Need of Designing Model for Screening of PPCPs (Ecopharmacology) and Therapeutic Drugs (Pharmacoenvironmentology) in Aquatic and Terrestrial Environment

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INTRODUCTION

Man consumes drug for various medical and non-medical reasons. There are innumerable incidents where these medicines played havoc on humankind. If they are boon to man, they are also afflicting diseases. But, what about human drugs when they have an effect on environment? There has been growing concern among scientists and environmentalists about the vast amount of drugs that end up in the environment one way or another. Adverse consequences of drugs may influence socio-economic environment. Low levels of human medicines (pharmaceuticals) have been detected in many countries in sewage treatment plant (STP) effluents, surface waters, seawaters, groundwater and some drinking waters. For some pharmaceuticals effects on aquatic organisms have been investigated in acute toxicity assays but the chronic toxicity and potential subtle effects are only marginally known.

The environmental occurrence of pharmaceuticals was first reported in Kansas City, US in 1976, where clofibric acid was detected in treated wastewater at concentrations ranging from 0.8 to 2 μg/L. Subsequently, presence of 25 pharmaceuticals in the river Lee (a source of potable water for North London) with concentrations up to 1 μg/L in 1981 was investigated. Since then, several studies have detected PPCPs in different environmental compartments across the globe. Despite the fact that reported concentrations of these PPCPs are low; many of them have the potential to persist in the natural environment for months to years. The detection of pharmaceuticals in the environment varies not only between countries but also between different regions of the same country. That is to say, detectable pharmaceuticals in one country or region may not appear in other countries_regions where they are not highly prescribed. This precludes meaningful global comparison of PPCPs levels due to variations of the targeted compounds and detected chemicals in each reported study. For instance, we compared the reported concentrations of NSAIDs in surface water from different countries. While this figure may provide indicative information on the global contamination levels, it should be studied carefully because studies from different countries have targeted and/or detected different members of the NSAIDs group.

A study by the United States Geological Survey found traces of many different pharmaceuticals and personal care products including steroids, insect repellents and many others in the American water supply. The effect of these traces is unknown, but the concern is about the unexpected! So much Cocaine is being used in London that traces of the narcotic can be detected in River Thames. An estimated 2 kg of cocaine gets into the river every day after it has passed through user's bodies and sewage plants. The Thames investigation, the first of its kind in Britain, was conducted by scientists using the latest technology. It is regarded as the most accurate large-scale drug-detection method available. It is extrapolated that 150,000 lines of the illegal drug are snorted in the British capital every day, or 15 times higher than the official figure given by the Home Office Statistics and equates to four out of every 100 people regularly taking cocaine, or up to 250,000 of the capital's six million residents.

Diclofenac is turning out to be a threat to ecological balance by its swipe at vultures, nature's scavengers, whose number is noticeably on the decline. Their population had crashed.
The vulture population drastically declining and are on way to extinction for many reasons and one of them is Diclofenac Sodium commonly prescribed by veterinarians but found in the carcasses of the cattle on which the scavengers feed. The reason of their death is visceral gout and renal failure. Such a drastic decline in the number of vultures meant an impending ecological disaster with the looming threat of outbreak of epidemics because of decaying carcasses. The vultures’ population crash has also led to an increase in the number of feral dogs which poses a range of disease threats such as rabies. Investigation, which began in 2000, was prompted by reports of a 95 percent drop in the number of Asian white-backed vultures (Gyps bengalensis), Indian vultures (Gyps indicus) and slender-billed vultures (Gyps tenuirostris). All three are now listed as critically endangered by the World Conservation Union, the international environmental agency based in Switzerland. Vultures are keystone species and their declines are having adverse effects upon other wildlife, domestic animals and people. The Peregrine Fund are all calling on the governments of countries with vulture populations, and the manufacturers of diclofenac, to ban the use of this drug in livestock. It is believed the recovery of vulture populations in southern Asia will not be effective until their exposure to diclofenac has been removed. The decline also threatened the traditions of the Parsis, a sect of Zoroastrians who have traditionally exposed their dead to the elements rather than burying or cremating them. In Bombay they had to stop putting their dead on the stone ‘Towers of Silence’ because the birds that once quickly consumed them were vanishing. Meloxicam could be an alternative medicine for veterinary use in India. Govt. of India now banned its use in livestock. Because of the high solubility of most PPCPs, aquatic organisms are especially vulnerable to their effects. Researchers have found that a class of antidepressants may be found in frogs and can significantly slow their development. The increased presence of estrogen and other synthetic hormones in waste water due to birth control and hormonal therapies has been linked to increased feminization of exposed fish and other aquatic organisms. The chemicals within these PPCP products could either affect the feminization or masculinization of different fishes, therefore affecting their reproductive rates.

Only very little is known about long-term effects of pharmaceuticals to aquatic organisms, in particular with respect to biological targets. One laboratory study suggested that antidepressants like Prozac (Fluoxetine) could trigger spawning in some shellfish, thereby disturbing more natural ecological balance. For investigated pharmaceuticals chronic lowest observed effect concentrations (LOEC) in standard laboratory organisms are about two orders of magnitude higher than maximal concentrations in STP effluents. For diclofenac, the LOEC for fish toxicity was in the range of wastewater concentrations, whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured STP effluent concentrations. In surface water, concentrations are lower and so are the environmental risks. However, targeted ecotoxicological studies are lacking almost entirely and such investigations are needed focusing on subtle environmental effects. This will allow better and comprehensive risk assessments of pharmaceuticals in the future. The environmental impact of pharmaceuticals and personal care products (PPCPs) is presently being widely studied and investigated. PPCPs include substances used by individuals for personal health or cosmetic reasons and the products used by agribusiness to boost growth or health of livestock. More than twenty million tons of PPCPs are produced every year. PPCPs have been detected in water bodies throughout the world. The effects of these chemicals on humans and the environment are not yet known, but to date there is no scientific evidence that they affect human health. Further research is needed to evaluate the risks of toxicity, persistence, and bioaccumulation. The term PPCPs encompasses environmental persistent pharmaceutical pollutants (EPPPs), and PPCPs are classified as pseudo persistent organic pollutants. They are not removed from wastewater by conventional methods. The European Union describes pharmaceutical residues with the potential of contamination of water and soil as “priority substances”.

RESEARCH QUESTIONS

1. Many questions are still to be answered as what kind of pharmaceuticals and what concentrations occur in the aquatic environment?
2. What is the fate in surface water and in STP?
3. What are the modes of action of these compounds in humans and are there similar targets in lower animals?
4. What acute and chronic ecotoxicological effects may be elicited by pharmaceuticals and by mixtures?
5. What are the effect concentrations and how do they relate to environmental levels?

Justification for the need of designing model for screening of PPCPs (Ecopharmacology) and Therapeutic Drugs (Pharmacoenvironmentology) in Aquatic and Terrestrial Environment:

In view of these newer expositions, the monitoring of adverse effects of drugs is an important component not only of good medical practice but also for environmental protection. Pharmacoenvironmentology is an extension of Pharmacovigilance as it deals specifically with the environmental and ecological effects of drugs given at therapeutic doses. It is a specific domain of pharmacology and not of environmental studies. This is because it deals with drugs entering into the environment through living organisms through elimination. Pharmacologists with this particular expertise (known as a pharmacoenvironmentologist) become a necessary component of any team assessing different aspects of drug safety in the environment. We must look at the effects of drugs not only in medical practice, but also at its environmental effects in terms of benefit and risk on environment. Any good clinical trial should look at the impact of particular drugs on the environment and the analysis of the outcomes of drug therapies. On the other hand, Ecopharmacology concerns the entry of chemicals or drugs into the environment through any route and at any concentration disturbing the balance of ecology (ecosystem), as a consequence. Ecopharmacology is a broad term that includes studies of “PPCPs” irrespective of doses and route of entry into environment.

1. Routes of PPCPs entering into the Aquatic and Terrestrial environment

The major route for pharmaceutical residues to reach the aquatic environment is most probably by excretion from patients undergoing therapeutic treatment. Since many pharmaceutical substances are not metabolized in the body they may be excreted in biologically active form, usually via
the urine. Furthermore, many pharmaceutical substances are not fully taken up from the intestine (following oral administration in patients) into their blood stream. The unabsorbed fraction not taken up into the blood stream will remain in the gut and eventually be excreted via the faeces. Hence, both urine and faeces from treated patients contain pharmaceutical residues. Between 30 and 90% of the orally administered dose is generally excreted as active substance in the urine.

An additional source to environmental pollution with pharmaceuticals is improper disposal of unused or expired drug residues. Proper destruction of pharmaceutical residues should yield rest products without any pharmaceutical or ecotoxic activity. Furthermore, the residues should not act as components in the environmental formation of new such products. Incineration at a high temperature (>1000 degrees Celsius) is considered to fulfill the requirements, but even following such incineration residual ashes from the incineration should be properly taken care of Pharmaceuticals used in veterinary medicine, or as additives to animal food, pose a different problem, since they are excreted into soil or possibly open surface waters. It is well known that such excretions may affect terrestrial organisms directly, leading to extinction of exposed species (e.g. dung-beetles). Lipid-soluble pharmaceutical residues from veterinary use may bind strongly to soil particles, with little tendency to leak out to ground water or to local surface waters. More water-soluble residues may be washed out with rain or melting snow and reach both ground water and surface water streams.

2. Impact of PPCPs on Aquatic Environment

While the full effects of most PPCPs on the environment are not understood, there is concern about the potential they have for harm because they may act unpredictably when mixed with other chemicals from the environment or concentrate in the food chain. Additionally, some PPCPs are active at very low concentrations, and are often released continuously in large or widespread quantities. In addition to being found only in waterways, the ingredients of some PPCPs can also be found in the soil. Since some of these substances take a long time or cannot be degraded biologically, they make their way up the food chain. Information pertaining to the transport and fate of these hormones and their metabolites in dairy waste disposal is still being investigated, yet research suggest that the land application of solid wastes is likely linked with more hormone contamination problems. Not only does the pollution from PPCPs affect marine ecosystems, but also those habitats that depend on this polluted water. Antimicrobials are found the most frequently detected PPCP, with gemfibrozil (a cardiovascular drug) the second most frequently detected. Other PPCPs detected were trimethoprim, naproxen, carbamazepine, caffeine, sulfamethoxazole, and fluoxetine. The data suggests that septic tank effluent is a probable source of PPCPs.

3. EMEA and USFDA Protocols for ERA

The EMEA risk assessment protocol is a tiered process that begins with a rough calculation of the aquatic predicted environmental concentration (PEC) of the new drug. During this Phase I prescreening, substances whose PEC is deemed too low to be of concern to environmental health are ruled out for further assessment. Vitamins, electrolytes, amino acids, peptides, and proteins are exempted by the guidance because they are not tailored active ingredients (unlike, a drug that interacts with a receptor) and thus are deemed unlikely to result in significant exposure of the environment. However, the guidance does note that certain substances that are likely to cause effects at very low concentrations, such as endocrine disruptors, may need to be addressed regardless of the quantity released into the environment.

Phase II begins with Tier A testing, which aims to determine the aquatic fate and effects of the drug. Its degradability, potential to bioaccumulate, adsorption on sewage sludge, and toxicity to sewage microbial populations are evaluated from the results of standard tests also used in the FDA risk assessment. Also included in Tier A of the EMEA protocol is the long-term testing of fish, Daphnia (water fleas), and algae to assess the predicted “no effect” concentration (PNEC) of the new drug for each of these species. The PEC is further refined at this stage in the EMEA assessment by taking into account the pharmaceutical company’s projected sales forecast for the drug. The risk assessment is terminated if the outcome of Tier A testing results in a PEC lower than the PNEC. However, if the PEC is greater than the PNEC in either water, sediment, the sewage treatment plant, or soil (where sewage sludge has been spread as a fertilizer), this indicates a potential risk, and further Tier B testing is initiated. These tests follow the protocol in the European Technical Guidance Document to further investigate the risk posed by the drug to the environment. For instance, where there is a potential risk to soil, tests would be conducted to determine the drug’s biodegradation in soil, its toxicity to soil invertebrates, and its acute effects on plants and soil microorganisms. At this stage, data on the drug metabolism and excretion profile may be consulted to allow a more accurate calculation of the PEC and determine whether metabolites need to be tested. The EMEA guidance recommends that metabolites exceeding 10% of the drug residue should be assessed for environmental risk. If this round of testing indicates that the PEC of the drug will be greater than the PNEC, then pharmaceutical companies following the European approach must propose recommendations to limit the drug’s impact on the environment.

There are two major differences between the proposed EMEA approach and the existing FDA approach. First, the FDA protocol turns to chronic testing only if acute testing indicates a risk or if there is an indication that the drug could bioaccumulate. The scientific research suggests that acute testing is not a reliable indicator of all chronic effects, however, and the EMEA document reflects this finding. Second, the trigger concentrations of pharmaceuticals that prompt risk assessment under the FDA and EMEA guidance differ by a factor of 10 when dilution is taken into account. These two separate guidelines trigger confusion. EMEA’s trigger of 0.01 microgram per liter (µg/L) reflects a surface water concentration, whereas the FDA’s 1.0 µg/L trigger reflects an “expected introduction concentration,” or the concentration of a compound in sewage effluent. The EMEA trigger of 0.01 µg/L is calculated from the maximum daily dose of the drug per patient and the assumption that 1% of the population is treated daily with the drug; this is divided by the amount of wastewater per person per day and a dilution factor of 10. The FDA trigger corresponds to a PEC in surface water of 0.1 µg/L, assuming a dilution factor of 10, and is calculated from manufacturers’ sales estimates.
The consideration given to metabolites and the provision for
the introduction of scientific experts into the risk assessment
process both part of the revisions to the 2003 guidance is a
welcomed initiative. It is also good that the guidance included
excipients as well as active ingredients in the risk
assessment process. For instance, phthalates such as
diethyl phthalate and dibutyl phthalate, used as plasticizers
in the coating of some site directed drugs, may be a potential
source of phthalates for people taking these drugs, as
reported in the May 2004 issue of EHP.

Limitations of the USFDA and EMEA Guidance
There are certain serious, though perhaps unavoidable,
limitations to the guidance. One is the fact that they are not
retroactive. The only thing that researchers are concerned
about is that the guidance only concerns those
pharmaceuticals that are not yet on the market. But even if
this future legislation required the environmental risk
assessment of drugs already on the market, the big question
would be who should do the testing since the originator of a
drug is often no longer the main manufacturer. Another major
problem is that monitoring may be difficult. There are
problems detecting certain substances that have been on the
market for years. Examples of such hard-to-detect drugs
include the antidepressants known as selective serotonin
reuptake inhibitors. So, the analysis can be quite difficult and
that’s one of the main stumbling features.

Further, it is not clear how drugs that pose risks will be
handled, apart from the addition of labels to recommend
appropriate disposal of expired drugs. Another emerging
area of concern in most of the countries is the disposal of
used birth control patches and hormone replacement
patches. Because pharmaceuticals can save lives, the
guidance does not suggest removing them from the market
even when a risk is found. There is going to be a lot of
emphasis on labeling, and also on treatment processes.
Drug take-back programs for expired pharmaceuticals are in
place in parts of Europe, so labeling drugs with instructions
to return unused portions to a pharmacy makes sense. By
comparison, in the United States, the Controlled Substances
Act complicates such schemes because it prohibits patients
from transferring controlled medicines to anyone other than
a law enforcement official. However, a drug return program
has recently been legislated but not implemented in Maine.
Another limitation, also difficult to avoid, is that the draft
guidance only briefly addresses the possibility of additive or
synergistic effects, noting that an assessment factor of 10 is
applied to the PNEC to account
for extrapolation from lab data to field impacts. It is worth
pointing out that the guidance is written as if the concern is
for a single drug in isolation. But if a drug shares a common
mechanism of action with other drugs, or even other
pollutants, there’s the possibility for additive effects. Some
scientists and drug companies are concerned that
assumptions in the guidance could lead to unrealistic PECs.
The initial calculation assumes the worst-case scenario: that
the drug is not metabolized or degraded at all, so the full dose
ends up in the environment.

It is also worrying for scientists that actual concentrations in
the environment could be higher than the calculated PEC
due to the guidance’s assumed 1:10 dilution factor for
sewage effluent entering rivers. In farming areas, water
levels drop precipitously in dry weather when water is drawn
for crops and cattle, so the 1:10 dilution factor could be too
high. In some areas, most of the river water comes from
returned sewage treatment plants, which is disturbing where
the influx of people stretches the capacity of sewage
Treatments plants. Another problem is that peak or seasonal
variations are not taken into account for example flu
epidemics, drought, or heavy snowfall could temporarily
increase drug concentrations in specific places to values
higher than the calculated PEC. Similarly, local use of
pharmaceuticals differs, reflecting, for example, recent visits
by pharmaceutical representatives telling doctors about new
drugs. This estimation that pharmaceuticals will be released
homogeneously across a particular region is mistaken.

One worry for pharmaceutical companies is that the
increased amount of testing required could translate into
costly delays for the release of new drugs. About 50 new
drugs come onto the market in the United
States each year, and approximately a dozen of those are
predicted to occur above the trigger concentration requiring
them to undergo the first level, or Tier A, of risk assessment
testing. But only one new drug in the last few years has gone
on to the next level to be tested for environmental risks
through chronic ecotoxicity tests. In fact, in the States, almost
all pharmaceuticals in the Tier A assessment will come out
at under one microgram per liter, whereas in the EU there will
be a fair number of pharmaceuticals which will move from the
Tier A to the Tier B as a result of their lower thresholds.

Since neither the EMEA guidance nor its U.S. sister
document addresses pharmaceuticals already on the
market, there is much research into whether wastewater
treatment can economically remove pharmaceuticals.
Increased retention time within treatment plants, chlorination,
zonation, and the natural reduction of a compound’s mass or
concentration over time due to processes such as
biodegradation all increase the removal of some drugs from
wastewater; more advanced treatments such as adding
activated carbon or reverse osmosis can remove even more.

The catch with ozone treatment is that it forms bromate,
which is a regulated disinfection by-product; with
chlorination, the catch is that chlorine combines with ammonia in the sewage treatment system to form chloramines, which are not strong oxidants and so cannot
break down compounds such as estrogens. However,
chlorination can destroy almost all the estrogens if ammonia
is removed first. But even with the use of reverse osmosis
(which removes pharmaceuticals down to parts per trillion)
and the addition of activated carbon, there is the problem of
what to do with the retained contaminants.

Although Europe has been at the forefront of recognizing and
addressing the potential environmental hazard posed by
pharmaceuticals, other countries are perhaps beginning to
catch up. In the United States, for example, the Federal
Interagency Task Group on Pharmaceuticals and Personal
Care Products was formed in September 2004. This group
comprises seven federal agencies and is chaired by the FDA.
One of the questions raised by this group was how much of
the estrogen in wastewater comes from synthetic sources.
In Canada, the Environmental Impact Initiative was formed in
2001 in response to growing evidence that pharmaceutical
substances are being found in the environment. The
initiative, which accepted public comments through
September 2005 on proposed options for regulating these
substances, may result in new rules for the environmental
assessment of substances in products regulated under the
Food and Drugs Act, according to Health Canada. Japan is
also in the process of formulating a plan for environmental risk assessment of pharmaceuticals with sales exceeding one ton per year. In the meantime, the EMEA draft guidance is seen as an appropriate response to an emerging issue which includes possible risks not just from pharmaceuticals but also from personal care products. What has come into the scientific literature is that most pharmaceuticals do not show acute ecotoxicity, so the whole mindset is shifting to chronic toxicity.

**Proposed Screening Models of the Effect of PPCPs in Aquatic and Terrestrial Environment:**
Drugs and their metabolites have a wide range of effects on environment. They induce damage to biological life forms everywhere including air, water and land. The known effects are just the tip of the iceberg, with majority of the effects of PPCPs and their metabolite (produced during interaction with physical and biological environment) are still unknown. At present there are no models to assess the effect of PPCPs in the aquatic and terrestrial environment. Designing a model for Pharmacoenvironmentology studies is always a challenge as it has to take into account the diverse food chains and their interaction with biological and physical environment. Therefore, we devised a template for designing the model for a particular food chain of a given environment. The template consists of 3 generation model of a laboratory animal with its interaction with the local niche. The drug is introduced into the system through water supply and the urine as well as excreta of the animal is then used to grow small herbs. These herbs serve as food for the second species of animals (which also includes the pregnant animals -3rd generation). The samples of soil, urine, excreta, herbs etc. are analyzed by using HPLC for drug and different metabolite levels. The levels are then compared with the pre-dose readings. The system is carefully evaluated for differences in levels of drug and appearance of new metabolite with reference to clinical, biochemical or histopathological anomalies in the test animals. This template model can be modified according to different food chain in a particular geographical region and gives us a tool to conduct more comprehensive Pharmacoenvironmentology studies.

**New screening method to study the effect of PPCPs and Therapeutic Drugs on Terrestrial Environment using Laboratory Animals**
The major drawbacks of present testing methods is because of the fact that more importance is given either to drugs or heavy metals as such, but and not its metabolite, which are actually major products excreted in the environment. The available Laboratory testing and modeling cannot completely represent the environment. As such no study design is conceptualized to study the effect of drug and its metabolite after ingesting the second-generation contaminated material. This new proposed model for screening of the effect of PPCPs and therapeutic drugs in terrestrial environment could be achieved in 6 steps. In step 1, Herbivorous animals could be given test drug for one month. In step 2, the Urine and excreta of test animals could then be collected on each day for one month. In step 3, the refuse could be mixed with soil. With this sort of manure, small fast-growing herbs and shrubs would be cultivated. In step 4, the parts of the plants would be fed to another group of herbivorous test animals for at least 10 days. In step 5, the toxicological studies of animals’ organs could be performed as per ICH guidelines. Finally, in step 6, the seedlings and plantation for second generation on normal soil could be grown (Figure 1). For above test trial, there would be a control test trial with similar plants and animals. The steps 1-6 would be repeated without giving test drug. For this ERA screening method, we need to include animals with following criteria: accessible, long survived, experimentally feasible, herbivorous, tolerant to environmental pollution i.e. should not die at therapeutic doses of the test drug given. Similarly, we can exclude animals which are sensitive to the drug. Likewise, we can include plants of having short life (Herbs / Shrubs), full cycle known, parts of the plants easily distinguishable, can easily be grown under laboratory conditions and has following parts: flower, leaves, fruits, seeds, roots. We can exclude plans which are having long life cycle. The test drugs could be selected Drug from essential medicines, excreted unchanged and / or as a metabolite, low toxicity to vital organs. The soil should be suitable soil for plant cultivation, e.g. for pea it has to be slightly acidic in nature with temperature above 10°C.

![Figure 1: Proposed screening method to study the effect of PPCPs and Therapeutic Drugs on Terrestrial Environment using Laboratory Animals](Image)
detection equipment and analytical methods. In the above mentioned model, drug concentration (ppm) in the specimens of soil (mixed with animals' urine and excreta) at step 2, plants (grown on contaminated soil) at step 3, animals (for toxicological studies on organs including blood examination) at step 5, productivity (for plants), plants analyzed for % proteins, carbohydrate and minerals compared with control group can be tested.

Gas chromatography with mass spectrometry (GC-MS) or tandem mass spectrometry (GC-MS/MS) and liquid chromatography with mass spectrometry (LC-MS) are advanced methods that are able to determine target compounds to the nanogram per litre level and are commonly applied for the detection of pharmaceutical compounds in water and wastewater. The selection of methods is dependent on the physical and chemical properties of the target compound. LC-MS/MS analysis is more suitable for measuring target compounds that are more polar and highly soluble in water, whereas GC-MS/MS is better for more volatile target compounds.

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