmental samples is high performance liquid chromatography (HPLC) combined with mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Before HPLC/MS or HPLC/MS/MS is adapted as routine techniques for antibiotic analysis, gas chromatography mass spectrometry (GC/MS) was used to measure pharmaceuticals in river and wetland (Gross et

al., 2004) and also to track the pathway of several pharmaceuticals including antibiotics from sewage treatment plant to watershed (Heberer, 2002). However, GC/MS method requires post-derivatization step that can cause more time, labor and contamination in the sample. In addition, interface of HPLC/MS or HPLC/MS/MS has been developed rapidly making enable to increase sensitivity and efficiency of antibiotic analysis.

Several variables need to be considered to optimize HPLC conditions for better separation of target antibiotics. Mobile phase composition, selection of analytical column, and injection volume are major consideration. Diluted acids or weakly acidic buffers composed with formic, phosphoric, or citric acid in deionized (DI) water is a common practice to avoid possible dissociation of antibiotics and normally combined with organic solvent (i.e. methanol and acetonitrile) (Thiele Bruhn, 2003). Analytical columns and injection volume can be varied depending on target antibiotics and sample matrix.

Two popular ionization techniques are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both methods have similar mechanism to produce protonated or deprotonated molecules for mass analysis (Zwiener and Frimmel, 2004). ESI technique is widely used to polar compounds while APCI is more suitable for medium or low polarity compounds (Alda et al., 2003). To resolve complex chromatographic peaks in environmental samples, MS or MS/MS is primarily adapted. Single MS method with selective ion monitoring (SIM) was used in early developing period for environmental samples but has been replaced with more sensitive and selective technique, MS/MS, for complex samples. Disadvantage of applying SIM method is that analysis can be interfered from co-eluting analytes and matrix components fragmented during analysis (Zwiener and Frimmel, 2004). Therefore, MS/MS techniques compensate this discrepancy to fragment molecules once more using collision energy to increase the selectivity of target compounds. Recently, more advanced mass analyze technique, time of flight (TOF), is available and adapted in analysis of environmental samples (Ferrer et al., 2004; Ferrer and Thurman, 2003). This method is superior to identify unknown or metabolites of compounds by avoiding false findings in process.

4. Pathways and Occurrence of Human and Animal Used Antibiotics

Anticipated exposure pathways of human and animal used antibiotics are different and schematic diagram of different pathways is shown in Figure 1. Exposure pathways of human and animal used antibiotics need to be identified because dose of antibiotics and duration of treatment are necessary for environmental loading calculation (Jorgensen and Halling Sorensen, 2000).

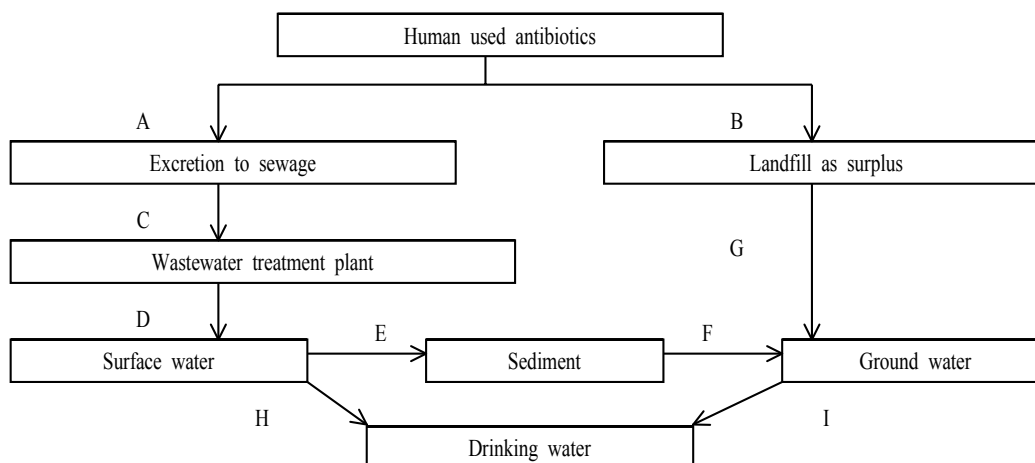


Figure 1. Anticipated pathway of human used antibiotics. Pathways are numbered for specific reference in the text

Administered human used antibiotics are excreted to sewage (route A) and mainly end up in wastewater treatment plants (WWTP, route C). Incompletely removed residuals of human derived antibiotics are released directly into surface water (route D). In certain cases, introduced antibiotics in surface water can be sorbed in sediment (route E) or leached into groundwater depending on physicochemical properties of different antibiotics. Eventually, surface water or groundwater contaminated with residual of antibiotics can be used as drinking water source and threaten human health risk.

From the late 1980s, occurrence of human derived antibiotics has been reported (Richardson and Bowron, 1985) and later more effort has been made to quantify the human derived antibiotics in different environmental compartments. Hirsch et al. developed analytical method to measure 18 antibiotics in water sample and measured concentration of studied antibiotics at the range of 0.2–6.0 $\mu\text{g}/\text{L}$ in WWTP effluent, surface water, and groundwater (Hirsch et al., 1998; Hirsch et al., 1999). Lindsey et al. introduced standard addition method to measure two classes of antibiotics and found concentration range of 0.06–1.34 $\mu\text{g}/\text{L}$ in surface water and groundwater (Lindsey et al., 2001). Kolpin et al. surveyed 95 organic waste contaminants including 22 antibiotics derived from WWTP and provided concentration of antibiotics measured in several streams of U.S (Kolpin et al., 2002). Until now, numerous studies have been documented to report occurrence of human used antibiotics in environment and measured concentration was generally less than 1 $\mu\text{g}/\text{L}$ with few exception (Barreiro and Lores, 2003; Miao and Koenig, 2002; McArdell et al., 2003; Wiegel et al., 2004; Gross et al., 2004; Heberer, 2002; Farre et al., 2001; Golet et al., 2002b; Stamatelatou et al., 2003; Vanderford et al., 2003; Gobel et al., 2004; Cahill et al., 2004; Kolpin et al., 2004; Glassmeyer et al., 2005).

Pathways of animal used antibiotics are somewhat unlike with human used antibiotics and anticipated pathway is shown schematically in Figure 2.

Antibiotics are normally administered to animals with medicated feed or external

application and excreted animal used antibiotics are contained in storage as manure (route J). After a certain period, stored manure containing residual of animal used antibiotics can be applied in field as fertilizer. Soil is the main environmental source to be exposed in animal used antibiotics (route M) and introduced contaminants in soil can be transferred to surface water and groundwater via surface runoff and leaching (route O and Q). For this reason, animal used antibiotics also have a potential to harm the human health after surface water and groundwater are used as source of drinking water.

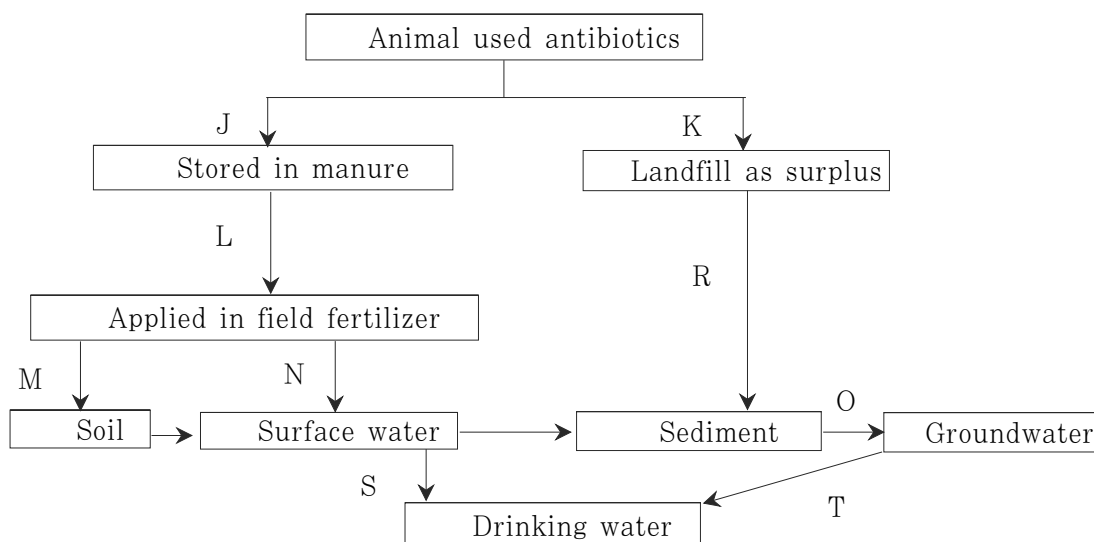


Figure 2. Anticipated pathway of animal used antibiotics. Pathways are numbered for specific reference in the text

Presence of animal used antibiotics is mainly reported in soil or manure matrix and much higher concentration compared to reported concentration of human used antibiotics in aqueous matrix was measured for both matrices. Previous studies measuring animal used antibiotics in manure revealed that significantly higher concentration up to 12.4mg/kg is present in manure (Christian et al., 2003;Haller et al., 2002;Liguoro et al., 2003;Schlusener et al., 2003). Hamscher et.al attempted to measure 4 veterinary antibiotics in soil previously fertilized with liquid manure and found 4.6–198.7 mg/kg of residuals within 30cm of soil depth (Hamscher et al., 2002). This study also concluded that tetracyclines could build up persistent residuals via repeated fertilization. Another studies also verified that fairly high concentration is present in soil amended with manure (Jacobsen et al., 2004;Hamscher et al., 2005;Liguoro et al., 2003;Blackwell et al., 2004a;Aga et al., 2005).

To demonstrate the occurrence of veterinary antibiotics in surface water or groundwater located in near animal feeding operations (AFOs), several studies reported the measured concentration of animal used antibiotics (Zhu et al., 2001;Blackwell et al., 2004b) and much lower concentration was detected in surface water and groundwater. This result might indicate that a natural attenuation mechanism (biotic and abiotic) may be involved in released antibiotics to environment.

5. Fate and Transport of Released Antibiotics in Environment

As stated in section 4, human and animal used antibiotics have been present in different environmental compartments with various concentration ranges. Consequently, understanding the fate of released antibiotics in environment is also important. Once antibiotics are introduced in environmental compartments, concentration of antibiotics in different matrices depends on biotic or abiotic processes including biodegradability, partitioning to solid matrix, and subsequent movement.

Biodegradation is the primary process to dissipate parent antibiotics released in environment. Microorganisms utilize the antibiotics as substrate and mineralize to decompose the antibiotics. Common experiment method for biodegradation is closed bottle test (CBT) and 18 selected antibiotics were evaluated to assess the biodegradability (Alexy et al., 2004). The result of this study showed that none of the studied antibiotics was readily biodegradable during study period (28 days) and concluded that co-metabolism, microorganisms do not obtain any energy from utilization of antibiotics, might lead to the slow degradation.

For human used antibiotics, fate and removal of released antibiotics was mainly focused on WWTP and drinking water system. In general, human used antibiotics cannot be fully eliminated in WWTP process and removal rate was varied depending on physicochemical properties of antibiotics and different operation process (Ternes et al., 2003; Clara et al., 2004; Kreuzinger et al., 2004a; Kreuzinger et al., 2004b; Castiglioni et al., 2006). Recently, fate and removal rate of introduced antibiotics were evaluated in drinking water treatment system (Ballard and Mackay, 2005; Westerhoff et al., 2005). In Westerhoff et al. study, adequate amount of 62 different endocrine-disruptors (ED), pharmaceuticals including antibiotics, and personal care products (PPCP) were manually injected in bench scale drinking water treatment (DWT) followed by general DWT process, coagulation, flocculation, and adding power activated carbon (PAC) to examine fate of target compounds. This study revealed that removal efficiency was varied polarity of each compounds and more elimination of compounds was observed as amount of PAC was increased.

For animal used antibiotics, sorption properties and mobility of compounds play an important role to decide the fate of released antibiotics and evaluating the fate of veterinary antibiotics in solid matrices is necessary to predict accurate behavior in environment for future environmental risk assessment (ERA). Laboratory scale experiment, batch test and column test, is the common practice to validate the sorption or mobility of antibiotics because parameters are easy to control and experiment can be repeated easily. Although, field experiment requires more attention to control the parameters, it can reflect a more realistic situation than laboratory experiment.

Sorption and mobility characteristic of antibiotics can be varied depending on several variables and soil texture, pH, and organic matter content are the major key parameters

(Hari et al., 2005). For instance, tetracyclines possessing acidic characteristic and strongly sorbed in solid matrix tend to decrease the sorption, as pH is increased and more sorptive in clay fraction rather than other texture in soil (Kulshrestha et al., 2004; Figueroa et al., 2004; Jones et al., 2005). In addition, metal complexation can be a part of sorption mechanism for tetracyclines (Gu and Karthikeyan, 2005).

In case of less sorptive antibiotics, sulfonamides, sorption to solid matrices is minimal and shows more mobility (Thiele, 2000; Boxall et al., 2002; Thiele-Bruhn et al., 2004; Gobel et al., 2005). More mobile antibiotics have the possibility to leach into groundwater and might contaminate the drinking water when groundwater is used as drinking water source.

6. Pharmaceutical management in EU, USA, and Canada

Recently, there has been an increased concern to emerging contaminants such as pharmaceuticals and hormones in the environment. For this reason, new regulation and environmental risk assessment (ERA) need to be considered to minimize adverse effect of emerging contaminants on human and ecology. Table 1 summarizes legal regulating authority, scientific decision/advisory body, action limit, guide of ERA systems in the EU, US, and Canada (Park, 2005).

Table 1. Outline of environmental risk assessment in the EU, US, and Canada
(adapted from (Park, 2005))

	EU	US	Canada
Legal regulatory authority	EMA (European Medicines Agency)	FDA (Food and Drug Administration)	Health Canada Healthy Environments and Consumer Safety Branch
Legal regulations	Directive 2001/83/EC	The national environmental policy act of 1969 (NEPA) FDA's regulations in 21 CFR part 2,62 FR 40569	The Food and Drug Act (CEPA 1999)
Scientific decision/ Advisory body	National authorities or national environmental agencies committee for properties medicinal products	Food and Drug Administration	The Environmental Assessment Unit
Action Limit	PEC surface water < 0.01 µg/L	EIC < 1 µg/L	-
Guide	Guidance on environmental risk assessment of medicinal products for human use	Guidance for industry-environmental assessment of human drug and biologics applications	Guide to New substances Notification for Products Regulated Under the Food and Drug Act

1) Regulations in EU

European Union (EU) introduced “Guideline on the environmental risk assessment of medicinal products for human use” in 2005 to protect environment against potential hazards of human pharmaceuticals. Human Medical Products (HMPs) evaluations are carried out through the Committee for Medical products for human use (CHMP) and veterinary medical products (VMPs) are assessed by the committee for Medicinal Products for Veterinary Use (CVMP).

The environmental risk assessment in the EU consists of two phases. Phase I assesses the degree of exposure in the environment and Phase II assesses the environmental risk with its physical, chemical, pharmacological and/or toxicological properties. Phase II consisted with two tiers (tier A and B) requires physicochemical properties, environmental fate and environmental effects data of pharmaceuticals. Table 2 summarizes phased approach of environmental risk assessment in EU.

Table 2. Phase approach in the environmental risk assessment

Stage in regulatory evaluation	Stage in risk assessment	Objective	Method	Test/Data requirement
Phase I	Pre-screening	Estimation of exposure	Action limit	No test requirement
Phase II Tier A	Screening	Initial prediction of risk	Risk assessment	Base set aquatic toxicology and fate
Phase II Tier B	Primary	Standard approach to ensure consistent decision making	Risk assessment	Extended data set on emission, fate and effects
	Secondary	Substance and site specific refinement		Case- by-case; Alternative approaches, TGD approach

1) TGD: Technical Guidance Document

2) Regulation in Canada

The Canada set the regulation named “Canadian Environmental Protection Act (CEPA)” in 1999 for all pharmaceutical substances. This regulation requires that all the substances are assessed for environmental and human health effects and risks prior to import or manufacture in Canada. Thus, risk assessments are performed to prevent potentially harmful substances from entering into the Canadian environment under the CEPA, *New Substances Notification Regulation (NSNR)*. After submitting a notification, new pharmaceuticals should be processed through screening, assessment, and decision steps.

3) Regulations in the US

In US, “Guidance for Industry: Environmental Assessment for Human Drug and Biologics Applications” was published at the FDA in July 1998. For environmental

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assessment (EA), concise document that provides sufficient information when determining whether an environmental impact statement or finding of no significant impact should be prepared (Park, 2005). Assessment procedures involve a toxicity evaluation including fate and effects of the pharmaceuticals. Approval of New Drug Applications (NDAs) or efficacy supplements when the approval will increase the use of the drug and concentration of drug expected to enter the aquatic environment (expected introduction concentration, EIC) is 1 ppb or greater.

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