Chapter 13

Risks of agricultural pharmaceuticals in surface water systems and soils

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Geographic and temporal distributions of agricultural pharmaceuticals measured in surface water at several locations in Grand River watershed in Southern Ontario, Canada showed 5 pharmaceuticals with pulses occurring between May and November at similar but varying times. Compared to species sensitivity distributions of acute toxicity values, pharmaceuticals detected in the surface waters presented small risks to aquatic organisms from acute effects. Effects on population and community responses in microcosms support the observation of low risk to the environment. In vitro bioassays of twelve pharmaceuticals on the arbuscular mycorrhizal fungi, *Glomus intraradices* grown on *Daucus carota* root organ cultures showed that, doxycycline, 17-α-ethinyl estradiol, and carbamazepine were selectively toxic to *G. intraradices* with 28-d EC50s less than 100 μg/L. Risks to plants and mycorrhizal fungi from estimated environmental concentrations were small.

Introduction

Increasing research and attention to human- and agricultural-use pharmaceuticals in the last decade have highlighted the extensive use of these products and their presence in the environment, particularly in surface waters, sediments and soils. Estimates of total pharmaceutical use are not readily available in

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many countries, especially North America and, as a result, it can be difficult to determine exactly what is being used and in what quantities. Some usage data for human prescription pharmaceuticals are available as sales information; however, this does not include livestock production or veterinary use. The United States and individual EU member states report primarily on the sales of antibacterial agents. Similar information is not readily available in Canada. Data on the sale and usage of veterinary medicines from Europe and the United States suggest that antimicrobial substances represent the highest amounts of pharmaceuticals used in livestock production with approximately half of the 22,700 tonnes of antibiotics produced annually in the US used in agriculture (*1,2*). The estimate for agricultural use in the United Kingdom for 2000 was 897 tonnes (*3*).

Since early reports drew attention to the issue of pharmaceuticals in the environment (*4*), a number of studies have reported concentrations in effluents from sewage treatment plants (STPs) (*5,6*) and surface waters (*7-9*). The application of manure and/or biosolids to agricultural fields, a recommended component of sustainable agriculture today, represents a potential non-point source for inputs of pharmaceuticals into aquatic environments (*10*). Although pharmaceuticals have been observed to degrade in manure (*11*), movement into the soil is also reported (*12*). Residues of chlortetracycline, tetracycline, oxytetracycline, and tylosin decreased to approx 1 µg/kg at 60 cm depth from 9-12 µg/kg in the upper 10 cm of soil in fields amended with manure slurry (*11*). Chlortetracycline, oxytetracycline, tetracycline, and tylosin were also reported at concentrations at the limit of detection (LOD) in the water samples near fields where manure had been applied (*13*). Agricultural-use antibiotics were reported from watersheds in Ontario (*14*) and temporal sampling over one season revealed the presence of several antibiotics and pharmaceuticals with large frequencies of detection and relatively large concentration ranges (Figure 1) in a subwatershed of the Grand River where intensive production of animals is practiced (data from *10*). While livestock production clearly represents a primary input to agricultural areas, contributions of human pharmaceuticals from application of biosolids to agricultural lands and seepage from septic systems cannot be disregarded. Leaching from biosolids spread on agricultural lands was suggested as the most likely source of carbamazepine in a portion of the Grand River watershed that received no inputs from STPs (*10*).

This paper presents an overview of environmental effects of pharmaceuticals in water systems and soil. Although the primary focus is on pharmaceuticals used in agriculture, studies on human-use products are also included. While we concentrate mainly on approaches and methods for assessing environmental effects and risks from agricultural pharmaceuticals, these principles apply to pharmaceuticals in general.

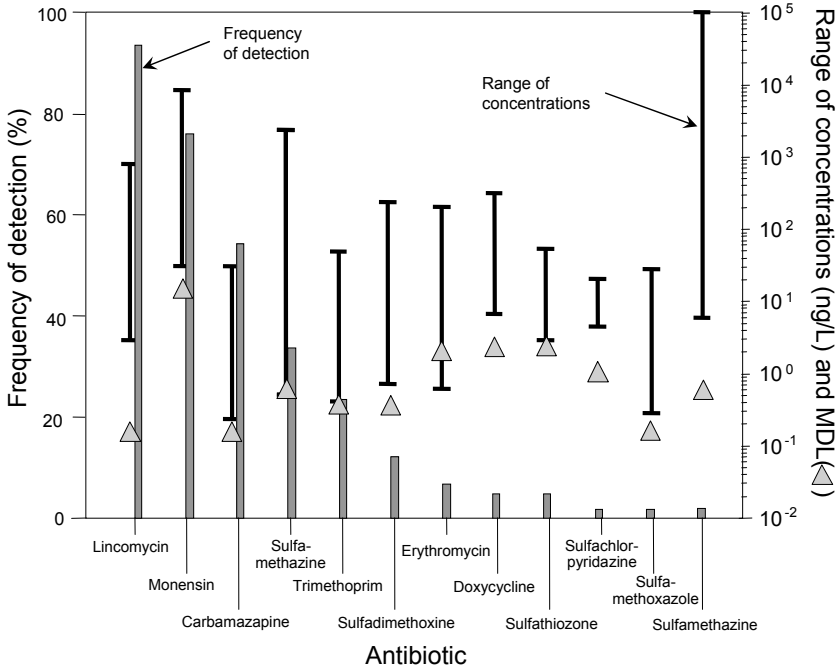

Figure 1. Frequency and range of concentrations of pharmaceuticals detected in surface waters a subwatershed of the Grand River, Ontario, Canada April 2003 and 2004 (data from 10).

Problem formulation and special considerations for pharmaceuticals

Surface water systems

As with all substances in the environment (*15,16*), assessing hazards and risks from pharmaceuticals requires knowledge of exposure as well as effects. Characterization of exposure is made more complex because pharmaceuticals from human sources (STPs) are frequently present as mixtures. These mixtures may change seasonally in response to pharmaceuticals used to treat seasonal infections but in the shorter term, consistent mixtures of components are a reality. For substances with a common mechanism of action, additivity is likely (*17,18*) but synergism and antagonism cannot be ruled out. Furthermore, continuous releases of pharmaceuticals result in chronic exposures, even for those substances that are not highly persistent in the environment. This pseudopersistence must be considered in developing tools to characterize effects.

Another issue is that, with a few exceptions such as parasiticides (*19*), pharmaceuticals are not designed to be highly acutely toxic to the organism in which they are used and therefore are usually not acutely lethal to non-target animals, although plants may be affected (*20*). The effects of pharmaceuticals may not be easily observed unless they produce clear biomarker responses, such as have been observed in fish exposed to estradiol, estrone, and ethinyl estradiol from STPs (*21*). In this latter case, lethality was not a suitable endpoint – reproduction and population structure were the most likely to be affected. Similarly, effects of diclofenac on vultures were not acutely lethal (*22*), although serious in the end. As a result, the environmental effects and subtle changes associated with pharmaceuticals in aquatic ecosystems remain relatively unknown.

In an attempt to address the issue of effects of mixtures (and single substances), we have used aquatic microcosms (12,000 L outdoor pools) as a surrogate for an ecosystem(*23-26*). Because microcosms contain many species in several trophic levels, most of which interact in a food web, subtle and non-lethal responses in one or more groups of organisms may be observed as changes in the structure (diversity and abundance of organisms) of the community. If a range of concentrations is tested in these systems, it is possible to use concentration-dependent changes in structure to identify causal relationships for further investigation. In a sense, these systems are hypothesis generating in terms of subtle effects (acute or chronic) but also have the additional advantage that they are also exposed to environmental factors, such as sunlight, thus providing the tools to assess fate processes, such as photolysis under more realistic conditions.

Chronic exposures are difficult to attain in large-volume microcosms because of the amounts of water that would be required to mimic a flowing system such as a river or stream. Chronic exposures may, however, be achieved through regular addition of the test substance as long as these amounts are small in relation to the total volume of the system (*23-27*).

If the proportions of the components of environmentally-relevant mixtures are known from measurements and the probability of occurrence can be estimated, these values may be used to extrapolate to greater concentrations than occur in the environment; responses to which may be used to determine margins of exposure and to identify possible biomarkers for use in field assessments (Figure 2). When these concentrations are based on distributions of values, probability of co-occurrence can be estimated. For example, if a mixture of chemicals is highly correlated and they always occur together in a fixed proportion, then a combination of these at the 99th centile will have a 1% probability of exceedence. If, at the other extreme, the components of the mixture are not correlated at all, the probability of co-occurrence is the product of the individual probabilities. Thus, for a three-component mixture at the 99th centile, the product of the individual exceedences (1% x 1% x 1%) is 0.0001%. In reality, as no mixture will always be completely correlated and complete independence also will not occur, the exceedence probability will be between these two values with the greatest (1% in this example) being the worst case.

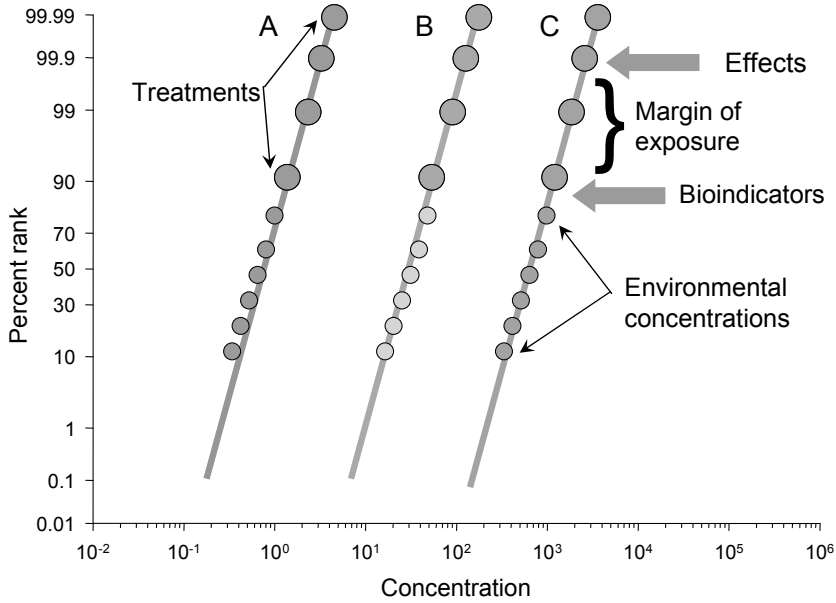

Figure 2. Illustration of the concept of testing mixtures of pharmaceuticals (A, B, and C) combined on the basis of cumulative distributions of measured or modeled values to allow the selection of treatment concentrations for microcosm studies.

Soils

Veterinary pharmaceuticals enter terrestrial systems through the common practice of amending soils with manure from intensive livestock operations or directly from grazing and free-ranging livestock. Human pharmaceuticals and personal care products may enter soils through the application of biosolids collected from the grit chamber, settling tanks, and waste activated sludge of municipal wastewater treatment systems. The application of pharmaceutical-containing manure and biosolids may lead to direct phytotoxic responses, resulting in a decrease in agricultural crop protection. Of equal importance is the indirect effect pharmaceuticals may have on soil health and productivity through effects on beneficial microbial and symbiotic processes that contribute to plant growth and yield. For example, most plants, including the majority of crop species, form a beneficial symbiosis with arbuscular mycorrhizal fungi (AMF), which improves plant nutrient uptake, tolerance to drought, pathogenic and toxic stressors as well as improved seedling establishment and soil stability (*28*). In addition, the below-ground mycorrhizal community has been shown to influence the plant community structure in terms of dominance and growth (*29*). Preferential feeding on AMF by soil invertebrates, such as Collembola, also highlights this plant symbiont as a key trophic level in soil food webs (*30*). Despite the fact that these specialized fungi provide ecological functions that are not duplicated by any other organism, within the fungal or any other kingdom,

they have been largely ignored in the evaluation of effects caused by the introduction of a chemical stressor to an agro-ecosystem.

Standardized plant growth and emergence experiments (*31,32*) were used to evaluate the impact of ten common pharmaceuticals and personal care products (PCPPs) directly on growth of three economically important agricultural crop species. A new technique using root organ cultures to host AMF in culture (*33*) was also used to evaluate the impact of 12 different PCPPs on root growth of carrot (*Daucus carota*) as well as growth and propagule endpoints for the AMF, *Glomus intraradices*.

Responses to pharmaceuticals in the environment

Surface water systems

A number of studies have reported on the acute toxicity of agricultural antibiotics and pharmaceuticals to aquatic organisms. In laboratory studies, the most sensitive non-target organisms to antibiotics are generally plants (*34*). Of the antibiotic classes tested, only members of the fluoroquinolone, sulfonamide, and tetracycline classes of antibiotics displayed significant phytotoxicity. The most toxic members of each of these classes tested were lomefloxacin, sulfamethoxazole, and chlortetracycline, with wet weight EC25 values of 38, 37, and 114 mg/L, respectively (*34*). These responses are most likely as a result of the evolutionary relationship between the chloroplasts and other key functions found in modern plants (the chloroplast, DNA gyrases, etc.) and similar processes in bacteria and the blue-green algae (*20,34,35*). Similar observations have been made in microcosms. For example, the antibiotic growth promoter, tylosin, promoted growth of the aquatic macrophyte, *Lemna gibba* in laboratory bioassays with a lowest observed effect concentration (LOEC) of 300 μg/L whereas, in the field, no effects were observed in this species at concentrations up to 3000 μg/L from a single treatment. However, exposures of the aquatic macrophyte *Myriophyllum spicatum* demonstrated a LOEC of 3000 μg/L in the microcosms. No effects were observed at higher trophic levels in microcosms treated with tylosin at concentrations up to 3,000 μg/L. Monensin, an ionophore antibiotic widely used in the poultry and beef industry was of low toxicity to plants (*34*). Effects on zooplankton community structure and population dynamics were evaluated in microcosms after a single treatment at concentrations ranging from 0.5 to 500 μg/L. Monensin did not significantly affect community structure within trophospecies (Rotifera, Cladocera, Copepoda adults, Copepoda nauplii, Ostracoda, and macroinvertebrates). However, significant changes within trophospecies groups were observed with decreases in the abundance of Rotifera and Copepoda nauplii and in the richness of Rotifera and Cladocera (*36*). A concentration-dependent increase in Ostracoda abundance was also observed.

Studies with mixtures of tetracyclines (oxytetracycline, chlortetracycline, tetracycline, and doxycycline) at total concentrations of 0.08, 0.22, 0.67, and 2.29 μM over a period of 35 days in microcosms showed no direct effects on

macrophyte growth, although they were toxic to *L. gibba* at EC50 concentrations ranging from 219 to 1010 μg/L when tested individually under laboratory conditions (because of differences in molecular weight, concentrations of mixtures of different pharmaceuticals have been expressed in molar units). In microcosms, a concentration-dependent decrease in the growth of *M. spicatum,* but not *L. gibba,* was observed at all treatment concentrations tested. This was ascribed to the formation of colored breakdown products in the microcosms which then reduced the penetration of light into the water column, thus reducing rates of photosynthesis and, as a result, growth (*25*). As *L. gibba* is a floating macrophyte with leaves above the surface, it was unaffected. Depths and volumes of water used in test systems with *M. spicatum* in the laboratory were such that any formation of colored compounds would not interfere significantly with availability of light (*25*). Effects on some phytoplankton endpoints were observed in the 0.22 μM and greater treatment concentrations. The largest responses were concentration-dependent reductions in total phytoplankton abundance and species richness (*26*). Abundance of phytoplankton recovered to control levels in all microcosms after treatment was terminated, and resilience (return to normal operating range before the removal of the stressor) was observed with respect to species richness of phytoplankton. Despite the effects on phytoplankton and rooted macrophytes and the potential for foodchain-driven interactions, zooplankton were generally unaffected by treatment with the tetracyclines. No-observed-effect-concentrations observed in microcosms were 40 to 100-fold greater than maximum concentrations measured in the environment (*26*).

Treatments of microcosms with more complex mixtures of pharmaceuticals have not shown effects that could not be explained by the action of one or more components of the mixture. Treatment of microcosms with a mixture of ibuprofen (a nonsteroidal anti-inflammatory drug), fluoxetine (a selective serotonin reuptake inhibitor), and ciprofloxacin (a DNA gyrase–inhibiting antibiotic) in the ratio of 6:10:10 at total concentrations of 0.09, 0.88, and 8.8 μM for 35 days resulted in few responses at 0.09 μM (*23*). However, mortality of fish occurred within the 35 d period at 0.88 μM and within 4 d at 8.8 μM. Fish mortality was attributed to fluoxetine and occurred at concentrations below therapeutic values for humans, suggesting enhanced sensitivity in aquatic vertebrates. Phytoplankton increased in abundance and decreased in diversity (number of taxa) at 8.8 μM, with consistent trends at 0.88 and 0.09 μM. Zooplankton increased in abundance but decreased in diversity at 8.8 μM with a similar trend at 0.99 μM, suggesting an interaction between phyto- and zooplankton. *L. gibba* and *Myriophyllum spp*. showed mortality at 8.8 μM with reduced growth of *L. gibba* at 0.88 μM. Effects on these macrophytes were attributed to sensitivity to ciprofloxacin, a response confirmed in laboratory studies (*34*). Treatment of microcosms for 35 d with a mixture of atorvastatin, acetaminophen, caffeine, sulfamethoxazole, carbamazepine, levofloxacin, sertraline, and trimethoprim) at total molar concentrations of 0, 0.044, 0.608, 2.66, and 24.5 μmol/L showed concentration-dependent effects on growth of *L. gibba* and *M. spicatum*, with EC25s of 0.5 and 0.6 μmol/L, respectively. Effects on plants were attributed to the combined response to levofloxacin (a DNA-gyrase inhibitor) and atorvastatin a lipid-reducing drug used in humans that also

inhibits an analogous target in plants, 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMGR) (*37*). These effects were observed at concentrations 10 to 100-fold greater than measured or predicted environmental concentrations.

A commonality to all of the laboratory and microcosm studies with agricultural antibiotics was that effects of individual compounds and mixtures of these were only observed at concentrations 10 to 100-fold larger than the greatest observed or estimated environmental concentration. Based on laboratory test data with individual pharmaceuticals, synergistic or antagonistic interactions were not observed with mixtures in the microcosms; however, the studies were not designed to specifically address this and low levels of interaction would not have been noticed.

Soils

Exposure of carrot (*D. carota*), alfalfa (*Medicago sativa*) and lettuce (*Lactuca sativa*) to ten antibiotics were found to elicit phytotoxic responses to seed germination, root, shoot and total growth, in vitro, typically at concentrations greater than 1,000 µg/L. Exceptions were the sulfonamide, fluoroquinolone and tetracycline classes of antibiotics where the results varied considerably between 10-1,000 µg/L dependent upon the tested plant species (*38*). These results are not unexpected as germination and radical growth are a highly conserved mechanism, with much of the carbohydrates, lipids, and nutrients needed for initial growth provided by seed reserves and mechanisms of action related to processes such as photosynthesis would have little effect. For example, the sulfonamide class of pharmaceuticals inhibits plant folate synthesis in a mechanism similar to that which causes its antibacterial activity (*39,40*). Folates are essential cofactors in one-carbon transfer reactions for all organisms. Initial folate concentrations in seeds have been shown to support root elongation for the initial growth period (*41*), indicating that longer duration plant-based experiments are required to evaluate effects. Plant emergence experiments in soil and root organ experiments in culture were conducted extending from 4 to 8 weeks. The majority of the tested compounds did not result in a decrease of any measured plant growth endpoint at exposure concentrations less than 1,000 µg/L. The most phytotoxic pharmaceutical observed was the sulfonamide antibacterial, sulfamethoxazole, with significant decreases in root growth observed at 10 µg/L. Exposure to atorvastatin, levofloxacin and chlortetracycline also resulted in significant decreases in plant growth. Measured environmental concentrations of these compounds in biosolids or manure, however, are typically an order of magnitude less than concentrations where phytotoxicity was observed. A lack of plant response to pharmaceuticals at environmentally relevant concentrations is consistent with the literature (*42-44*).

Concurrent to the root organ culture phytotoxicity tests, the mycorrhizal endpoints of hyphal growth and spore production were measured using the AMF, *G. intraradices,* in root-organ culture (*45*). In general, if effects were observed on the plant root organ culture-AMF system, three characteristic plant-microbe responses were noted. Some pharmaceuticals resulted in primarily

phytotoxic responses such as the previously described sulfamethoxazole and atorvastatin. Mycorrhizal fungi are obligate symbionts with the plant host and consequently decreases in hyphal growth and spore production were observed following the phytotoxic response. Other pharmaceuticals elicited a narcotic or generalized toxicity where the plant and mycorrhizal fungi were impacted at a similar rate of toxicity. This response type, which is characterized by parallel reductions in the measured endpoints, occurred with the fluoroquinolone, levofloxacin, and chlortetracycline. Of greater interest, were the few pharmaceutical compounds which resulted in little or no phytotoxicity but resulted in significant reductions in mycorrhizal growth. These compounds were the antibacterial, doxycycline, the synthetic estrogen, 17-α-ethinyl estradiol and the anti-epileptic, carbamazepine (Figure 3). As was reported with the evaluation of the potential for phytotoxicity due to pharmaceuticals in soil, the concentrations required to elicit a negative response in AMF were in general, much greater than that measured in manure and biosolids. The importance of the observed negative effects to beneficial microbes without showing evidence of effect through an immediate phytotoxic response should, however, be further considered. The vast majority of pharmaceuticals are antibiotics and effects of these compounds on bacterially regulated soil mineralization processes as well as the symbiotic relationships between legumes and rhizobacteria still require examination.

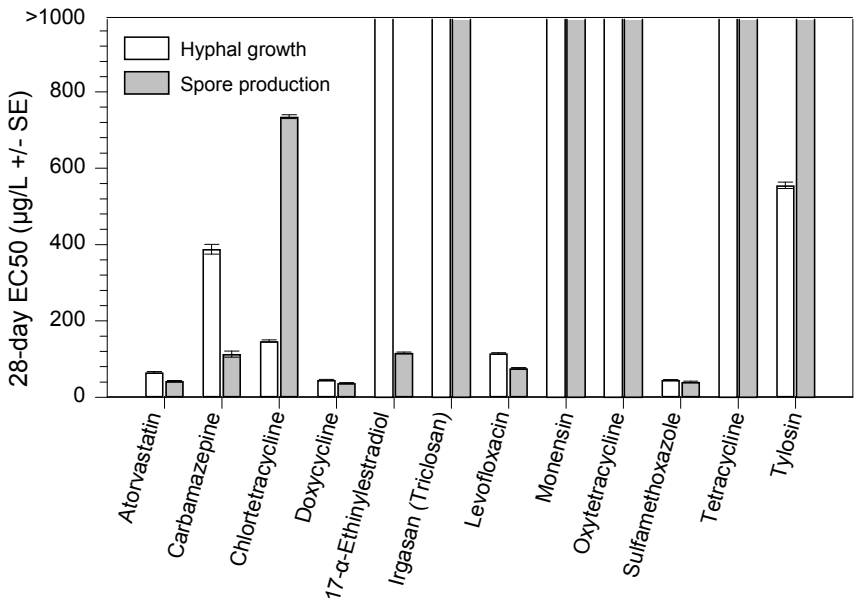

Figure 3. EC50 (28 d) values for pharmaceuticals and antibiotics in Glomus intraradices, in root organ culture (redrawn from data of Hillis et al (45)).

General conclusions

Some pharmaceuticals have been observed to have adverse effects in the environment; however, these are few in number. Natural and synthetic estrogens have been observed to have effects in surface waters with a high proportion of inputs from STPs (*21*) and the veterinary drug diclofenac has been shown to be the cause of decline of Indian vultures (*22*). Other pharmaceuticals, such as the selective serotonin reuptake inhibitors have been shown to be toxic to algae (*17*), affect behavior in pelagic animals (*46*), and reproduction (*18*) but the relevance of this at the population level or in the field is still uncertain. The antiparisiticide, avermectin, (also used an insecticide) has been demonstrated to cause changes in ecosystem structure and function at concentrations ≥30 ng/L, below the predicted environmental concentration (*19*), suggesting the potential for adverse effects, however, the current paucity of measures of environmental concentrations prevents the use of a full environmental risk assessment.

As discussed above, although there are more data on ecological effects for antibiotics used in agriculture, not all antibiotics have been tested at this time; only those used in large amounts or detected frequently in the environment have been characterized. Based on responses to acute bioassays in the laboratory and currently reported concentrations from surface waters, there is a low acute risk of adverse environmental effects for individual antibiotics. At large concentrations of mixtures of antibiotics, significant acute and chronic effects have been observed in microcosms at all levels of biological organization. At this time, these responses were observed at concentrations 10 to 100-fold above currently measured environmental concentrations and risks from exposures to individual substances and mixtures of these appear to be low (*10*). Although there are few data for sediment-dwelling organisms, responses appear to occur at concentrations greater than those measured in the environment (*47*), consistent with observations for water-dwelling organisms. Overall, risks from agricultural use of antibiotics and pharmaceuticals appear to be small.

Other considerations which influence the potential risk of PCPPs to soil systems is that reported biosolid and manure concentrations are often reported before aging or anaerobic digestion of human and animal waste, which typically results in further reductions of the active pharmaceutical parent compound. In addition, the dilution of the pharmaceutical concentration which occurs from the application of manure and biosolids across an agricultural landscape should also be considered. There is a paucity of measured concentration data in the literature for pharmaceuticals in soil following amendment with biosolids or manure.

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