DO PHARMACEUTICALLY ACTIVE COMPOUNDS HAVE AN ECOLOGICAL IMPACT?

by

Oana V. Enick
Bachelor of Science, University of Regina, 1992
Post-Baccalaureate Diploma, Simon Fraser University, 1998

PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ENVIRONMETAL TOXICOLOGY

In the
Department of Biological Sciences
of
Faculty of Science

SIMON FRASER UNIVERSITY

Spring 2006

APPROVAL

Name: Oana V. Enick

Degree: Master of Environmental Toxicology

Title of Project:

Do pharmaceutically active compounds have an ecological impact?

Examining Committee:

Chair: Dr. F. Breden, Associate Professor

Dr. M. Moore, Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. F. Gobas, Professor
School of Resource and Environmental Management, S.F.U.

Mr. G. van Aggelen, Head
Environmental Toxicology Section, Environment Canada
Public Examiner

Apr. 11/06
Date Approved

ABSTRACT

Pharmaceutically active compounds (PhACs) are used in human and animal medicine as therapeutics and in agriculture as growth-enhancing agents. They are discharged directly to the environment following animal excretion, or indirectly via wastewater treatment plants following human use. This thesis evaluates the current theory and practice of environmental impact assessment of PhACs: specifically, the process and methods to assess bioaccumulation, environmental persistence and risk management strategies, and the value of acute and chronic toxicity tests. PhACs impact the environment to a degree determined not only by their actual concentration, but also by the ways we define and characterize impact, risk assessment and environment. In turn, these definitions evolve according to socially constructed values. By acknowledging the inherent uncertainty and value-laden nature of risk assessment, I assert that the precautionary principle and the integrated risk assessment approaches should direct further research and policy development.

Keywords:

Drugs – environmental aspects

Environmental toxicology – social aspects

Environmental risk assessment – values, uncertainty

DEDICATION

To the next generation of thesis writers: Tristan, Calista, Julia and Violetta

and,

To my husband Chris, for his unending support and patience.

ACKNOWLEDGEMENTS

I would like to acknowledge my debt to the community of teachers, scholars, students and other collaborators within which I learned many of the concepts discussed in this work.

I would like to express my thanks to Dr. Margo Moore for her continued guidance and encouragement. I owe her a great debt of gratitude as well for the patience and insightfulness with which she reviewed several versions of this work.

I would like to acknowledge Dr. Frank Gobas for his direction and support at various times throughout my MET project and course work.

I would like to acknowledge Mr. Graham van Aggelen for taking the time to discuss the issue of pharmaceutical contamination of the environment with me. I would also like to thank Mr. Brent Moore for introducing me to this topic.

I would also like to acknowledge the support of staff and faculty in the Department of Biology, and staff and students in Dr. Moore's Lab.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ABR	Antibiotic resistance
AMR	Antimicrobial resistance
ASA	Acetylsalicylic acid
BaP	Benzo-a-pyrene
BCF	Bioconcentration factor
BMF	Biomagnification factor
CA	Concentration addition
CBZ	Carbamazepine – an antiepileptic drug
CEPA	Canadian Environmental Protection Act
Cyt P-450	Cytochrome P-450 enzyme system
DES	Diethylstilbestrol
DHS	Department of Health and Safety
E1	Estrone
E2	Estradiol
EC	Effect concentration
ED	Effect dose
ED	Endocrine disruption
EDC	Endocrine disruptive compounds
EE2	17α-ethinylestradiol
EMEA	European Agency for the Evaluation of Medicinal Products
ERA	Environmental/Ecological risk assessment
EU	European Union
FDA	Food and Drug Administration
HHRA	Human health risk assessment
HI	Hazard Index
HRT	Hydraulic retention time
IRA	Integrated risk assessment

LC	Lethal concentration
LD	Lethal dose
LRP	Lung resistance protein
MF	Membrane filtration
MFO	Multifunction oxidase
MIC	Minimum inhibitory concentration
MR	Methicillin resistance
MR	Microbial resistance
MRP	Multidrug resistance protein
MVP	Major vault protein
MXR	Multixenobiotic resistance
NADPH	Nicotinamide adenine dinucleotide – reduced form
NF	Nanofiltration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
NP	Nonylphenol
NPE	Nonylphenol Ethoxylate
NSAID	Non-steroidal anti-inflammatory drug
P2	Pollution prevention
P450	Cytochrome P450 detoxification system
PAH	Polyaromatic hydrocarbons
PCB	Polychlorinated byphenols
PEC	Predicted environmental concentration
PgP or P-gp	P-glycoprotein
PhAC	Pharmaceutically active compounds
PNEC	Predicted no effect concentration
POP	Persistent organic pollutants
PP	Pollution prevention
PP	Precautionary principle
QPAR	Quantitative property-activity relationships
QSAR	Quantitative structure-activity relationships

RO	Reverse osmosis
RPF	Relative Potency Factor
SIM	Sexually immature
SM	Sexually mature
SSRI	Selective serotonin reuptake inhibitors
STP	Sewage treatment plant
TEF	Toxic equivalency factor
USEPA	United States Environmental Protection Agency
UV	Ultra violet
VMP	Veterinary medicinal products
VR	Vancomycin resistance
VTG	Vitellogenin
WHO	World Health Organization
WWTP	Waste water treatment plant

CHAPTER 1: BASIC CONCEPTS

Overview

Pharmaceutically active compounds (PhAC) are chemicals that have been specifically designed to treat or alter animal physiological conditions (Cunningham, 2004). PhACs are used primarily in human and veterinary applications as either medication or growth enhancing/pesticidal substances (Daughton and Ternes, 1999). Following excretion, these chemicals, or their metabolites may find their way to the aquatic environment. PhACs have been detected in soils, sediments and surface and ground water (Halling-Sorenson et al., 1998; Heberer, 2002). Some of these products enter the environment via direct application at fish farms, others via excretion from animals following agricultural therapeutic and prophylactic use, and/or liquid manure application. Yet others may enter via sewage treatment plant effluent discharge after human therapeutic use (Hamscher et al., 2004). In the receiving environment, these contaminants may sorb to suspended particles, soil and sediments, remain in the aquatic phase or sequester to the organic-lipid compartment and enter the food chain via biomagnification (Kümmerer, 2004). Acute effects attributed directly to pharmaceutical contamination of the environment have been largely limited to the feminization of some fish exposed to natural and xenobiotic endocrine disruptors (Ayscough, 2000). Chronic effects as a result of exposure of aquatic animals to these and other human pharmaceuticals have also been reported (Ayscough et al., 2000; Cleuvers, 2004). However, chronic effects associated with PhAC are difficult to detect, evaluate and

manage under the current risk assessment process which is based on acute toxicity testing and high (acute) action limits. Efforts to include chronic, multi-route, multi-chemical exposure in the risk assessment process are reflected in the advent of toxicogenomics and *in-silico* modelling. Originating from an acknowledgement of the inherent uncertainty and value-laden nature of risk assessment, the precautionary principle (and later the integrated risk assessment system), provides a direction for further research and policy development. A number of mitigation strategies have been advanced, including remedial, end-of-pipe and preventative, manufacturer-user controls. However, successful mitigation of environmental PhACs requires a more integrated approach to both the research and policy aspects of this issue.

Specific Attributes of Pharmaceuticals

Pharmaceutical compounds constitute one of a myriad of chemical classes discharged into the environment. Is there any reason to study drug residues more intensely than any of the other type of contaminants? The weight of evidence currently suggests that pharmaceutical substances are of special concern for four reasons:

1. PhAC can cause chronic toxicity even at low concentrations

Pharmaceutical compounds and/or their residues have been detected in the environment in concentrations in the nano- to low microgram range (Halling-Sorensen et al, 1998; Daughton and Ternes, 1999; Heberer, 2002; Kümmerer pp7, 2004). In the vast majority of cases, these amounts are orders of magnitude below those shown to induce acute toxicity. However, current evidence suggests that for some agents, these concentrations are sufficient to elicit chronic effects (Velagaleti, 1997; Bendz et al.,

2005). For example, Ferrari et al. (2003) reported 7-day reproduction inhibition on ceriodaphnids associated with environmentally relevant concentrations of the antiepileptic drug, carbamazepine. Similarly, Foran (2004) showed that Japanese medaka (fish) exposed to ng/L levels of another psychoactive drug, fluoxetine, exhibited increased rates of developmental abnormalities and altered levels of plasma estradiol. Detecting chronic effects is notoriously more difficult than detecting acute toxicity and requires focussed and intense (and more expensive) exploration (Ruden, 2005). Unless specifically investigated, the effects of pharmaceutical compounds on exposed wildlife could easily be overlooked, or confused with 'natural population variation' (Daughton and Ternes, 1999; Bound and Voulvoulis, 2004).

2. PhAC are globally distributed

As pharmaceutical compounds are used in every reach of human habitation, their release into the environment is equally ubiquitous (Halling-Sorensen et al., 1998; Kolpin et al., 2002; Sanderson et al., 2004). Thus, the effects of drugs and their metabolites may be expected even in pristine and sheltered environments such as national parks (Daughton and Ternes, 1999). Areas at highest risk are highly populated, highly industrialized and highly agriculturalized watersheds where the effects of pharmaceutical residues and other compounds present in municipal wastewater can combine with the effects of other traditional pollutants (Isidori et al., 2004). The results of a 1998 study of the genotoxic potential, expressed in B(a)P equivalents, of Montreal area municipal wastewater treatment plant (WWTP) effluents support this assertion. The authors concluded that discharges form WWTPs servicing large municipalities were more genotoxic than those receiving inputs from some commercial activities (textile and mining industries) (White

and Rasmussen, 1998). In fact, the authors stated that "over 85% of the genotoxic inputs into the St. Lawrence river at Montreal are non-industrial in origin".

As most pharmaceutical compounds are targeted to specific receptors, and the lowest effective dose is generally used, one might conclude that they are manufactured in relatively low amounts. To some extent, this suggestion may be true, but it is dependent on one's frame of reference and comparison standard. A number of measurements place pharmaceuticals' manufacturing volumes at quantities rivalling those for some agrochemicals (Daughton and Ternes, 1999; Jones et al., 2002). For example, Stan and Heberer (1997) reported that the volume of prescribed pharmaceuticals per annum in Germany is of the same order of magnitude as agricultural pesticides applied in that country. Similarly, the 200 tonnes of antibiotics applied annually as veterinary therapeutic and growth promoting agents in Denmark are equivalent to the 185 tonnes of insecticides used in that country annually (Bound and Voulvoulis, 2004). The relationship between PhAC and pesticides seems to be reflected in the actual measured concentrations of each class of contaminants in the WWTP effluent as well. Comoretto and Chiron (2005) concluded that the pesticide and pharmaceutical loads in WWTPs are comparable, with ratios ranging from 0.2 to 0.7 for three sites in southern Italy. The authors calculated that under specific conditions such as low rainfall, the pharmaceutical load exceeded that of pesticides, at least for one of the sample sites (Comoretto and Chiron, 2005).

3. PhAC are associated with many, and often unexpected side effects

The well-known incidents with thalidomide and diethylstilbestrol (DES) are good examples of the potential for severe and unexpected side effects of pharmaceuticals.

DES, originally prescribed to prevent miscarriage, is now recognized for its ability to enhance tumour formation in female offspring whose mothers received it during pregnancy (Herbst et al., 1971; Stillman, 1982; Schechter et al., 2005). Thalidomide, originally prescribed as an antiemetic during pregnancy, was later found to have teratogenic effects (Schardein, 1993; Ostraff et al., 2000). It is often difficult to identify these side effects even on the target species – and pharmaceutical developers actively search for them. Adverse effects in non-target organisms are even more difficult to predict. Nevertheless, numerous researchers have reported such findings. For example, at environmentally relevant concentrations, the human lipid regulator gemfibrozil has been shown to reduce testosterone levels in the goldfish *Carassius auratus* (Mimeault et al., 2005). Likewise, a series of seven antibiotics including metronidazole, erythromycin, oxytetracycline and clarithromycin have shown toxicity against non-target algae, rotifers, microcrustaceans and fish at either environmental or fish therapeutic concentrations (Cavas, 2005; Isidori, 2004).

4. PhAC are designed specifically to alter some biological function

Pharmaceuticals are biologically active compounds that have been designed or serendipitously discovered to alter biological systems including those in humans, mammals and other vertebrates (Cleuvers, 2003; van der Ven et al., 2004; Sanderson et al., 2004a; Sanderson et al., 2004b; Issidori et al., 2004; Halling-Sorensen et al., 1998). Uptake, distribution and effect of pharmaceuticals within the target organism depends upon their ability to cross biological membranes and remain chemically stable long enough to meet their therapeutic target Thus, pharmaceuticals resemble other intensely regulated environmental pollutants such as pesticides and herbicides (Sanderson, 2004;

Cleuvers, 2003; van der Ven et al., 2004), and the argument that pharmaceutical contamination of the environment is innocuous needs to be re-evaluated (Drillia et al., 2005; Daughton and Ternes, 1999).

Pharmaceuticals: Basic Concepts of Toxicity Testing

Toxicity testing is used to determine the effect/s of substance/s on target organism/s. It can be performed on various organisms, organs, tissues, cells or even molecules. Potential effects can be observed over various time spans, ranging from seconds to years. Single or multiple exposures can be employed, with high, low or intermediate doses on individuals at various stages of development. Endpoints may range from death or severe illness to subtle changes in immunological competence, energy consumption and reproductive success. Toxicity tests can be classified according to effects on various organs, effects at various stages of development and effects of varying time and dose of exposure. This discussion will focus on the latter. Varying the time and dose of exposure essentially leads to the design of acute versus chronic toxicity testing (DiPasquale and Hayes, 2001; Wilson et al., 2001). Because the data is obtained from such tests used to formulate management strategies, there is considerable debate concerning the type, quality and relevance of toxicity testing, and its applicability to policy decisions (see chapter 3).

Acute toxicity testing

One definition of acute toxicity is "the adverse changes occurring immediately or a short time following a single or short period of exposure to a substance or substances" (Rhodes, 2000). To elucidate the effects of acute toxicity, several standard testing

methods have been designed. These tests aim to define the "intrinsic toxicity of a chemical, assess the susceptible species, identify the target organs of toxicity, and provide information for risk assessment and/or dose levels for future studies" (Ballantyne et al., 1995). For those chemicals where no other toxicology data is available, the results of acute testing are used to set standards for classification, labelling and transportation, as well as for safety and monitoring.

A common endpoint of acute toxicity testing is the LD_{50}; the dose that is lethal to 50% of test organisms. Variations on the LD_{50} include the LC_x, ED_x, MIC and NOAEL/C defined, respectively, as the concentration lethal to x percent of respondents, the effective dose for x% of respondents, the minimum inhibitory concentration, and the no-observed-adverse-effect-level/concentration. Acute toxicity values are usually given in conjunction with additional information concerning the length of exposure and the type of organism tested. For example, bacitracin, an antibiotic often used as a livestock growth promoter, has a 24hr LC_{50} of 126 mg/L on the aquatic invertebrate *Daphnia magna,* and a 48hr LC_{50} of 30 mg/L (Brambilla, 1994, Migliore et al., 1997). Another example is the antiparasitic agent ivermectin. It has a 96-hr LC_{50} in *Salmo gairdneri* (Rainbow trout) of 3.0 mg/L and a NOEC (No Observed Effect Concentration) at 48 hr of 0.9 mg/L (Halley et al, 1989).

Acute toxicity testing is performed over a relatively short period of time, often between 24 and 96 hours, and has pathologically gross, easily identifiable endpoints such as severe illness and death. It is a preferred test for many environmental policy analysts and a benchmark of regulatory toxicology (Daughton, 1999). Acute toxicity testing is

also readily amenable to the development of substance-specific dose response curves. These are described in more detail in the following section.

Linear Dose-response or Dose-effect Relationships

Several textbooks (Zakrezewski, 2002; Hayes, 2001; Ballantyne et al., 2000) offer comprehensive coverage of all aspects of dose-response relationships. The following is a summary of the points most relevant to the environmental toxicology of pharmaceutical compounds.

Dose-response relationships are based on the intrinsic variability of response both within an individual and within a population. In general, as the amount of material given to an individual or a group increases, so does the magnitude of the response or the number of individuals responding (Ballantyne et al., 1995 pp11). These connections are usually presented visually as a dose-response or dose-effect curve. If the effects investigated relate dose to the severity of response in an individual or population, the result is as dose-effect curve. If they relate dose to the proportion of subjects showing a specific, pre-determined effect, the result is a dose-response curve (Rhodes, 2000). Dose-response/effect curves can be presented in various forms that yield different types of information. Through a series of mathematical manipulations, a linear function relating dose and response can be obtained (Figure 1). Generally, biological responses to a range of doses tend to be skewed such that individuals showing resistance do so over a larger range of doses than do individuals showing sensitivity (Figure 1a) (DiPaswuale and Hayes, 2001). For example, expressing the dose as log dose will transform the skewed distribution into a normal distribution (Figure 1b), while expressing frequency of response as cumulative percentage will further transform the results into the well-known

sigmoidal response curve (Figure 1c). A significant value on this graph is the dose corresponding to 50% response. If the measured effect were mortality, that value would denote the LD_{50} and would describe the average response to the test chemical. This graph also shows the existence of hyper- and hypo-susceptible groups at the lower and upper ends of the curve respectively. In the context of environmental toxicology, this means that the hypersensitive individuals within a species and the hypersensitive species within an ecosystem may be at higher risk of injury. Lastly, the range of doses eliciting an effect is represented by the slope of the curve, i.e., a steep curve denotes a narrow effective range, and a flat slope indicates a much wider range. For chemicals with a steep slope, a very small change in dose can result in a large change in response.

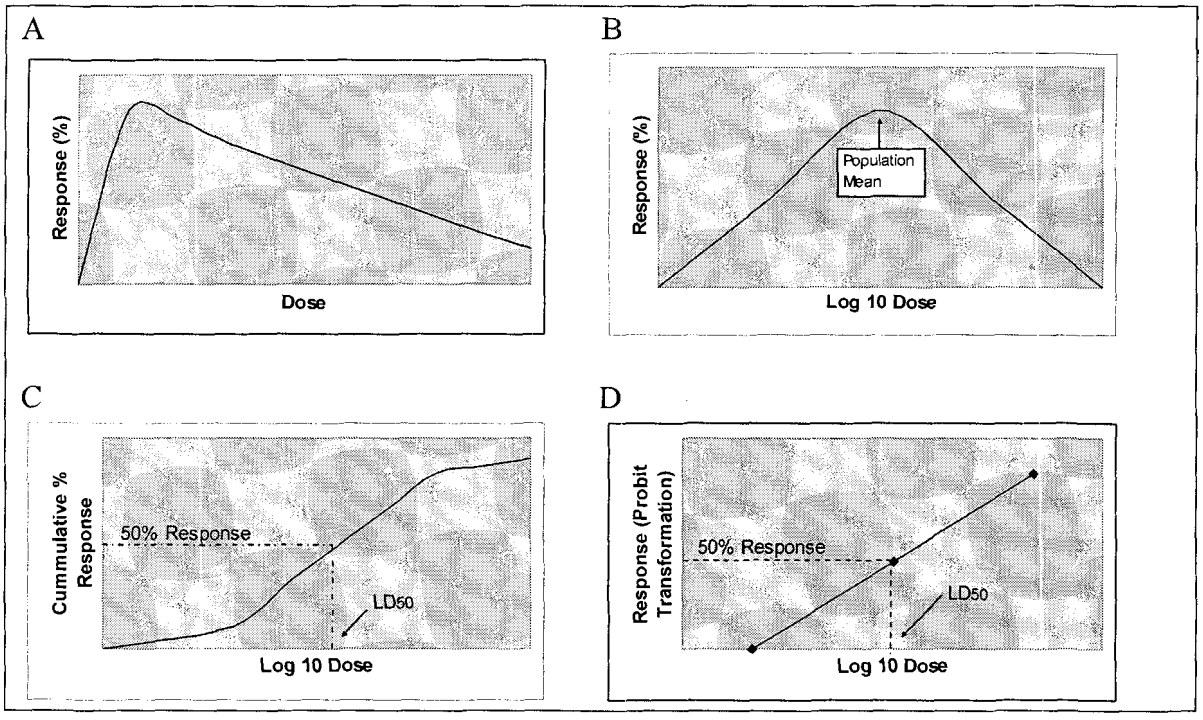


Figure 1: Dose-Response Curves: Representation of mathematical transformation from a typically non-normal response distribution (A) to normalized (B and C) linear function (D).

Transforming the percentile cumulative response on the y axis into probits (normal equivalent deviant ranges), and maintaining the $\log_{10}$ dose on the x axis will convert the normally distributed sigmoid response into a linear function (Figure 1d) (Bliss, 1935, 1940, 1941 for the development and refinement of the probit model). Probit transformations are performed because linear functions are more amenable to statistical calculations such as median values and confidence intervals and because probits allow for the examination of data over a larger range of values (Ballantyne etal., 2000).

There is an important relationship between median effect values (ED_{50}, LC_{50}, LD_{50}, etc) and the slope of the dosage-response/effect curves. First, the median values reflect the point of highest statistical confidence in the results, as the confidence intervals at this point are narrowest (Rhodes, 2000). Median values are often reported and used for regulatory purposes, but they are not meant as stand-alone parameters; they should be reported with confidence intervals and slope values (DiPasquale and Hayes, 2001). Second, any effects indicated by median values are heavily influenced by the slope of the dose-response curve. For example, consider the four chemicals in Figure 2. Chemicals A and C have the same LD_{50} values, but different slopes. Thus, while both chemicals induce similar median toxicity, chemical A is much more toxic at lower doses relative to chemical C. Another implication is that chemical C may have a narrower range of safety or rapid onset of action and faster absorption (Ballantyne et al., 2004).

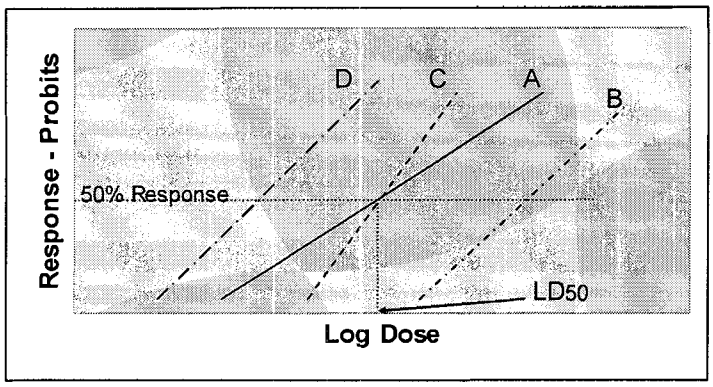


Figure 2: Relationship between slope and LD_{50}. Chemicals with similar slopes (D and B) can have very different LD_{50} values. Chemicals with similar LD_{50} values, can have dissimilar slopes (C and A).

For example, while investigating the mixture effects of three β-blockers, Cleuvers found that at doses below EC_{30}, the measured effects exceeded the predicted effects by 36%. He attributed this discrepancy to the sharp dose-response slope of one of the drugs, propranolol (Cleuvers et al., 2005). (Further discussed in the section Handling Mixtures at the end of this chapter.)

Non-linear Dose-Response Relationships

The behaviour of many chemicals may be studied using the general principles of dose-response relationships explained above. However, there are some exceptions. One of these is the case where the sigmoid curve does follow a normal distribution but there is a higher-than-normal incidence of effects in the hyperreactive zone (small proportion of the population responding to very low dosages). This situation is indicative of a genetic variant in a proportion of the population, causing the enhanced sensitivity (Ballantyne et al., 1995). In terms of the low environmental concentrations expected for pharmaceutical contaminants, these populations would be expected to be the first to show effects.

Another exception to the sigmoidal response is the case of hormesis in which biphasic or U-shaped dose-response curves are obtained. Although the concept has only recently been debated in mainstream environmental toxicology, some authors claim that these relationships are actually the rule and that the sigmoid curve is the exception (Calabrese, 2005b). Hormesis is defined as "an adaptive response characterized by biphasic dose responses of generally similar quantitative features with respect to amplitude and range of the stimulatory response that are either directly induced or the result of compensatory biological processes following an initial disruption of homeostasis" (Calabrese and Baldwin, 2002). At very low doses, hormesis results in a mild (30 – 60%), usually stimulatory effect relative to controls. When represented graphically, this phenomenon results in U- or inverted U-shaped graphs (Figure 3). The direction of the curve is dictated by the endpoints measured. For example, U-shaped graphs occur when the measured endpoints include mutation rates, birth defects and cancer incidence and inverted U-shaped graphs occur when growth rate, fecundity and longevity are measured (Calabrese, 2005a; Calabrese, 2005b; Beck et al., 2001).

A critical feature of hormesis is that it is only apparent at doses below NOAEL – with higher dose responses being indistinguishable from the traditional sigmoid relationships (Calabrese, 2005b). The hormetic response is believed to represent a repair overcompensation following a minor disruption in homeostasis (Beck et al, 2001). For example, a minor chemical insult induces a generalized repair response in the affected organism. To ensure adequate clearance, the response is slightly overcompensating. However, as the dosage (and the damage) increases, the intrinsic repair capacity decreases, eventually equalling the level of damage. This is the zero-equivalent-point

(ZEP) or the NOAEL. Above this level the more familiar 'frank toxicity' occurs (Beck et al., 2001).

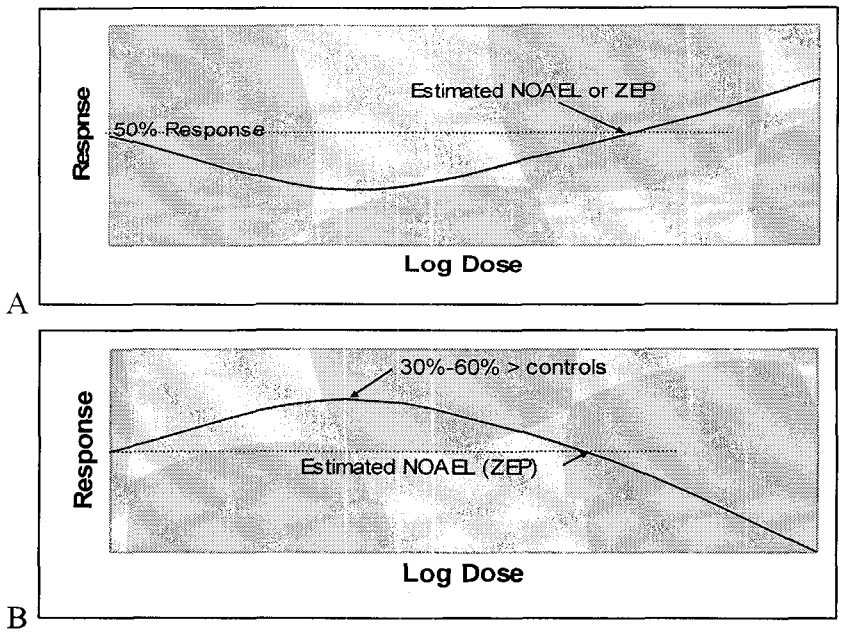


Figure 3: Hormesis graph showing the typical U-shaped (sometimes referred to as J-shaped) curve (A) or the inverted U-shaped curve (B). NOAEL = No Observed Adverse Effect Level; ZEP = Zero Effect Level

Another critical attribute of hormesis is that the process does not necessarily imply beneficial effects (Calabrese, 2005b; Chapman, 2002). For example, while retinoic acid has been shown to suppress the development of some epithelial tumours (at therapeutic doses), lower doses actually encourage cell proliferation (Bentel, et al., 1995; Hurnanen, 1997). This point merits repetition as even the mildest connotation of innocuousness at low doses can be misleading (Thayer, 2005). In human medicine, biphasic responses have been observed for antibiotic, antiviral and non-steroidal anti-inflammatory drugs (Bentel, et al., 1995; Hurnanen, 1997; Calabrese and Baldwin, 2001). In environmental toxicology, biphasic dose-response curves have been reported or suspected for estrogenic effluents and xenoestrogens acting on snails' reproductive output

and for 17α-ethinylestradiol acting on fathead minnows' egg production level (Jobling et al., 2004). The paucity of published biphasic dose-response curves for environmental contaminants may be interpreted as indicative of a paucity of biphasic relationships. However, many authors suggest that non-sigmoidal dose-responses are not unusual but that few investigators employ an appropriate design of dose-response experiments that would identify hormesis (Cleuvers, 2005; Daughton, 2004; Calabrese, 2005a; Calabrese, 2005b). For example, experiments may be performed at an inappropriate number, spacing or range of doses and/or temporal measurements relative to endpoints or endpoint selection may be unsuitable for detecting hormetic effects (Beck et al., 2001).

In a series of recently published articles, Edward Calabrese and colleagues make a strong case for the inclusion of hormesis as the default assumption for toxicity testing (Calabrese, 2005a; Calabrese 2005b; Calabrese and Blain, 2005; Calabrese and Baldwin, 2001). In the context of pharmaceutical contamination, it is important to note that hormesis functions in the same range of concentrations as those found for pharmaceutical compounds in the environment, i.e., 3 to 5 orders of magnitude below the NOAEL (Calabrese, 2005b). This fact may make hormesis especially relevant to the toxicological evaluation of pharmaceutical contaminants. However, the application of hormetic concepts to environmental contamination with PhACs must be implemented with caution so that in setting minimum acceptable contaminant levels, regulators do not anticipate unfounded hormetic effects (thereby setting dangerously high limits of exposure). These issues and concerns have been addressed in detail by Thayer et al., (2005). Furthermore, hormesis is inherently difficult and very expensive to establish because the effects are very mild and the endpoints not easy to establish (Calabrese, 2005a; Calabrese, 2005b).

Regardless of the eventual approach to hormesis, Calabrese and others refer to a virtual 'paradigm shift' in the way scientists handle low-dose effects to reflect the U-shaped dose response curves (Calabrese, 2005a).

Repeated Exposure and Chronic Toxicity Testing

Repeated exposure studies (performed over up to 10% of an animal's lifespan) and chronic toxicity studies (performed over 2 years for small laboratory animals), both use repeated, sub-acute exposure levels. Repeated exposure studies investigate the effects of a compound's accumulation over a period of time and/or its possible latency periods (Ballantyne et al., 1995). Chronic toxicity is particularly relevant to aquatic contamination, as organisms have the potential to undergo life-long exposure (Daughton and Ternes, 1999; Weigel et al., 2002). Chronic toxicity testing is usually more demanding in terms of time, effort and financial commitments than acute testing. Changes in energy consumption, reproductive success, immune function, tumour formation and behaviour can be monitored. These subtle endpoints may also be more difficult to establish and interpret than those for acute exposure. For example, in a 90 day reproductive study of the effects of dietary exposure of male rats to 17β-estradiol, some of the endpoints chosen for study were decreases in epididymal weight and sperm number in the F1 (Cook et al., 1998). The length of time required for the experiment, the constant attention and work associated with this set-up and the significant technical requirements add to the difficulty of this experiment.

Scientific Approaches to Eco-Toxicological Assessments of Pharmaceuticals

Compared to the wealth of toxicological information available for products such as pesticides and persistent organic pollutants (POPs), information pertaining to the environmental toxicology of pharmaceutical products is relatively scarce (Jones et al., 2002; Sanderson et al., 2004a; Lange and Dietrich, 2002; van der Ven et al., 2004). In fact, the open peer-reviewed literature and ecotoxicological databases contain data for less than 1% of pharmaceuticals (Sanderson et al., 2004a). It was not until the 1990s that two events coincided and raised awareness of pharmaceutical contamination of the environment: advances in technological capabilities that allowed the quantification of low concentrations of pharmaceuticals in aquatic systems, and publication of a number of comprehensive reviews which documented the presence of these contaminants in various bodies of water (Velagaleti, 1997; Halling-Sorenson et al., 1998; Daughton and Ternes 1999). In subsequent years, scientists began to investigate the toxicity of pharmaceutical compounds to non-target organisms and to collect information for environmental risk assessments. Their efforts resulted in a series of environmental risk assessments performed for specific drugs or drug classes; for example antibiotics (Andreozzi et al, 2004; Jones et al., 2002), psychoactive drugs (Cunningham et al., 2004) and lipid regulators (Emblidge and De Lorenzo, 2005). The effect of mixtures of a limited number of pharmaceutical compounds has also been assessed (Cleuvers, 2005) as has the effect of some municipal sewage treatment plants (STP) effluent on some species of fish, mussels, algae and aquatic invertebrates (Porter and Janz, 2003; Quinn et al., 2004). From a regulatory standpoint, the result of all this research is that many jurisdictions (Canada, United States, European Union, Australia) now require various levels of environmental

impact assessments for new pharmaceuticals seeking market approval (Straub, 2002; Sanderson et al., 2004a; Schwab, 2005).

Adequacy of Toxicity Testing and Risk Assessment Information

Although the assessment approach varies between Canada, the United States and the European Union, the information required is similar: pharmaceuticals are assessed for ecotoxicity based on their measured and predicted acute toxicities to non-target organisms, and their chemical and physical properties as these relate to environmental persistence, bioaccumulation and biomagnification (Daughton and Ternes, 1999; Straub, 2002). For example, the European Union uses a tiered assessment system based on the ratio of the predicted environmental concentration (PEC) to the predicted no-effect concentration (PNEC). The PNEC is an experimentally determined value based on standard acute toxicity tests to i.e., algae, *Daphnia magna* and fish (Straub, 2002; Sanderson et al., 2004b). However, these tests evaluate the effects of individual substances on individual species (e.g., *Daphnia magna,* zebrafish (*Danio rerio),* African clawed frog *(Xenopus laevis)* or fat head minnow (*Pimephales promelas)*), often overlooking mixture or multi-species effects (Lange and Dietrich, 2002). Also often overlooked in these tests is the potential chronic and generational toxicity associated with low-dose, continual exposure (Daughton, 2004). Similar concerns have been advanced regarding information for the chemical's physical and chemical properties. For example, the models used to determine basic chemical properties (such as partitioning coefficients), are based on laboratory information obtained from structurally unrelated compounds such as neutral industrial chemicals and pesticides (Cunningham, 2004). For these reasons, the appropriateness of current assessment practices and data for

pharmaceutical ERAs has been questioned (Daughton, 2004; Lange and Dietrich, 2002). Responding to such criticisms, researchers are starting to incorporate some changes into the data gathering/assessment systems. Some of these changes are outlined below.

Changes on the Horizon

Assessing the validity of models employed to determine chemical properties

Environmental persistence, partitioning preferences (as denoted by individual partition coefficient values, log P) and toxicity data are used in various predictive mathematical models. The two models "ranked best overall" (Moore et al., 2003) QSAR (Quantitative Structure-Activity Relationship) and ECOSAR (Ecological Structure-Activity Relationship) use measurements of acute toxicity, log P, solubility and predicted environmental persistence amongst a multitude of other physico-chemical properties of the contaminant and the receiving environments (Sanderson et al., 2004a; Sanderson et al., 2004b). These models attempt to integrate a wide range of information, but some of the numerical values used have been developed for chemicals quite unlike pharmaceutical substances (Cunningham, 2004). For example, many relationships describing chemicals' partitioning preferences have been modelled on smaller, neutral and highly hydrophobic molecules (Lyman et al., 1990). Some large (cyclosporine for example, has a molecular weight of 1203), ionisable molecules would not follow the expected mechanism of sorption to organic matter by hydrophobic partitioning as it discounts the more complex ionic and ion-pairing mechanisms of this molecule (Cunningham, 2004). Similarly, some authors are questioning the methods used to characterise drug sorption to soil particles (Tolls, 2001; Diaz-Cruz 2003). They point out that sorption is driven by a number of different mechanisms – surface adsorption to

minerals, ion exchange, complex formation, etc. – and that exclusive reliance on hydrophobic interaction for risk assessment purposes is misguided (Diaz-Cruz, 2003). Some (Monforts, 2005) even suggest that cut-off values on sorption properties (used for assessing and categorizing environmental persistence) are not warranted. Pharmaceutical substances are sufficiently variable in their sorption behaviour to justify a re-evaluation of the models employed. For example, Scheytt et al. (2005) found that hydrophobic sorption is the main sorption process for carbamazepine but that ionic interactions are more important for the sorption of diclofenac and ibuprofen. For the specific case of veterinary pharmaceuticals, Montforts (2005) suggests that the screening and mechanistic models required for pesticide registration may be more appropriate for determining the distribution of PhAC in soil.

Alternatives to animal toxicity tests

Acute toxicity testing has traditionally relied heavily on the use of laboratory animals. Escalating public pressures for humane animal treatment and for reducing animal testing has led scientists to look for alternatives to tests relying on *in-vivo* studies. Consequently, improved *in-vitro* and *in-silico* models continue to be refined and to find scientific and regulatory acceptance (Bhogal et al., 2005). These approaches are based on Russel and Burch's (1959) proposed 3Rs of humane animal treatment – replacement, reduction and refinement, with the contemporary addition of a fourth R – responsibility for the safety of the products tested (Gad, 2000). The alternative systems available range from relatively simple *in-vitro* subcellular fractions and tissue slices to the more complex 3D organotypic cultures such as EPISKIN™ or EpiDerm™ (see Bhogal, 2005 for a review of current *in-vitro* systems). The information obtained from such models can be

used in conjunction with information derived via the so-called 'omics' and computer modelling technologies in a manner depicted in Figure 4.

'Omic' technologies include genomic, proteomic and metabolism profiling approaches to determining toxicity. These are based on the premise that toxicological events change the protein composition, structure and function of the affected cell in a detectable way (Bhogal et al., 2005). Gene expression profiles at either the transcriptional or the translational level, of individuals exposed to new chemicals are compared to those of individuals exposed to known toxicants. Analysis of these results should provide information for identifying the mechanism of toxic action as well as biomarkers for individual variation in susceptibility, exposure and effect (Bhogal et al., 2005). Figure 4 depicts the intimate relationship between the traditional and new *in-vitro* testing/*in-silico* applications. The 'omics' technologies operate on a structure-toxicity axis extrapolating effects of new chemicals based on those of known chemicals much in the same way that some in silico approaches do. For example, QSARs extrapolate properties of new chemicals based on the properties of known chemicals. QSARs and modelling techniques will be further discussed in chapter 2, and the simplicity of the integration between toxicity assessments will be revisited in chapter 3 as an example of an epistemic value.

Figure 4: A scheme for integrated toxicity testing. Adapted from Bhogal et al., 2005

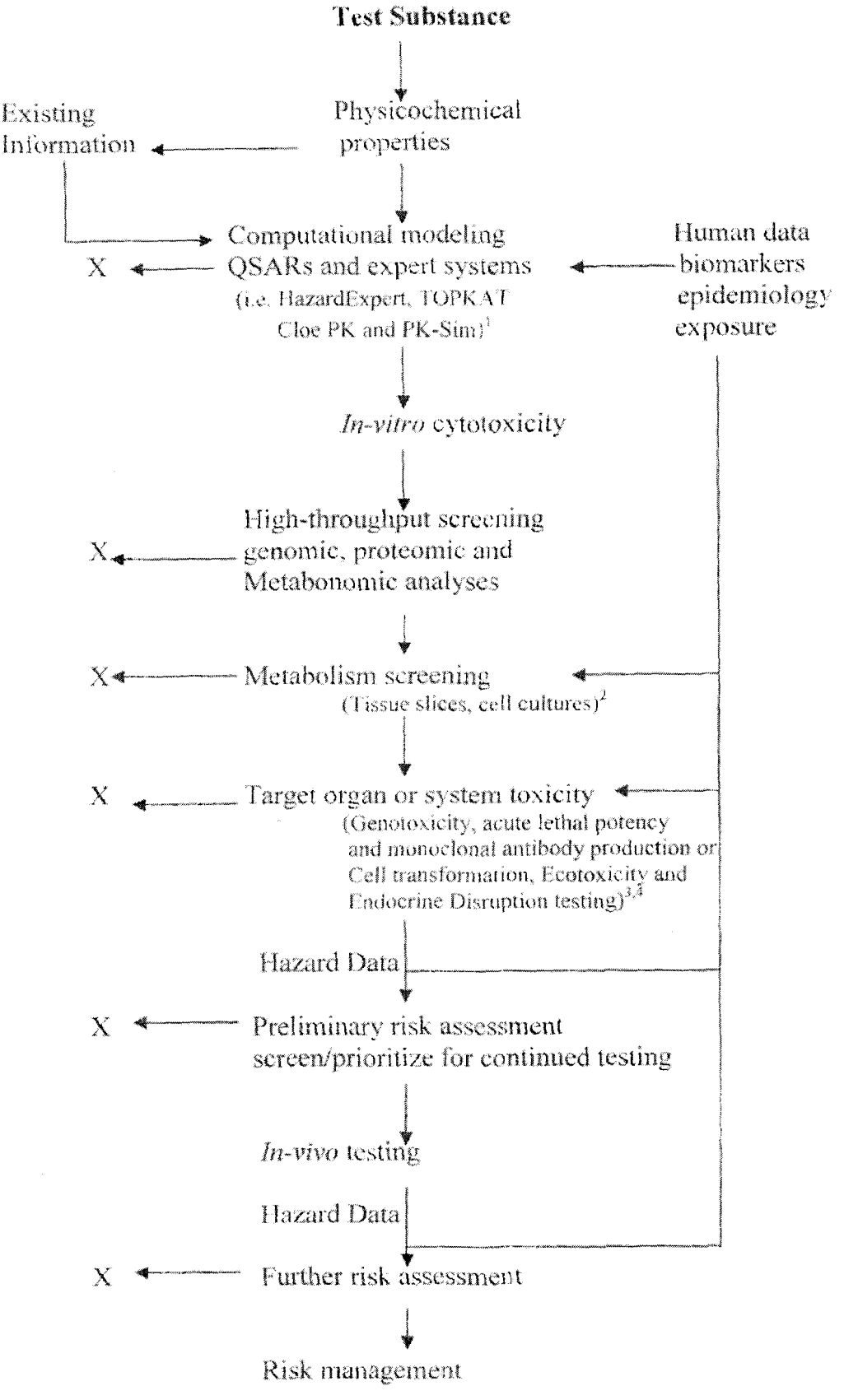
X = substance not required to undergo further testing/evaluation.

[1] TOPKAT and HazardExpert are two of the commonly used expert systems (computer models which can predict the most relevant types of toxicity based on available data). Each program predicts various endpoints of toxicity. For example, TOPKAT (http://www.accelrys.com) predicts carcinogenicity, mutagenicity, developmental toxicity, skin sensitization, eye irritancy, biodegradability, acute toxicity and chronic toxicity. HazardExpert (http://www.compudrug.com) can predict bioaccumulation, immunotoxicity and neurotoxicty among other endpoints. CloePK and PK-Sim are two of the commonly used models for predicting biokinetic properties. For example, Cloe-PK (http://www.cyprotex.com) can predict potential exposure, GI absorption, plasma, tissue and organ concentrations, renal excretion and hepatic metabolism.

[2] Tissue slices such as liver or kidney or cultures of hepatocytes for incorporating metabolizing enzymes and metabolic screening

[3] Examples of *in-vitro* (or refined *in-vivo*) methods validated or accepted for regulatory use.

[4] For further information on the abilities, advantages and disadvantages of using new technologies for assessing toxicity *in-vitro* and *in-silico* see Bhogal et al., 2005


Test Substance
Physicochemical properties
Existing Information
Computational modeling
QSARs and expert systems
(i.e. HazardExpert, TOPKAT Cloe PK and PK-Sim)[1]
X
Human data biomarkers epidemiology exposure
In-vitro cytotoxicity
High-throughput screening genomic, proteomic and Metabonomic analyses
X
Metabolism screening
(Tissue slices, cell cultures)[2]
X
Target organ or system toxicity
(Genotoxicity, acute lethal potency and monoclonal antibody production or Cell transformation, Ecotoxicity and Endocrine Disruption testing)[3,4]
Hazard Data
X
Preliminary risk assessment screen/prioritize for continued testing
In-vivo testing
Hazard Data
X
Further risk assessment
Risk management

Toxicogenomics describes the application of omic technologies to toxicology. For example, recent work combined Gene Chip™ technology with chemical profiling and traditional toxicological exposure assessments to characterize the molecular responses of fish exposed to STP effluent (van Aggelen et al., 2005). They found changes in the regulation of genes of effluent-exposed fish (including up-regulation of the VTG gene – an indicator of estrogenic exposure). Further, chemical profiling analysis will enable them to detect changes associated with a suite of pharmaceuticals including estrogens, sterols, and non-steroidal anti-inflammatory drugs (NSAIDs) (van Aggelen et al., 2005). In a more generalized manner, toxicogenomics can provide a wealth of mechanistic information on cellular and molecular responses to chemical stressors. For example, Moggs (2005) reviewed different toxicogenomics approaches used to acquire mechanistic data for molecular responses to xenoestrogens. These include rodent uterotrophic response profiling (i.e., gene ontology and pathway mapping of E2-responsive gene functions and phenotypic anchoring of gene expression changes during E-2 induced uterine growth (Moggs et al., 2004; Moggs, 2005)) or analysis of diethylstilbestrol (DES) induced carcinogenesis (Huang et al., 2005). The new data on molecular responses to xenoestrogens will impact hazard and risk assessment most likely by facilitating the development of short- and long-term exposure biomarkers (Moggs, 2005).

Integrated Assessments

As will become apparent throughout this paper, predicting, assessing and monitoring the effects of xenobiotic contaminants on non-target organisms is a multifaceted endeavour. The ultimate effect of a substance on an exposed organism depends not only on the contaminant's physical and chemical properties, but also on

those of the receiving environment, the overall status of the organism and the extent of exposure to other stressors. Figure 5 is a diagrammatic representation of the interactions that lead to the generation of a response in a species. The concept is equally applicable to a subgroup of a species, or to one individual.

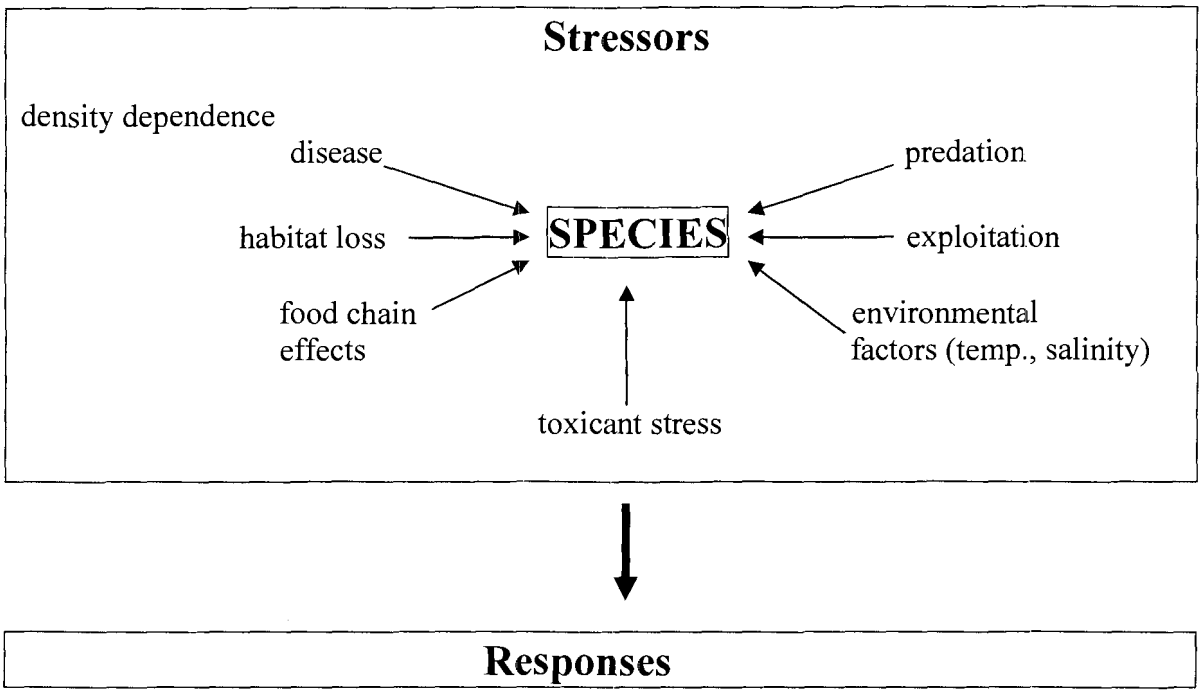


Figure 5: Biologic interaction for the development of a response. Adapted from van der Oost et al., 2003

Predictions of effects based on chemical-specific, physico-chemical and dose-response interactions integrate some of the aspects of response depicted in Figure 5. However, as Power and McCarty (1997) point out, the current paradigm does not correctly account for a majority of potential stressors experienced by animals in their natural environments. This view is mirrored also by Olden (2004) in his assessment of the current 'simplistic' models used in our understanding of toxicology. He continues his argument by suggesting that the integration of toxicogenomics with conventional techniques may provide a better approximation of natural systems. The integration

between various assessment systems discussed above may address some of these concerns. The current process is 'simplistic' and accounts only for stress from toxicants. However, the *in-vivo – in-vitro – in-silico* integration may also account for environmental factors and food-chain effects. Furthermore, the more general integrated risk assessments discussed in chapter 3 could integrate stress from the other areas depicted in Figure 5 such as exploitation, disease, habitat loss and predation, and may even extend to consider human-animal welfare interactions.

Handling Mixtures

Terms such as synergism and potentiation may be applied to the effects elicited by a mixture of two or more chemicals. Both of these terms imply at least additive effects, but these effects are part of a larger spectrum of interactions possible within chemical mixtures. The effects of mixtures have been classified Ballantyne (1995) as follows:

- *independent effects* – toxicity exerted independent of each other, both quantitatively and qualitatively
- *antagonistic effects* – toxicity of each interferes with the other and reduces the toxicity of one or more components.
- *additive effects* – toxicity of chemicals with similar qualitative action, produce a response which is quantitatively equal to the sum of the effects produced by the individual constituents
- *potentiating effects* – one substance (often of low toxicity) enhances the toxicity of another; resulting in higher than expected toxicity
- *synergistic effects* – two materials administered simultaneously result in toxicity greater than that expected from either each one alone, or both additively.

Mixture effects would be expected to be significant when contaminants are at exceedingly low concentrations in a multi-media, multi-chemical environment, as with pharmaceutical contaminants.

Selected Properties of Simple and Complex Mixtures

Researchers distinguish between simple mixtures, which contain less than 10 compounds, all with available toxicity data, and complex mixtures, which have a larger number of constituents, some without readily available toxicity data (Groten et al., 2001). According to most literature, including US EPA procedures (US EPA 2000), complex mixtures are evaluated based on either the 'mixture toxicity data' or, when such information is not available, on the data of a 'sufficiently similar mixture' (US EPA 2000). This method is often used to evaluate the effects of well-characterized mixtures such as diesel fuels, polychlorinated biphenols and natural flavourings (Groten et al., 2001; Monosson, 2005). A variation on this theme is the practice of whole effluent testing, which evaluates the effects of treatment plant effluents on organisms living in impacted areas (Folmar, 1996; Harries, 1997; Porter 2003).

All other means of calculating mixture effects are designed for simple mixtures. Within this category, researchers distinguish between mixtures exhibiting some level of interaction and those exhibiting zero interaction, where interaction is defined as the deviation from results expected on the basis of "additive interactions of the individual mixture components" (Silva et al., 2002). The concept of 'deviation from expectations' is important as those expectations are the sole basis of differentiation between various types of responses (i.e. synergism and potentiation). This idea has been discussed at length by Kortemkamp and Altenberger (1999).

Simple mixtures exhibiting interaction – those showing synergistic or antagonistic effects – are evaluated using the method of interaction-based hazard index. This method assumes cumulative and interactive effects. Interaction-based hazard index used alone on

in combination with the weight-of-evidence approach (which involves an understanding of routes, timing, duration of exposure, binary mixture kinetics, etc.) incorporates toxicity data from binary mixture testing (Groten et al., 2001; Monosson, 2005). Synergistic relationships between pharmaceutical contaminants have been reported or suspected in several studies (Halling –Sorensen et al., 1998; Routledge et al., 1998; Jones et al., 2002; Cleuvers, 2003). For example, several groups have evaluated the inhibitory effects on bacterial growth of anti-neoplastic, 5-fluorouracil and several antibiotics including β-lactams, cephalosporins and norfloxacin (Ueda et al., 1983; Gieringer et al., 1986).

Simple – No Interaction Mixtures

Simple mixtures not exhibiting interaction are studied using one of three models: dose addition or effect summation, response addition or independent joint action and concentration addition (Silva, et al., 2002; Monosson, 2005).

Dose addition / effect summation

This method is based on the expectation that the net effect of a mixture is the arithmetic sum of the effects of its components (Silva, 2002). This approach applies to chemicals exhibiting a similar mode of action with similar endpoints. It assumes that individual potencies can be 'normalized', in such a way as to be expressed relative to an index chemical (Monosson, 2005). The toxic equivalency factor (TEF), the relative potency factor (FPF) and the hazard index (HI) are different means of expressing results of mixture toxicity based on effect summation (discussed in Monosson, 2005). A serious limitation of dose addition models is that they are only applicable to those chemicals with a linear dose-response curve (Payne et al., 2000; Kortenkamp and Altenburger, 1998).

However, many pharmaceuticals do not necessarily show this dose-response relationship (Kortenkamp and Alterburger, 1999; Rajapakse, 2002). Consequently, calculations based on dose addition/effect summation tend to underestimate the total effects.

Response addition / independent joint action

This approach is used with chemicals that act on independent subsystems of an organism and have different end-points (Silva, 2002; Monosson, 2005). Consequently, there is an assumption of no interaction between the effects of the chemicals in the mixture. Response addition is preferably applied to chemicals that are present in low, but not sub-NOEL concentrations. In fact, a major assumption of this model is that chemicals present in sub-NOEL concentrations do not contribute to the overall toxicity of the mixture (Silva, 2002). This view is being challenged as evidence of chronic exposure to sub-NOEL chemicals shows adverse environmental effects (Rajapakse, 2002; Silva, 2002). To date, response addition has primarily been applied (or accepted) to the estimation of risk associated with chemical carcinogens (Monosson, 2005).

Concentration addition

Similar to dose addition, concentration addition applies to chemicals with a similar mode of action having similar end-points. It assumes that the same effects can be produced by replacing one constituent with another and that each component of the mixture contributes to the final toxicity in proportion to its concentration (Payne, 2000). Unlike the response addition model, concentration addition recognises each chemical's effect on the final toxicity, even if the chemical's concentration is below the zero-effect level (Payne et al., 2000; Faust et al., 2001). Concentration addition can be used to

evaluate a more accurate TEF for chemicals with sigmoidal dose-response curves (Safe, 1990) and is currently the preferred means of evaluating overall mixture effects (Payne et al., 2000; Faust et al., 2001; Silva et al., 2002; Cleuvers, 2004).

For estrogens and some common environmental xenoestrogens (bisphenol A, 4-nonylphenol, 4-octylphenol, *o,p'*-DDT – synthetic chemicals mimicking the action of estrogens), concentration addition was used to demonstrate two key concepts: low concentration does not imply low risk, and low potency does not imply low risk. It has been suggested that since most xenoestrogens are present in quantities well below their individual NOECs, they would present little or no risk to exposed organisms (Safe, 1995). However, several studies have shown conclusively that combinations of xenoestrogens do produce effects even at individual concentrations below the NOEC (Kortenkamp and Altenburger, 1999; Payne et al., 2000; Silva et al., 2002; Rajapakse, et al., 2002). It has also been suggested that since many xenoestrogens are orders of magnitude less potent than endogenous estrogens (Cargouet et al., 2004), the former will pose comparatively no harm to the environment (Safe, 1995). This view has also been refuted. Using CA, Rajapakse et al., (2002) showed that combinations of xenoestrogens at below NOEC can modulate the action of the more potent natural steroid hormone, 17β-estradiol.

Cumulative mixture effects are not restricted to endocrine-disrupting compounds. Effects greater than predicted from individual drug toxicities have been observed for binary mixtures of diclofenac/ibuprofen and clofibric acid/carbamazepine in *Daphnia* tests (Cleuvers, 2003). Mixtures of 3 cardiovascular β-blockers (atenolol, metoprolol and propranolol), showed a markedly increased toxicity to *Daphnia* compared to individual

effects (Cleuvers, 2004), as did mixtures of 4 nonsteroidal anti-inflammatory drugs (NSAID) (Cleuvers, 2005). Using the model of concentration addition, Cleuvers concluded that, for both of these studie, the effects were at least additive. The use of concentration addition (CA) in this case is intriguing, as in the test animals these drugs do not share a similar mode of action (as they do in humans, and as would be required for the conventional application of CA), but rather act by non-specific narcosis (Cleuvers, 2004; Cleuvers, 2005). These results corroborate earlier findings that CA can be applied to chemicals that produce toxicity via narcosis (van Loon et al., 1997; Deneer et al., 1988).

Narcosis describes non-specific, non-receptor mediated, anaesthetic-type toxicity, thought to function by a disruption of the hydrophobic interactions in the cell membrane (Ren, 2002; Schultz et al., 2003). The narcosis mechanism of action has been observed in various aquatic species exposed to environmentally-relevant concentrations of pharmaceuticals (Cleuvers, 2004; Cleuvers, 2005). Several mechanistic theories for narcosis exist; one proposal is that chemicals accumulating at the lipid bylayer interface increase lateral pressure within it thereby changing the ratio between open and closed ion channels (Cantor, 1997; Schultz et al., 2003). Several classes of narcoses have been described; the two most prominent ones being polar and non-polar. Non-polar narcotics are usually inert compounds such as halogenated gaseous anaesthetics, for which log K_{ow} relationships demonstrate *baseline toxicity* such that the chemical's toxicity is never less than that predicted by the log P-based models. Polar narcotics include aromatic compounds with strong electron-donating constituents whose potency exceeds that estimated on the basis of their log P values (Schultz et al., 2003; Ren, 2002). For

example, phenols and anilines are polar narcotics, while pharmaceuticals such as ibuprofen and naproxen demonstrate non-polar narcosis in non-target organisms (Ren, 2002; Cleuvers, 2004). The type and applicability of narcosis to observed effects in wildlife is an area of avid research as it provides a basis for the determination of mechanistic information (Borgert, 2004). Mechanistic data is incorporated in both in-vivo/in-silico modelling and in structure-activity systems in order to predict toxicity (Olden, 2004; Cunningham, 2004).

Cleuvers suggests that many pharmaceuticals (non-steroidal anti-inflammatory and cardiovascular drugs) can produce narcosis even if they act on specific targets in humans (Cleuvers, 2004; Cleuvers, 2005). The application of concentration addition to chemicals not sharing a common mode of action accounts for the effects of substances present in concentrations below their NOECs without any apparent drawback in the model's predictive powers. For that reason, it is gaining in acceptance over other means of modelling mixture effects.

Aspects of bio-transformation of pharmaceuticals

Most pharmaceuticals undergo some level of metabolic transformation in the target organism, at least for some fraction of the administered dose although the extent of metabolism can vary widely. For example, the psychoactive drug carbamazepine undergoes substantial metabolic transformation, while the anaesthetic drug propofol resists metabolic degradation and is excreted largely as the parent compound (Kümmerer, 2004). Generally, metabolic processes increase a chemical's water solubility thereby facilitating its excretion and minimizing potential toxicity (Vermeulen, 1996). This situation is depicted in pathways 3 and 4 in Figure 6. Occasionally, the opposite is true,

where the molecule actually becomes more hydrophobic and excretion is delayed. For example, metabolism of sulphonamides via acetylation decreases their solubility causing them to accumulate in the kidney tubules (Ballantyne, 1995). This situation is represented by pathway 1A in Figure 6. Lastly, metabolic activation to more reactive or toxic compounds is also possible (pathways 2 and 6C). The anti-neoplastics cyclophosphamide and ifosfamide undergo metabolic activation leading to the formation of isophosphoramid mustard and acrolein (Christensen, 1998; Sanderson et al., 2004a). Compared to the parent compounds both of these products have considerably increased cytotoxicity thereby accounting for their anti-neoplastic activity (Sanderson et al., 2004a).

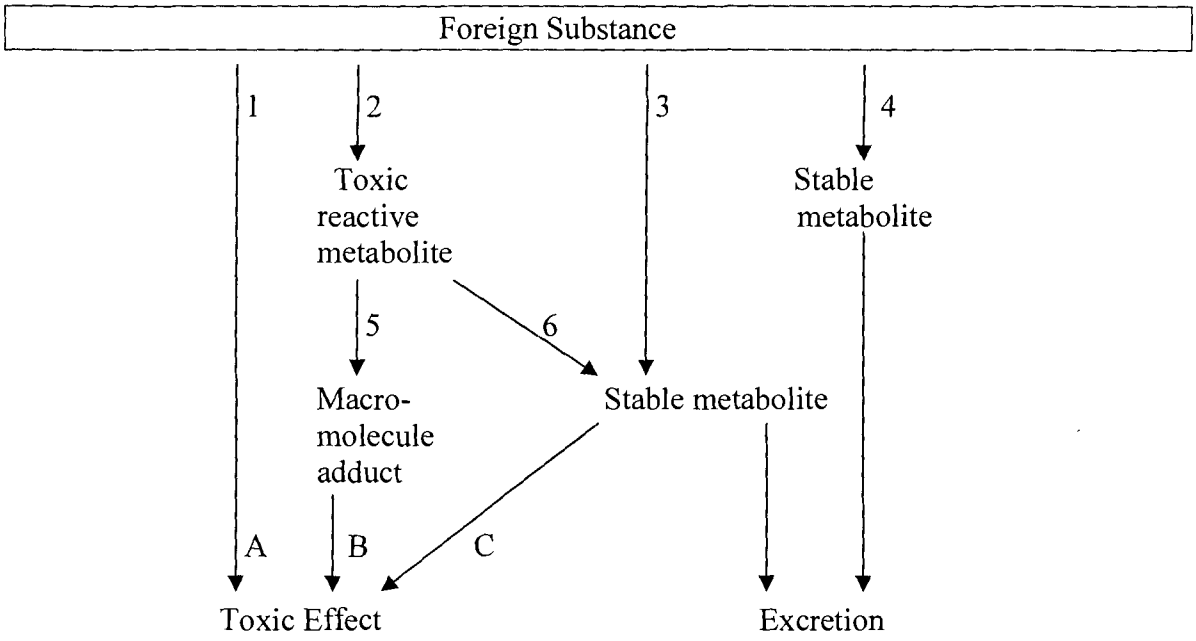


Figure 6: Xenobiotic transformation within the body. Adapted from Bimbrell, 1991

(1) direct toxic effect (A); (2) metabolic activation to a toxic product; (3) formation of a stable metabolite which may cause a toxic effect (C); (4) detoxification via the formation of a stable metabolite and subsequent excretion (note that the foreign compound may be excreted directly. However, that situation does not involve any transformation, thus it is not included in the diagram). The reactive metabolite formed by bioactivation (2) may cause a toxic effect (B) through reaction with critical targets (5) or be detoxified through reaction with a protective agent (6). Adapted from Timbrell (1991), slightly modified.

Types of Pharmaceutical Biotransformation Reactions

Phase I and II reactions are metabolic processes whose major purpose is to facilitate the excretion of xenobiotics, particularly hydrophobic xenobiotics (Zakrzewski, 2002). This often involves transforming the chemical from its hydrophobic form to successively more hydrophilic structures.

The primary purpose of phase I reactions is to unmask or add reactive functional groups, primarily but not exclusively via hydroxylation (Goeptar eta l., 1995). The overall reaction of phase I is depicted below (R is the substrate).

$$RH + O_2 + H_2 \rightarrow ROH + H_2O$$

Phase I reactions are mediated by a group of enzymes known as the mixed-function oxidase (MFO) system which includes the cytochrome P450 heme proteins (cyt P450) and NADPH cytochrome P450 reductase (Stegeman et al., 1992; von der Oost et al., 2003). In animals, the primary site of phase I metabolism is the liver, therefore, assessment of hepatocyte function is commonly used in environmental monitoring. Furthermore, as the MFO system is inducible and has therefore shown considerable environmental sensitivity (Sijm and Opperhuizen, 1989), it has been investigated as a biomarker for contamination of aquatic systems (reviewed in Goksoyr and Forlin, 1992).

In phase II reactions, the xenobiotic or its product of phase I metabolism is covalently bound to an endogenous polar molecule such as a sugar or amino acid, in a process called conjugation. This is catalyzed most often via glycosyltransferases, sulfotransferases, glutathione S-transferases and acetyl- and aminoacetyltransferases (Timbrell, 1991; van der Oost, 2003). The primary purpose of conjugation is to further increase the water solubility of the xenobiotic. It occurs when the target chemical

contains an available functional group, usually -OH, -COOH, $-NH_2$ or -SH (Cunningham, 2004). The compound is conjugated to an endogenous ligand, usually a sugar or an amino acid. (Lech and Vodicnik, 1985). Perhaps owing to the ready availability of sugars in the body, a common drug conjugate is glucuronide, involving the condensation of the target moiety with D-glucuronic acid (Cunningham, 2004). Several types of drugs undergo primarily glucuronide transformation including phenols, carboxylic acids, amines and steroids (Ballie et al., 2002; Cunningham, 2004). Phase II are not necessarily permanent; reversal to the original structure has been observed in a process called de-conjugation (Arcand-Hoy, 1998) and is often catalyzed by microorganisms. De-conjugation is relevant to PhAC contamination in that it provides a means by which the un-altered parent compound is made bio-available (further discussed in Chapter 2).

Phase III reactions function primarily in the efflux of conjugates, but can also act as a first line of defence against xenobiotics (hence, they are also known as Phase 0 reactions) by preventing their intracellular accumulation (Luedeking and Koehler, 2004). They are mediated by transporter proteins including P-glycoprotein (P-gp), multidrug resistance-related protein (MRP) and the lung resistance protein (LRP) which is the major vault protein (MVP) (Luedeking and Koehler, 2004). This transporter system (including the above-mentioned proteins) was first identified in cancer cells recalcitrant to multi-drug therapy and later observed in various marine invertebrates (Edicott and Ling, 1998; McFadzen et al, 2000), including sponges *T. aurantium* (Kurelec, 1992) *G. cydonium* and *V. aerophoba*, mussels (Kurelec and Pivevi, 1991), snails (Kurelec, 1995), and clams (Waldmann et al, 1995). The transporter mechanism is known as the multi-xenobiotic resistance system (MXR) (Euphemia and Epel, 2000). This detoxification system has

been identified in normal cell lines as well, including human liver, kidney, intestine and pregnant uterus cells (Bard, 2000) and in trout epidermal cells (Shuilleabhain et al., 2005). The MXR system is inducible by various environmental contaminants, thus the expression of the MXR protein may function as a very sensitive indicator of exposure (Bard, 2000). However, the system can be easily overloaded by fairly innocuous environmental chemicals (for example, synthetic musks used as fragrances in cosmetics and household products) allowing for intracellular accumulation of other potentially harmful xenobiotics (Kurelec, 1997; Smital et al., 2004). Studies on various agrochemicals, pesticides, fragrances and pharmaceuticals have shown that these substances can be potential inhibitors of the MXR system (Lanning et al., 1996; Lanning et al., 1997; Luckenback et al., 2004). MXR inhibitors are typically referred to as chemosensitisers and include ATPase inhibitors (Sharom, 1997), some pesticides, e.g. pentachlorophenol (Kurelec et al., 1998), and some household and pharmaceutical products (Smital et al., 2004). For example, as selective inhibitors of Pgp in fresh water mussels (*Dpolymorpha* and *C. fluminea*), the PhAC cyclosporine and saturosporin reduce the active efflux of metabolites from cells leading to the intracellular accumulation of potentially toxic compounds (both endogenous such as cellular waste products and exogenous such as cyclosporine and saturopsporin) (Lange and Dietrich, 2002).

Owing to its ability to prevent xenobiotic accumulation within the cell, the MXR system has important implications for many aspects of toxicity evaluation, e.g., exposure assessment, uptake, bioavailability/bioaccumulation, internal dose determinations and synergism (Smital and Kurelec, 1998). Kurelec (1997) suggested that MXR induction could be an indicator of exposure. However, MXR induction has been shown to be a

non-specific mechanism. P-gp, an MXR effector protein, was activated in the presence of compounds that are not its known substrates, or in the presence of non-specific physical stressors such as high temperatures (Eufemia and Epel, 1999). These authors concluded that (a) P-gp may be a useful indicator of a general response to cellular stressors and that (b) P-gp induction is not substrate-specific and thus can only be used to assess a general response to stress. Their results were corroborated several years later when another study found that MXR activity could be used as a general stress indicator in bivalve populations of *Dreissena polymorpha* (Pain and Parant, 2003).

There is sufficient data to conclude that the MXR system is protective against environmental factors (natural and anthropogenic) in aquatic organisms. Furthermore, the data also indicates that the MXR system can be inhibited by natural and synthetic stressors (Smital et al., 2004). However, further work is needed to evaluate the effects of PhAC on the MXR system, particularly to identify whether mixtures of PhAC show toxicity greater than expected due to MXR inhibition. Likewise, the consequences of MXR inhibition at the population and ecosystem level have not yet been evaluated (Smital et al., 2004).

Biotransformation of Pharmaceuticals

Drug metabolism within the target organism is important in the context of environmental contamination with pharmaceutical substances for several reasons. First, the type and amount of metabolic transformation controls the amount of drug excreted as parent compound into the environment. For example, it was calculated that 5 – 35% of the administered anti-cancer drug cyclophosphamide (CP) and 20% of ifosomide are excreted as un-metabolized parent compounds (Cohen et al., 1971; Kummerer, 1997).

Excretion of un-metabolized penicillin V is estimated to average 25% (Cole et al., 1973) while the anaesthetic propofol is almost entirely (90%) excreted as parent compound (Guitton et al, 1997).

Second, metabolic transformation of pharmaceutical products does not necessarily equate to a reduction of toxicity. This fact highlights the inadequacy of investigating the fate and effects of the parent chemicals only (Cunningham, 2004). For example, clofibrate (a widely used lipid regulator) is not detectable in the environment, but its active metabolite clofibric acid is one of the most common pharmaceutical contaminants (Emblidge and DeLorenzo, 2005). The statement that that clofibrate does not reach the environment is technically true, but it is also misleading.

Third, the results of detoxification may be reversed by microbial action either in the human colon or in the sewage treatment plant. This process is called de-conjugation and is apparent in the detection of biologically active pharmaceuticals in sewage effluent and in the receiving environments (Sumpter, 1998; Ternes et al., 1998). For example, the frequent detection of carbamazepine in surface waters has been attributed in part to de-conjugation of metabolites (Ternes, 1998). Ninety-eight percent of administered carbamazepine is excreted as active metabolites but parent CBZ continues to be detected in surface water in quantities that could only be attained if de-conjugation was occurring (Jiemba et al., 2005; Ferrari et al., 2003). Another convincing example is that of estrogens. Studies show that more than 80% of administered dose of estrogens (E1, E2 and EE2) undergoes urinary excretion as conjugated products (Arcand-Hoy, 1998). However, de-conjugation has been shown to occur both in the human colon and in sewage treatment plants (Desbrow et al., 1996).

The exact pathways that drugs follow in the environment and the potential effects associated with the movement of PhAC through various ecosystems will be discussed in Chapter. 2.

CHAPTER 2:
SOURCES, FATE AND EFFECTS OF SELECTED PHARMACEUTICALS

Sources of Pharmaceuticals in the Environment

Pharmaceutical residues enter the environment via one of three sources: intentional or accidental discharges at drug manufacturing plants, discharges of veterinary and agricultural pharmaceuticals after therapeutic and/or commercial use and discharges of human pharmaceuticals most often via sewage effluent. The latter two sources and the possible pathways to various compartments of the aquatic environment are depicted in Figure 7.

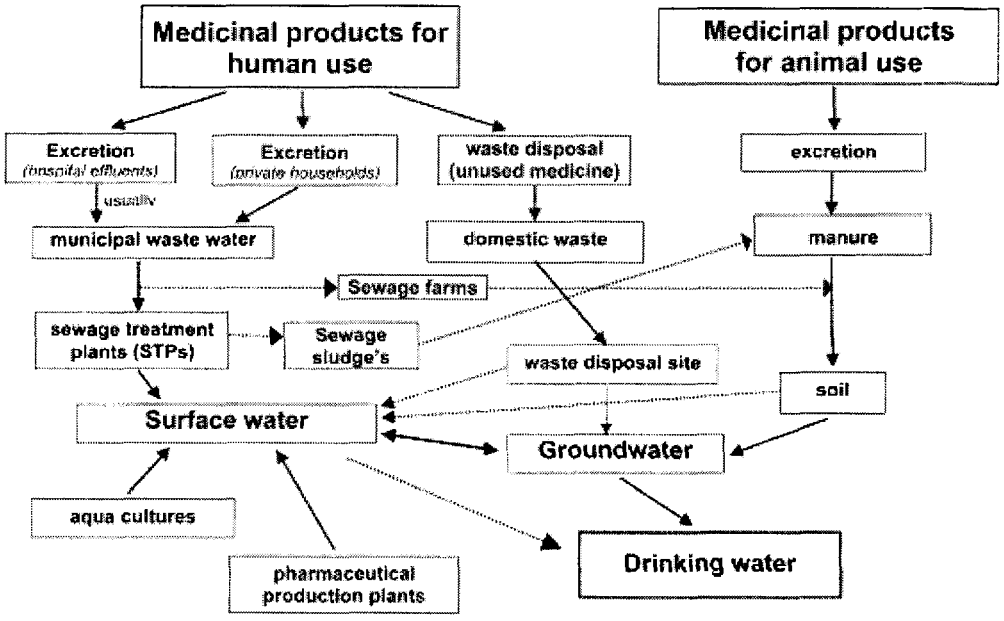


Figure 7: Sources and pathways of pharmaceuticals in the environment

Re-printed from Toxicology Letters 131:5-17 **Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data**, with permission from Elsevier.

Contamination from manufacturing plants

Manufacturing plants are associated with a relatively low rate of environmental contamination as their releases tend to be localized and controlled. Local environmental regulations usually prevent any intentional release of contaminated material into the environment. When accidents do occur, introduction of contaminants into the surrounding environment is usually a point release and attempts at remediation are generally prompt and effective (Daughton and Ternes, 1999). Consequently, these discharge events will not be discussed further here.

Contamination from agricultural use

Agricultural pharmaceuticals are used as growth promoters in livestock production, cocciostatics in poultry production, feed additives in fish farms, veterinary therapeutic agents, or as pesticides (Halling-Sorensen et al., 1998). These substances comprise several classes of antibiotics and heavy metal-hormone cocktails. Agropharmaceuticals are used extensively, constituting some 40% of the 22,680 tonnes total annual US antibiotic production (Levy, 1998; Nawaz et al., 2001). Similar annual distribution of antibiotics occurs in the European Union, with some 4,700 tonnes being administered to animals and 8,500 tonnes to humans (Sanderson, et al., 2004b). Antibiotics are used extensively in the animal husbandry industry as they provide immediate commercial benefits. For example, the prophylactic use of antibiotics minimizes losses to disease and contamination allowing for high density farming practices (McEwen et al., 2002; Barza, 202). Further, the practice results in quicker weight gain, reducing the time required for housing and feeding the animals (swine) by an average of 5 days (Hayes et al., 2002; Hayes and Jensen, 2001).

Concerns associated with agricultural pharmaceuticals

Pharmaceuticals applied in the farming industry enter the environment when they are excreted in the urine and faeces of animals or as constituents of animal manure fertilizers (Koschorreck et al., 2002). In either case, the antibiotics pass directly from the animal to the environment, without possible transformation into less active metabolites in the sewage treatment plant. Biodegradation within the animal may occur but, not for all substances. It has been estimated that 30% - 90% of the administered antibiotic dose enters the environment unaltered (Rang and Dale, 1991; Halling-Sorenson, 1998). The total antimicrobial concentration in swine manure storage lagoons in the USA for example, frequently approaches 1 mg/L (Campagnolo et al., 2002). Similarly, the actual concentrations of some drugs in soils exposed to manure can be considerable: the level of tetracycline in the top 20 cm of soil fertilized with liquid animal manure was approximately 200 μg/kg (Hamscher et al., 2002).

A second source of concern with the agricultural use of pharmaceuticals is the development of antibiotic resistance (ABR). Resistance develops via three mechanisms: (1) continuous exposure of humans to antibiotic residues in food products, (2) transfer of resistance genes between animal and human microbial flora, and (3) transfer of resistance genes within the ecosystem via direct contamination and foodchain relationships (Klein, 1998; Teuber, 1999). Antibiotic resistance is the ability of a microorganism to resist the effects of antibiotics targeted to its control. Consequently, therapeutic agents traditionally used to control these (often pathogenic) organisms become ineffective against them. This phenomenon is the microbes' normal defence mechanism against inhospitable environmental conditions (Schwartz et al., 2003). However, when

antibiotics are over- or mis-used, resistance can escalate to levels well above those expected to occur naturally. The statistics in support of such a phenomenon are convincing: in 1987, less than 5% of *Streptococcus pneumoniae* isolates were resistant to penicillin. By 1997, that figure had increased to 44% (Hellinger, 2000). Less than 5% of *Staphylococcus aureus* isolates were resistant to methicillin between 1975 and 1982. By 1991, 29% were methicillin-resistant (Panhho et al, 1992). The incidence of vancomycin resistant enterococci rose from less than 0.5% in 1989 to a staggering 30-50% in 1998 (Hellinger, 2000). Scientists continue to establish the connection between antibiotic growth promoters (AGPs) and the development of human and veterinary bacterial resistance (Teuber, 1999; Kelly, 2004; ARAD - Health Canada, 2004). For example, a 1995 report by the American Society for Microbiology concluded that "due to increased drug resistance in animal pathogens and changes in food production practices, there is a growing threat to food, the food industry and hence the US economy" (American Society for Microbiology, 1995). Similarly, in 1997, the World Health Organization formulated the impacts of antimicrobials used in food production. The main threats were: a) an increase in bacterial resistance in animals and transfer of resistance to humans, b) an increase in human infections caused by resistant bacteria, c) a high potential for therapeutic failures in animals and humans and d) a largely increased resistance of specific animal bacteria on farms (WHO, 1997). Antibiotic resistance surveillance programs in the United Kingdom (MAFF, 1998), Denmark (Teuber, 1999), France (Teuber, 1999) and Denmark (Dermot, 2003) continue to provide evidence that agricultural antimicrobials contribute to the development and perpetuation of antimicrobial resistance. Whether the use of antimicrobials as growth promoters is the

major cause of bacterial resistance or merely a contributing factor will be revisited in chapter 3.

Resistance genes have been identified in both effluent and environmental samples. A 2003 German study on antibacterial resistance in WWTP effluent and potable water found that hospital effluent contained vancomycin-resistance (VR) and methicillin-resistance (MR) genes from enterococci and staphylococci respectively as well as *Enterobacteriaceae* β-lactam-hydrolysing genes (Schwartz et al., 2003). The authors were able to culture VR enterococci and the β-lactam-hydrolysing *Enterobacteriaceae* from all municipal WWTP effluents sampled and found their resistance genes in the two potable water samples as well. (It is notable that although the resistance genes were amplified from potable water biofilms, the *Enterobacteriaceae* organisms themselves were not. This suggests that simple bacterial testing may not detect bacterial resistance, as these genes would have been part of a viable but non-cultivable species (Schwartz et al., 2003)).

Contamination from sewage treatment and solid waste disposal plants

The two principal sources of active human pharmaceuticals to the STP effluent are (1) used and unused, legal and illicit drugs from residential users, and (2) used and unused drugs and their metabolites from clients of hospitals, clinics and other medical facilities (Daughton and Ternes, 1999).

The concern with the direct disposal of unused drugs into the sewer system is that these substances enter the treatment plant in their fully potent form, having bypassed potential biotransformation within the body. While some pharmacies provide their

clients with the option of returning unused drugs (Government of British Columbia, 1997 http://www.env.gov.bc.ca/epd/epdpa/ips/meds/reports/meds1998.html), some 35% of US clients choose to dispose of expired or no longer necessary drugs at home (Kuspis and Krenzelok, 1996). In fact, there is evidence that vast quantities (the authors do not specify exact amounts) of prescription and non-prescription drugs are distributed, but never consumed (Bosch, 1998; Daughton and Ternes, 1999).

A myriad of drugs including analgesics, stimulants, NSAIDs, carbamazepine (CBZ), artorvastatin, gemfibroxil and EE2 have been identified in sewage effluent in the range of ng/L to μg/L (Kümmerer 2001; Metcalfe et al., 2003). These concentrations are found in the treated effluent despite various levels of treatment, suggesting that traditional microbial degradation-coagulation/flocculation treatment does not provide sufficient protection against contamination (Stuer-Lauridsen et al., 2000; Ternes et al., 2003; Bound and Voulvoulis, 2004). Table 1 lists levels of selected pharmaceutical compounds in domestic sewage treatment influent and effluent and in various environmental compartments.

Table 1: Concentrations of selected pharmaceuticals in various compartments

Name of pharmaceutical substance	Compartment or matrix	Conc.[1] (µg/L)	Comment	Reference
Antibiotics				
Sulfamethoxazole	Effluent	0.24	Median, 8 Canadian STPs[2] in 2003	Miao et al., 2004
	Surface waters	0.008	4 Ontario rivers	Metcalfe et al., 2004
	Surface waters	1.9 – 2.0	max. concentration	Kolpin et al., 2002 and Hirsch et al., 1999
Tetracycline	Effluent	0.151	Median, 8 Canadian STPs in 2003	Miao et al., 2004
	Surface waters	0.016	1 Ontario river	Metcalfe et al., 2004
	Liquid manure from pigs/calves	66000	Mean; n=181	Winckler and Grafe, 2001
	Farmed soil under conventional fertilization	310	Mean; n=60 Conventional fertilization with liquid manure	Hamscher et al., 2002
Oxytetracycline	Effluent	Nd	Median, 8 Canadian STPs in 2003	Miao et al., 2004
	Surface waters	Nd	4 Ontario rivers	Metcalfe et al., 2004
Clarithromycin	Eflfuent	0.087	Median, 8 Canadian STPs in 2003	Miao et al., 2004
	Surface waters	0.009	4 Ontario rivers respectively	Metcalfe et al., 2004
Ciprofloxacin	Effluent	0.118	Median, 8 Canadian STPs in 5 cities in 2003	Miao et al., 2004
	Surface waters	0.011	4 rivers in Ontario in 2003	Metcalfe et al., 2004
Lincomycin	Surface waters	0.73	19% of surveyed US streams – Maximum concentration	Kolpin et al., 2002
Lipid Regulators				
Clofibric acid	Influent	0.46	Berlin STP	Heberer, 2002
	Effluent	0.48	Berlin STP	
	Ground water	7.3	Germany	
	Drinking water	4.0	Germany	Metcalfe et al., 2004
	Effluent	0.03	Canadian STPs between 1999-2002	
	Surface waters	0.015	Ontario rivers	

Name of pharmaceutical substance	Compartment or matrix	Conc.[1] (μg/L)	Comment	Reference
Gemfibrozil	Influent Effluent Effluent Effluent Surface waters	0.71 0.18 0.071 0.043 and 2.174 0.012 and 0.112	Kallby STP, Germany Mean, Canadian STP Median and Maximum respectively; Canadian STPs between 1999-2002 Median and Maximum respectively; Canadian STPs between 1999-2002	Bendz et al., 2005 Gagne et al., 2005 Metcalfe et al., 2004
Psychoactive Drugs				
Carbamazepine	Effluent Surface waters	0.107 and 2.30 0.02 and 0.65	Median and maximum concentration in 5 Canadian STPs Median and maximum respectively in 4 Ontario rivers	Metcalfe et al., 2004
Fluoxetine	Effluent Surface waters	0.05 0.013	Canadian STPs Ontario rivers	Metcalfe et al., 2004
Analgesic and Non-Steroidal Anti-Inflammatory Drugs				
Diclofenac	Influent Effluent Surface water Effluent Surface waters	3.02 2.51 0.0062 0.359 0.026	Berlin STP Berlin STP Berlin STP Median, 5 Ontario STPs Median, 4 Ontario rivers	Heberer, 2002 Weigel et al., 2002 Metcalfe et al.,2004
Ibuprophen	Influent Effluent Effluent Surface waters	3.59 0.15 0.786 0.064	Kallby STP, Germany Mean, Canadian STP Median, 4 Ontario rivers	Bendz et al., 2005 Gagne et al., 2005 Metcalfe etal, 2004
Carboxy-Ibuprophen	Influent Effluent	10.75 0.43	Kallby STP, Germany	Bendz et al., 2005
Naproxen	Influent Effluent Effluent Surface waters	3.65 0.25 0.27 0.094	Kallby STP, Germany Mean, Canadian STP Median, 4 Ontario Rivers	Bendz et al., 2005 Gagne et al., 2005 Metcalfe etal, 2004
Hormone Regulators				
17β-estradiol	Influent Effluent	0.0156 0.0018	Mean for II, III and lagoon Canadian Municipal Plants	Servos et al., 2005
	Surface waters	0.00083	Acushnet River estuarine	Zuo et al., 2005

Name of pharmaceutical substance	Compartment or matrix	Conc.[1] (μg/L)	Comment	Reference
			seawater, Eastern USA	
Estrone	Influent Effluent	0.0490 0.0170	Mean for II, III and lagoon Canadian Municipal Plants	Servos et al., 2005
	Surface waters	0.002	Acushnet River estuarine seawater, Eastern USA	Zuo et al., 2005
17α-Ethynyl estradiol	Surface waters	0.00467	Acushnet River estuarine seawater, Eastern USA	Servos et al., 2005
Anti-neoplastic drugs				
Ifosfamide (anti-neoplastic)	Hospital effluent Communal influent Communal effluent	0.109 0.0085 0.0093	Median values	Kummerer et al., 1997
Other pharmaceuticals of interest				
Triclosan (antimicrobial disinfectant)	Surface waters	2.3	Maximum concentration in US streams	Kolpin etal., 2002

[1] Concentration
[2] Sewage Treatment Plant

Almost all of the documented cases of adverse effects associated with exposure to pharmacologically-active substances have occurred in animals that either reside in or feed on aquatic environments. Guillette et al. (1994) reported endocrine disruption in alligators in Florida, and Bortone and Davis (1994) have reported similar endocrine effects in fish in the USA. Purdom et al. (1994), Harries et al. (1997), and Jones et al. (2004) documented similar effects in fish in British sewage lagoons and rivers. The well-researched incidents of eggshell thinning in nesting birds are evidence of endocrine disruption in organisms feeding on exposed animals such as fish (Peakall and Lincer, 1996; Lundholm, 1997). These observations support the generally held belief that the aquatic environment is the ultimate sink for both synthetic and natural chemicals (Sumpter, 1998; Fent et al., 2005).

The actual concentration of pharmaceuticals in various media depend on several factors: (1) rates and pattern of use, (2) physical and chemical properties of the receiving

environment (3) physical and chemical properties of the contaminant(s), and (4) processes in and operating conditions of the treatment plant (Byrns, 2001; Daughton and Ternes, 1998).

Fate of PhACs in the Environment

Treatment plant: process and operating conditions

In a sewage treatment plant (STP), two processes affect the subsequent distribution of pharmaceutical contaminants: adsorption/absorption and bio-degradation (Fent et al., 2005). Both processes are discussed in more detail below.

Adsorption results in sequestration of pharmaceutical contaminants either via colloidal-facilitated transport as suspended particulates or via deposition to sewage sludge (Bendz et al., 2005). Adsorption correlates directly with hydrophobic and electrostatic interactions between the pharmaceutical and particulates/micro-organisms and, to a more limited extent, with plant operating conditions such as pH and hydraulic retention time (Fent et al., 2005). For example, polar pharmaceuticals such as ibuprophen, naproxen, diclofenac and indomethacin (with pK_a values from 4.9 to 4.1), occur mainly as dissolved ions in the aqueous phase and show little tendency to sorb to sludge (Fent et al., 2005). Similar behaviour has been observed for bezafibrate, gemfibrozil and ifosfamide (Kummerer et al., 1997; Buser et al., 1998). Alternatively, basic, non-polar drugs such as fluoroquinolone antibiotics or steroid hormones (with log K_{ow} values exceeding 3 – 3.5) are removed from the STP plant primarily via adsorption to sludge (Byrns, 2001; Golet et al., 2003; Ternes et al., 2003; Fent et al., 2005). Further degradation while bound to sludge is not a significant biotransformation process for these

compounds (Huang et al., 2001; Ternes et al., 2003). The process of coagulation and/or flocculation in secondary treatment may succeed in removing contaminants from the free water, but it does not necessarily reduce the potential environmental hazard posed by those contaminants. The highly organic sludge component is then landfilled in a solid waste disposal (SWD) facility. Subsequent leaching into the aquatic environment can and does occur. For example, Ahel and Jelicic (1999) reported concentrations of the analgesic prophyphenazone of 3.7 – 60 μg/L in samples of leachate from a municipal landfill site.

Biodegradation mediated by microbial action is one of the two means of biotransformation in the treatment plant (the other being photo-degradation, discussed in a separate section) (Daughton and Ternes, 1999). Biodegradation is the primary process for eliminating dissolved substances. Bio-degradation occurs aerobically during activated sludge treatment and anaerobically during digestion (Fent et al., 2005). It is dependent on factors associated with the environment (temperature and pH), the microbial community, the chemical properties of the target compound and the operating conditions of the plant. For example, as pH decreases, adsorption for many polar, dissolved NSAID may increase thereby reducing their potential for bio-degradation (Fent et al., 2005). Bio-degradation rates have also been correlated to hydraulic retention times (HRT): for example, diclofenac was significantly transformed only when residence time exceeded 8 days (Kruzinger et al., 2004). In contrast, HRT had no effect on the extent of degradation of other drugs, particularly carbamazepine (Metcalfe et al, 2003b).

Microbial activity is sensitive to a number of variables including physical and chemical environmental conditions and the presence or absence of agents toxic to

microorganisms. Environmental conditions affect the rate of microbial degradation of contaminants by affecting the type and rate of bacterial metabolic activity. Municipal sewage effluents often contain relatively high concentrations of antibiotics and disinfectants. For example, the mean concentration of antibiotics in municipal treatment plants can reach 50 μg/L (Kümmerer, 2001). The concentration of the disinfectant benzochloride was measured in hospital effluent at 6 mg/L, and that of quaternary ammonia compounds in municipal sewage at 0.05 – 0.1 mg/L (Kümmerer, 2001). These classes of compounds are specifically designed to inhibit the growth of microorganisms. In this case, microbial inhibition can lead to a decrease in the operating specifications of the treatment plant and subsequent decrease in treatment efficiency (Servos et al., 2005). Furthermore, many antibiotics (β-lactams, for example) employ the bacteriostatic rather than the bacteriocidal effects on target microbes. The effect of bacteriostasis on the STP microflora has not been studied (Kummerer, 2004a; Kummerer, 2004b).

Lastly, the process of chemical and ultraviolet oxidation, comprising tertiary treatment in most facilities, is designed for the removal of pathogens rather than that of a toxicological hazard such as PhACs (Daughton and Ternes, 1999; Auriol et al., 2005). In fact, it may have the opposite effect in that some oxidized products may show increased toxicity. For example, both naproxen and carbamazepine form oxidation products that have toxicities equal to or higher than those of the parent compounds (Isidori et al., 2005). Similarly, clofibric acid often present in environmental samples is an active metabolite of clofibrate, etofibrate and etofyllinclofibrate used as blood lipid regulators.

Removal Efficiencies

A common approach to tracing environmental transport of drugs is to measure their influent and effluent concentrations. This approach has not been standardised so results vary widely according to construction and treatment technology, HRT, season and weather conditions, and measurement techniques (Stumpf et al., 1999; Carballa et al., 2005). Generally, removal rates range from 7% for the antiepileptic carbamazepine to 96% for the beta-blocker propranolol, with most removal efficiencies of PhACs averaging 60% (Ternes, 1998; Clara, 2004). For the plants investigated by Ternes in 1998, an average of 40% of the pharmaceutical load reached the environment directly via effluent discharge. In general, secondary treatment was more efficient removing 51 – 99% of studied pharmaceuticals, while primary treatment eliminated only 0 – 44% (Ternes and Hirsch, 2000). Even tertiary treatment did not guarantee adequate hazard neutralization. In a study measuring pharmaceutical concentrations under ice cover, incomplete removal during (III^{ry}) treatment, absence of photo- and microbial degradation and lack of dilution potential, led to mean concentrations of ibuprofen, naproxen and gemfibrozil ranging from 2129 to 5893 ng/L (in the summer, these concentrations do not exceed 68 ng/L) (Donald and Sverko, 2005).

Measuring influent and effluent contaminant concentrations even under standardised conditions can be somewhat misleading. First, pharmaceuticals may be removed from the effluent, but removal is often to the sludge component (via adsorption, as discussed above). For example, in laboratory-scale experiments, Kummerer et al., (2000) was able to recover 78% of the 'removed' ciprofloxacin from the sludge component (the model STP removed 65% of the influent amount from the system, while

allowing 35% to pass through). Second, the degradation products are not considered. These products may include equally hazardous lower molecular weight compounds or biotransformation conjugates, which may subsequently be de-conjugated and released as the parent compound (Reddersen et al., 2002). For example, Bendz and his co-workers (2005) suggest that the high concentrations of carbamazepine and gemfibrozil consistently detected in their study are due to the deconjugation of the glucuronide metabolites. Conclusions made regarding removal in the absence of information pertaining to the type and fate of degradation products may offer a false sense of security by underestimating the potential risk.

Quite aside from all the variables affecting the environmental residence of drugs, some compounds are intrinsically difficult to degrade. For example, the anti-cancer agents ifosfamide, cyclophosphamide, epirubicin hydrochloride, treosulfane, mitozantrone were not degraded in laboratory tests (Kummerer, 1997), nor were the contrast agents diatrizoate, iopamidol and iopromide (Ternes and Hirsch, 2002). Quinolones, nitroimidazoles and sulphonamides are also included in this category (Al Ahmad et al., 1999). In fact, (in similar environmental conditions) sulphonamides were degraded even less than pentachlorophenol – a persistent and toxic pesticide (Ingerslev and Halling-Sorrensen, 2000). For this reason, some authors believe that some pharmaceutical compounds may undergo negligible degradation in conventional sewage treatment systems (Kummerer, 2001; Jones et al., 2002). When various rates of adsorption, incomplete mineralization and degradation to more toxic by-products are considered, it becomes evident that biodegradation of pharmaceuticals to environmentally

benign compounds is the exception rather than the rule (White and Rasmusen, 1998; Stuer-Lauridsen et al., 2000).

Landfills and Solid Waste Disposal Facilities

Municipal facilities contain household solid waste as well as some commercial residues including sludge from local wastewater treatment plants. The two major sources of pharmaceuticals to the disposal site are the household and the treatment plant sludge disposal (Metzger, 2004). As discussed above, pharmaceuticals tend to accumulate and concentrate in the sludge compartment, so land filling the sludge may supersede household disposal as a main source of drugs to the landfill site. Although a direct comparison of measured concentrations of drugs in leachate is difficult (as the few available studies do not correct for rainfall dilution events), most authors report PhAC concentrations in the μg to the low mg per liter (Metzger, 2004). For example, Breidenich (2003) found clofibric acid, ibuprofen and carbamazepine in the leachate of five disposal facilities in Germany in concentrations of 10, 9.5 and 5.2 μg/L respectively.

Physical and Chemical Attributes of the Contaminant

It is convenient to refer to the group of pharmaceutically active contaminants as 'pharmaceuticals' and to regard them as a quasi-homogenous class of chemicals. This tendency is reminiscent of the use of 'dioxins' or 'polyaromatic hydrocarbons – PAHs' to refer to chemicals often differentiated only by the number, type or position of chemical substitutions (Kummerer, 2004a; Kummerer, 2004b). However, pharmaceuticals are grouped together not because of some chemical similarity, but because of their clinical use which depends on their ability to interact with biological systems. As such,

pharmaceuticals come in a wide variety of molecular weights, structures, polarities, activities and salt forms (Kümmerer, 2004a). The lack of homogeneity amongst pharmaceuticals may explain the confusion regarding some basic attributes of these compounds. They have been variously referred to as 'mostly lipophilic with a low biodegradability" (Christensen, 1998), "poorly soluble in water with a high partition and adsorption coefficient" (Velagaleti, 1997) or "usually lipophilic and often with a low biodegradability" (van der Ven et al., 2004). Others see most pharmaceuticals as "polar" (Miao et al., 2002; Koutsouba et al., 2003), as "polar, non-biodegradable" (Bendz et al., 2005), as "water-soluble and biodegradable" (Beausse, 2004) or as compounds with "low lipophilicity whose log K_{ow} values typically do not exceed 3.5" (Jjemba, 2005). In fact, pharmaceutical compounds can be hydrophilic or lipophilic, biodegradable or persistent, polar or non-polar. The applicability of these terms to drugs in general, depends more on the frame of reference of each product and on the point of view of the speaker, than on any measurable, intrinsic quality of pharmaceuticals as a class of chemicals. One approach to understand the fate of these compounds in the environment is to study them through various lenses: persistence, hydrophobicity, bioavailability, bioconcentration and biomagnification.

Environmental Persistence

The persistence of a chemical in the environment is determined by the substance's photo-stability, rate of biotransformation, binding and adsorption capabilities, and extent of transport within the aquatic or terrestrial spheres (Diaz-Cruz, 2003). Consequently, environmental persistence is closely connected to the compound's intrinsic properties, including its chemical stability and partitioning preferences. This connection has often

led to the observation that hydrophobic chemicals tend also to be highly persistent. One view was that since most pharmaceuticals are hydrophilic and biodegradable (Schwab et al., 2005), sustained environmental persistence of PhACs should not be of concern. However, new paradigms of environmental persistence are becoming accepted within the scientific community. These views integrate factors other than hydrophobic partitioning in the definition of persistence. Firstly, not all drugs are readily degradable: the environmental half-life of clofibric acid, the main metabolite of the blood lipid regulator clofibrate, is 21 years (Diaz-Cruz, 2003). Secondly, pharmaceuticals are continuously discharged into the environment, creating what some authors call 'pseudo-persistence', so that even hydrophilic and biodegradable drugs can act as persistent pollutants (Daughton, 2004; Bendz et al., 2005). Thirdly, as discussed above, the transformation products may be equally or even more hazardous to the environment. For the remainder of this thesis, I will use the term environmental persistence as I have defined it here inclusive of all of the above-mentioned factors.

Hydrophobicity and octanol-to-water coefficients

From the Greek *hydros* meaning water, and *phobos* meaning fear, the term hydrophobe is applied to a molecule that is repelled by water. The Oxford dictionary states that hydrophobic or lipophilic species "tend to be electrically neutral and non-polar, thus preferring other neutral and non-polar solvents or molecular environments". Hydrophobicity is measured in terms of octanol-to-water distribution or partition coefficients. The value of these parameters influences other important eco-toxicological attributes of contaminants including bioavailability, bioconcentration and biomagnification.

The octanol/water distribution coefficient (D_{ow}) is the ratio of the concentration of a compound in n-octanol (lipid surrogate) and water when the two are in equilibrium. As noted by Cunningham (2004), the D_{ow} measures a chemical's ability to partition into lipids, sorb to particulates and biomass and distribute among various environmental compartments – all critical factors in determining its environmental fate (van der Oost, 2003). Thus, chemicals with $D_{ow} < 1$ are unlikely to sorb to organic particulates and bio-concentrate, and those with $D_{ow} > 1$ are likely to show significant organic sorption and bio concentration (Cunningham, 2004). When the distribution coefficient is corrected for ionization so only the non-ionized portion of the chemical is considered, the term partition coefficient (K_{ow}) is used. The ionization-corrected log D_{ow} becomes the more familiar log K_{ow}, a value now starting to be represented as log P (Cunningham, 2004; Cleuvers, 2005). Considering that many pharmaceuticals occur as ionized species (e.g., the NSAIDs), log D_{ow} may be a better indication of the degree of solubility than log P, as it accounts for both ionized and neutral species (Cunningham, 2004). However, log K_{ow} or log P remains the value of choice for many environmental studies. Log P values often serve as a screening tool indicative of bioavailability and persistence. For example, pharmaceuticals with log P values of 1.72 or greater were associated with higher bioavailability in clinical settings (Kasim et al., 2004) owing to their enhanced ability to cross biological membranes. In fact, the critical value for significant environmental bioaccumulation potential is currently accepted at log P values equal to or greater than 3 (Sanderson et al., 2004a). The regulatory implications of this cut-off are further discussed in chapter 3.

Bioavailability

Environmental bioavailability is defined in terms of the fraction of contaminant in soil and water available for potential uptake in an organism's tissues during life-long exposure (Belfroid et al., 1996). It is expected that the contaminant concentration in fish be connected to that in water, as predicted by log P modelling. When deviations occur, they can be connected to variations in bioavailability, suggesting potentially site-specific differences in bioaccumulation patterns (Kristensen and Tyle, 1991; Van der Oost et al., 1996). Bioavailability is partially determined by the compound's sorption kinetics, mobility within various environmental compartments and degradability, which in turn depend on its physico-chemical properties including sorption coefficient (K_d), charge transfer, H-bonding and hydrophobic forces (Dorado and Almendros, 2001; Jjemba, 2005). Bioavailability is also influenced by particle size, amount of organic matter, residence time, and clay, moisture, oil and metal content in the immediate environment (Belfroid et al, 1996). Inasmuch as bioavailability influences exposure of non-target organisms to pharmaceutical compounds, it is an important variable in assessing risk. Generally, substances with low polarities and high log P values tend to sorb onto soil particles (Beausse, 2004). Steroid estrogens, with log P values between 2.5 and 5 are a good example: sediments appear to be reservoirs for natural and synthetic estrogens (Petrovic, 2001).

Bioconcentration

Bioconcentration is the uptake of contaminants from water via the gills or skin. It is often expressed in terms of the bioconcentration factor (BCF) which is the ratio between the concentration of a chemical in the organism and that in water at equilibrium

(Opperhizen, 1991). The concentration of pharmaceuticals in water is primarily driven by the molecule's partitioning preferences (log P), and controls the rate of uptake of the chemical from the water into the organism. Generally, the rate of uptake will be higher for more hydrophilic substances (Gobas et al., 1993) and lower for more hydrophobic substances. However, the rate of uptake of hydrophobic substances increases as the lipid content of the biological membranes increases (Spacie and Hamelink, 1982).

Some investigators have suggested that the potential for bioconcentration of pharmaceuticals in fish is low as ionic, water-soluble chemicals are metabolized and do not show appreciable accumulation (Schwab et al., 2005). These authors classified all PhACs as ionic, water-soluble chemicals. However, evidence to the contrary exists based on the fact that not all PhACs are water-soluble and the fact that high water solubility does not preclude bioaccumulation. Lai et al. (2002) reported significant bioconcentration of 17β-ethynylestradiol (EE2) in fish, later supported by corroborating results from Cargouet et al. (2003). The BCF for the anti-inflammatory drug diclofenac was calculated at between 12 and 2700 in various tissues of rainbow trout (Schwaiger et al., 2004). For the lipid regulator gemfibrozil, BCF values were 500 and 92 in goldfish plasma for low and high treatment concentrations, respectively (Mimeault, et al., 2005)

Biomagnification

Biomagnification, the uptake of contaminants via food exposure, is the ratio between contaminant intake and its rate of clearance from the organism (Sijm et al., 1992). It is often expressed in terms of a biomagnification factor (BMF) defined as

$$BMF = F_F(E_F/k_B)$$

where F_F is the amount of food transported through the intestines per gram of fish per day, E_F is the efficiency of uptake of the chemical from food and k_B is the depuration rate constant (van der Oost et al., 2003).

Biomagnification can be explained in terms of fugacity (Gobas et al., 1988). Fugacity denotes a chemical's tendency to escape from its current phase and represent the driving force for passive transport of chemicals between different phases that have different fugacity capacities (Clark et al, 1988; van der Oost, 2002). Field and laboratory experiments performed in the 1990s showed the critical relationship between the amount of digestion and absorption of food, and the BMF and dietary uptake efficiency: as food digestion increased and food volume decreased due to gastrointestinal absorption, the chemical fugacity increased by a factor of 5 – 8 (Gobas et al., 1993; Gobas et al., 1999). Biomagnification may play a major role in the transport of chemicals with slow release from sediments via uptake by benthic organisms and subsequent predation by larger animals (Farrington, 1991). Furthermore, the larger fish may feel the effects of biomagnification more than smaller ones as relative gill ventilation volumes decrease with size while feeding rates remain constant (Opperhuizen, 1991). When biomagnification becomes a major route of exposure, site-specific eco-system variations (community make-up, micro-geography, stream conditions, diet etc) also become important factors controlling bioaccumulation (Van der Oost et al, 1996a).

Physical and Chemical Attributes of the Receiving Environment

Effluent discharge is essentially a point source contaminant in the receiving stream. Therefore, the stream's physical and chemical attributes play an important role in determining the transport and fate contaminants (Hemond and Fechner, 1994). The

following is a synopsis of the stream properties most important for the fate of pharmaceutical compounds.

Stream size

An issue of paramount importance pertaining to stream size is its ability to dilute the effluent plume. In general, a large dilution factor is preferable as it will effectively reduce the overall concentration of contaminants in the stream (Daughton and Ternes, 1999). Large dilution factors are often achievable, but are by no means guaranteed even at the same wastewater treatment facility. Periods of drought lead to low dilution, as would discharges into small stream systems. For example, in the United Kingdom and many other highly-populated areas, effluent recharge may constitute 50% to 90% of total flow volume (Sumpter, 1998; Routledge et al.; 1998; Jones, 2002). In Canada, water volume to Wascana Creek, SK is 100% effluent during the winter months for 63 km from the plant (Donald and Sverko, 2005). The river's dilution capacity is an important variable in assessing total toxic potential of discharged effluents. A highly toxic effluent discharged in a large reservoir may be less harmful to the resident organisms than a mildly toxic effluent discharged in a very small creek (White and Rasmussen, 1998). Even though the effects of dilution on the potency of discharged effluent seem self-evident, experimental evidence to prove them does exist. For example, a 1996 study showed that negative effects on male fish caged downstream of effluent discharge points decreased with distance from the output source (Harries et al., 1996). Similarly, Vethaak et al. (2005) noted that fish in large waters (Rhine River) suffered less from the negative impacts of estrogen contamination than those in smaller waters (the Meuse River).

Particle size

Suspended particle size, depends to some degree on the stream-bed composition, micro-geography and stream velocity (Hemond and Fechner 1994). However, particle size (and type) is also an important determinant of the ultimate fate of contaminants. For example, studies show that hydrophobic contaminants tend to aggregate on particles with a high organic content (Belfroid et al., 1996; van der Oost, 2002), a predictable behaviour based on partitioning preferences and fugacity. Furthermore it has been suggested that "organisms preferentially ingest smaller particles" (van der Oost, 2002). For example, it has been shown that blue mussels (*Mytilus trossulus*) can increase its organic matter uptake by 20% by selectively feeding on smaller particles (MacDonald and Ward, 1994; Arifin and Bendell-Young, 1997). Based on surface area to volume ratios for the load of contaminants on the particles, preferential ingestion of the smaller particles leads to increased contaminant uptake. If the organism were able to digest the material or to otherwise alter the gastrointestinal fugacity relationships between the particle and the contaminant, the overall chemical body burden would be increased (van der Oost, 2002).

pH and temperature

pH has a profound influence on the stability, polarity and ionization state of many pharmaceuticals (Cunningham, 2004) and therefore on their subsequent solubility and bioavailability. For example, Scheytt et al. (2005) found divergent calculated and measured k_{oc} values (organic carbon partitioning coefficient, very similar to k_{ow}, the octanol/water partitioning coefficient) for diclofenac and ibuprofen. They concluded that since these compounds are negatively charged weak acids, partitioning behaviours could not be estimated based solely on the K_{ow} values. Such estimations must also take into

account the chemicals' ionic character (Scheytt et al., 2005). As discussed in an earlier section, this result supports the contention that D_{ow} values should be used instead of log P in modelling the environmental behaviour of pharmaceuticals (Cunningham, 2004).

As the solubility of a chemical changes so does its bioavailability and consequently its potential toxicity. This phenomenon was described by Cleuvers in 2004. He found that the acute toxicity of acetylsalicylic acid (ASA) to *Daphnia* was higher than expected based on ASA's log K_{ow} value. Cleuvers suggested that the increased toxicity was due to the pH-lowering effects of ASA and its derivative salicylic acid which increased the solubility and bioavailability of ASA, and consequently its toxic effects (Cleuvers, 2004).

Suspended Solids and Turbidity

The principal physical degradation process for pharmaceutical contaminants in the environment is photo-degradation. This process depends on the chemical characteristics, intensity of solar irradiation, and the presence of photo-sensitizers and/or light-blocking agents (Fent et al., 2005). In general, human drugs are sensitive to photolysis. Direct and indirect photodegradation has been shown to be the main removal process for diclofenac (Buser et al., 1998), sulfamethoxazole, ofloxacin and propranolol (Andreozzi et al., 2003), as well as for clofibric acid and carbamazepine (Andreozzi et al, 2003). Nitrates and humic acids may act as photo-sensitizers by generating hydroxyl radicals and singlet oxygen (Fent et al., 2005). In contrast, some pharmaceuticals, tetracycline, for example, can act as light-scattering agents, thereby decreasing photo-degradation (Brain et al., 2005).

The effect of seasonal variation on the environmental persistence of some drugs is exemplified by results obtained by Donald and Sverko (2005). They found that concentrations of some drugs including ibuprofen and naproxen (both highly dependent on photolysis for environmental elimination) varied by two orders of magnitude between summer and winter: mean concentrations of naproxen were 66 and 3988 ng/L in summer and winter, respectively. The study was performed in Regina, Saskatchewan, where during the winter months volatilization and photodegradation are reduced due to ice cover, and microbial degradation is slowed due to zero or sub-zero temperatures (Donald and Sverko, 2005).

Predicting the Environmental Fate of Pharmaceutical Contaminants

A large volume of work has been dedicated to the development of accurate predictive models of the environmental and subsequent biological effects of pharmaceutical residues. These efforts occur at the interface between biology, chemistry, statistics and chemo- and bio-informatics (Cronin, 2004). An earlier section on basic concepts of toxicity testing provided some detail on the biological aspects involved in predicting environmental behaviour of drugs. The preceding two sections focussed on the chemistry. The following section will examine some of the prediction and modelling aspects of determining drug behaviour in the environment.

The fate and transport of contaminants can be followed by instituting a mass balance relationship where the input and output rates are equated (Pollard and Davidson, 2001). Terms for storage (to account for lake phosphorus for example), advection and diffusion (e.g. accounting for riverine/free product flow and bottom sediments), dispersion (e.g. air quality modelling) and exposure, amongst a myriad of others can be

added to create more accurate predictions (Pollard and Davidson, 2001; see also Mackay, 1992 and Schoor, 1996 for details on modelling). For pharmaceuticals in the environment, these models can be roughly divided into fugacity modeling and quantitative structure activity relationship (QSAR) analysis.

Fugacity-based models

Fugacity-based multimedia fate models are often used as screening tools to predict the fate of chemical contaminants within various environmental compartments. They aim to establish a general environmental behaviour profile for the modelled substance. They can be relatively simple (Level I fugacity models where fugacities in all compartments are equal and constant) or more complex (Level II where reactions and advection are considered, and Level III where chemical equilibrium is not assumed and inter-compartment fugacities are not equal). For example, Khan and Ongerth modelled the behaviour of 50 pharmaceutical compounds in various compartments of Australian wastewater treatment plants (2004). Their (Level III) model accounted for national dispensing and ingestion rates, excretion and metabolic transformation rates, deconjugation and photo- and bio-transformation within the treatment plant, and loss to sludge and air. Figure 8 depicts their model.

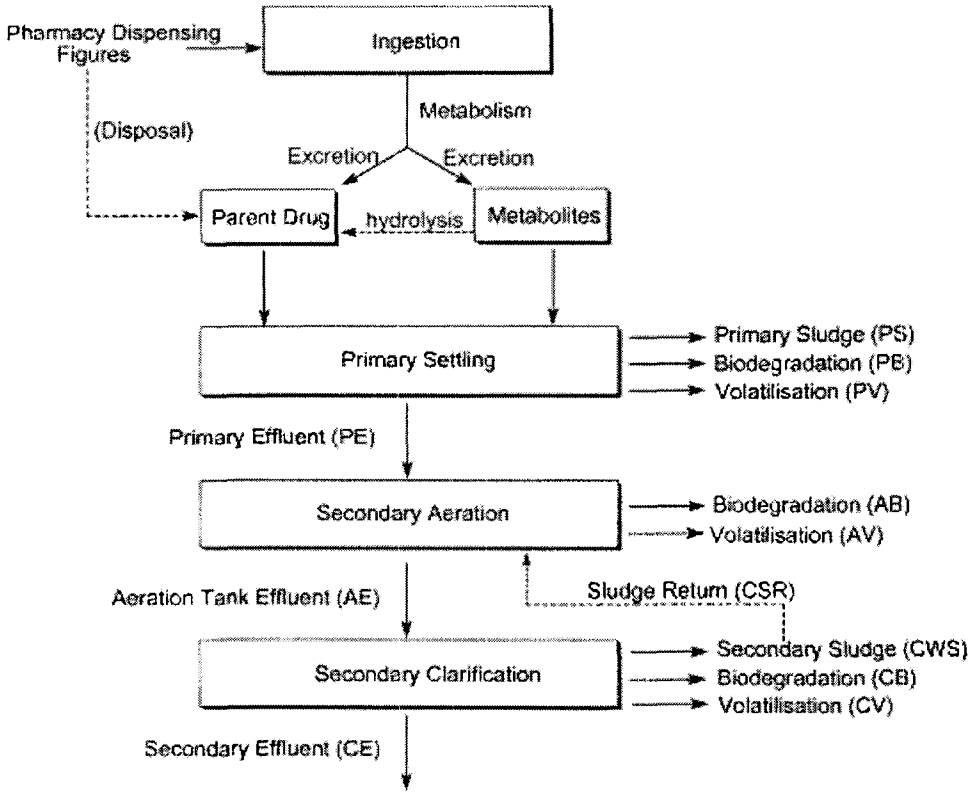


Figure 8: Level III fugacity model for pharmaceuticals in Australian STPs

Reprinted from Chemosphere 54:355-36, Khan and Ongerth, Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations, with permission from Elsevier.

The Level III fugacity-based model employs a non-equilibrium steady-state multimedia mass balance approach to determine ultimate contaminant concentrations in each of its four compartments – air, water, soil and sediments. It assumes equilibrium within but not between the four compartments. Level III modelling includes parameters for advective and diffusive transport and some degradation processes. It requires data describing environmental characteristics, emission rates, degradation rates and physico-chemical properties of the contaminant. The model computes concentrations in each of the four compartments as well as overall environmental persistence and associated transport, advective flow and degradation rates (Mackay and Paterson, 1991; Zukowska et al., 2005). A possible source of concern with fugacity-based modelling is its apparent

reliance on single-parameter linear free energy relationships (SP-LFERs – an analysis tool) to predict phase (air, water, land, sediments) partitioning. It has been noted in both toxicology and chemistry, that no single parameter can accurately reflect the complex molecular interactions that determine phase distribution of chemicals (Goss and Schwarzenbach, 2001). (Recall the discussion on integrated modelling and data analysis from chapter 1). Consequently, the original level III model has been modified to include a poly-parameter analysis, thereby increasing its predictive powers and decreasing its reliance purely on hydrophobic interactions of neutral molecules (Breivik and Wania, 2003). The poly-parameter linear free energy relationships (PP-LFER) analysis is characterized by five equations depicting chemical and phase partitioning parameters (see Zukowska et al., 2005 for further details), thereby increasing the model's predictive powers (Zukowska et al., 2005).

Such a modified Level III analysis was recently applied to polar pharmaceuticals by Zukowska et al. (2005). This group found that many pharmaceuticals were not significantly transported amongst the various compartments but remained in the media into which they were emitted. For example, the results for testosterone and progesterone depicted in Figure 9 indicate that these substances remain predominantly in the aqueous compartment.

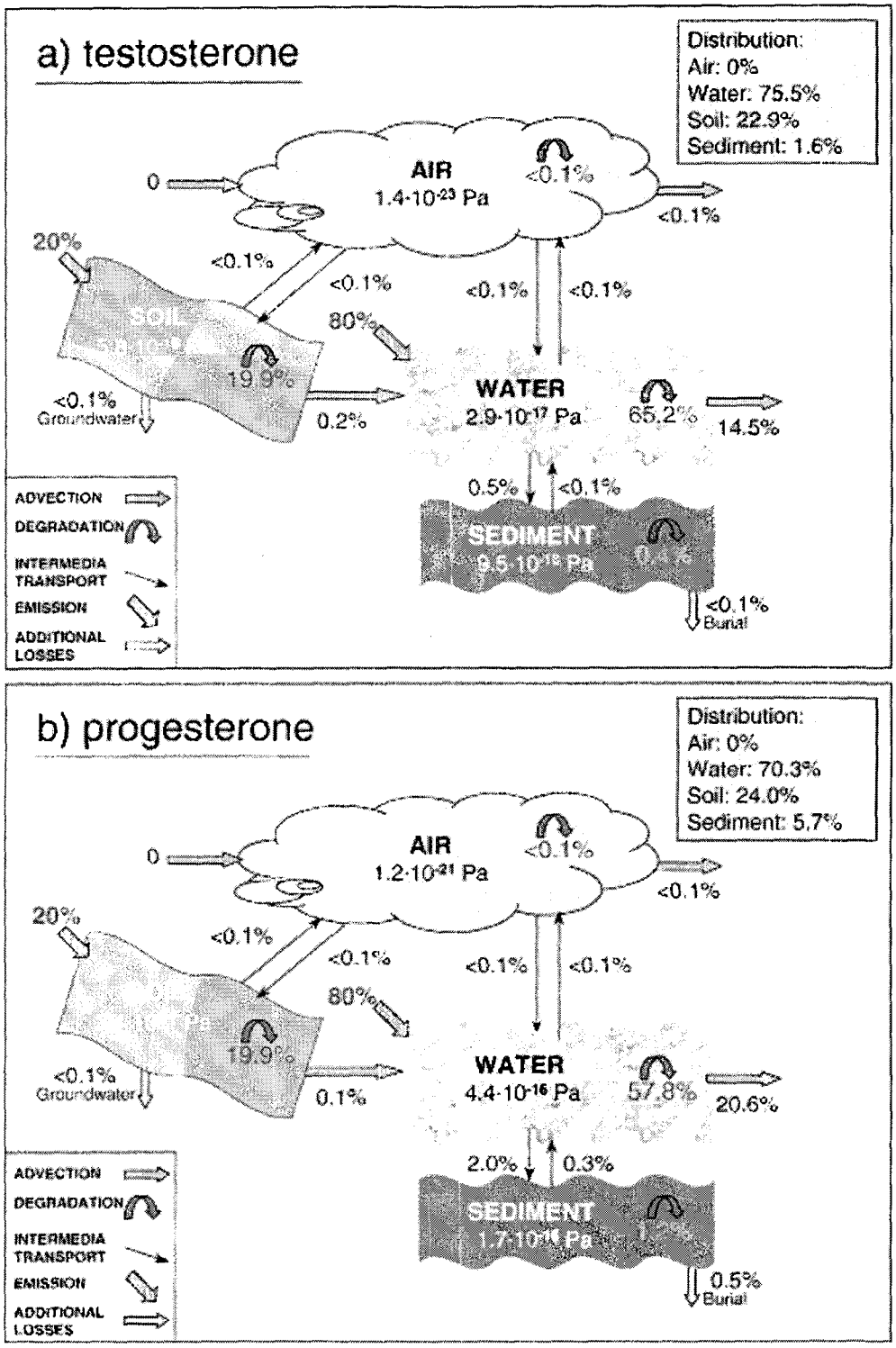


Figure 9: Output of Level III fugacity modelling.

Reprinted from Science of the Total Environment 359:177-187, Zukowska and Breivik, Evaluating the environmental fate of pharmaceuticals using a level III model based on poly-parameter linear free energy relationships, with permission from Elsevier.

Quantitative structure-activity relationships – QSARs

Quantitative structure-activity relationship (QSAR) modelling is a computer-aided toxicological prediction method based on the concept that a compound's biological activity can be inferred from the molecular structure and/or properties of similar compounds with known biological activity (Cronin, 2004; Schultz, 2003). A number of textbooks and review articles have been published on the subject of QSARs in general (Ford et al., 1996; Holtje and Sippl, 2001) and environmental QSARs in particular (Turner et al., 1987; Hermens and Verhaar, 1996). The purpose of QSARs is to relate molecular structures to consequent biological activities, existing on the so-called structure-property-toxicity axis – as introduced in chapter 1. This concept was expressed as early as 1869 by Crum-Brown and Fraser who stated that a change in the chemical 'constitution' of a molecule will be reflected in a change in its biological activity (Schultz et al., 2003). QSARs operate on the assumptions that (1) the structure of a molecule contains all elements responsible for its physical, chemical and biological properties (2) the chemical can be represented by numerical descriptors (i.e. physical, chemical and/or structural properties) (3) structurally similar compounds share similar mechanistic elements (i.e. rate-determining steps and free energy requirements for activity) and (4) differences in reaction rates will result in measurable differences in activity and/or potency (Schultz et al., 2003).

The three major elements of QSARs are the toxicity data, the descriptor data and the statistical method linking the two sets of information (i.e., regression analysis for quantitative results or pattern recognition for qualitative results) (Schultz and Netzeva, 2004). As is the case with other modelling techniques, QSARs are severely limited by

the nature and quality of data used in their development. Ideally, a good model should include data on a large number and variety of structurally-related molecules. Information on polar, ionisable compounds with large molecular structures should also be included. The model should use a wide range of toxic potency information (i.e. activity over a large range of doses, perhaps even at sub NOAEL levels – i.e. biphasic toxicity) and it should allow for a mechanistic interpretation of the results (Schultz et al., 2003).

Mechanistic interpretation reflects the assumption that similar physical and chemical properties result in a similar mechanism of biological action. Consistent with this approach, toxicity can be classified as non-covalent narcosis, covalent narcosis via interactions with biological molecules, or receptor-mediated toxicity, as discussed in chapter 1 (Schultz et al., 2003). Current parameters used in the determination of structure-activity relationships are derived based on the 2-dimentional structure of molecules. However, toxic effects occur in a 3-dimentional environment, where the mechanisms of actions are heavily dependent on the correct 3-D molecular structure. For this reason, properties-based models (QPARs) may be used in conjunction with structure-based evaluations, as the former encompass more information relevant to interaction with target molecules, e.g. electron distribution, ionization constants, binding properties and energy values (Schultz et al., 2003).

Environmental QSARs are also based on the relationship between chemical structures, and type/potency of toxic action. Their specific approach involves determining (1) the limits of chemical variation consistent with a particular effect and (2) the manner in which the structure-potency relationship occurs (Schultz et al., 2003). Structure/property-activity relationships (QSARs or QPARs) have been applied to a

variety of situations ranging from the general (substance persistence, soil sorption, bioaccumulation) to the specific (benzene response or estrogen receptor binding) (Cronin and Livingstone, 2004). For example, using environmental QSAR, Sanderson et al. (2003 and 2004) screened the toxicity of some pharmaceuticals to various aquatic animals. They found that the overall susceptibility to environmental pharmaceuticals was daphnids > fish > algae (2004). However, due to the complexity of pharmaceutical chemicals and the complexity of their interaction with environmental and biological systems, current QSARs or QPARs, even when used in conjunction with expert systems can only serve screening or prioritizing purposes (Kümmerer, 2004b). As outlined in chapter 1, these models can also be used in assessing toxicity, thereby decreasing reliance on *in-vivo* testing.

Environmental Concentrations and Effects of Selected Pharmaceuticals

Antibiotics

Main concerns: antibiotic resistance and sewage treatment plant operation

Despite the fact that many antibiotics are natural products or derivatives of natural products, they are considered environmental contaminants for two reasons: first, for their potential to elicit toxic effects in aquatic and terrestrial organisms, and second, for their ability to increase selection pressure for antibiotic-resistant strains of bacteria (Daughton and Ternes, 1999). The European Union and Switzerland have estimated the 1997 production of antibiotics for human and animal therapeutic use to be 5.46 million kilograms and 5.04 million kilograms for animal growth promotion. It is further estimated that 30-90% of administered dose enters the aquatic environment unaltered by

physiological processes within the target organism (Rang and Dale, 1991; Halling-Sorenson, 1998).

The environmental distribution of antibiotics varies widely as it depends on frequency and type of use. Areas in close proximity to fish or livestock farming operations, hospitals effluent discharge points or agricultural terrain enriched by manure fertilizer show the greatest accumulation of antibiotic residues. For example, macrolides, sulphamides and quinolones were detected in sewage effluent in Germany in concentrations up to the low µg/L (Hirsch et al., 1999 and Andreozzi et al, 2003). Swiss primary and tertiary effluent wastewater contained 249 and 45 ng/L quinolones respectively (Golet et al., 2001), while concentrations of lincomycin, erythromycin and clarithromycin in the range of 10 – 100 ng/L were detected in Italian rivers (Castiglioni et al., 2004; Calamari et al., 2003). Similar results were reported for Canadian domestic/municipal effluent and surface waters. For example, maximum effluent concentrations of clarithromycin, sulfamethoxazole and tetracycline were detected in the range of 536 to 977 ng/L (median 80 to 240 ng/L), and surface water concentrations of the same drugs ranged from 16 to 99 ng/L (Miao et al., 2004; Metcalfe et al., 2004).

Predictably, the main toxic effects occur against bacteria, fungi and algae, with effects on higher trophic levels occurring at concentrations 2 to 3 orders of magnitude higher (Wollenberger, et al., 2000). In general, acute effects on higher trophic levels are rare. Chronic or reproductive effects have been detected against some non-target bacteria in surface waters. Thomulka et al., (1993) determined that toxic effects of novobiocin, tetracycline, chloramphenicol, ampicillin and streptomycin on the reproduction of *Vibrio harveyi* could be detected at environmentally relevant concentrations. This is not

surprising as one would expect an antibacterial compound to be toxic to bacteria. The intent of the research was to show that non-target organisms can be affected by pharmaceutical substances. Further, the ecological significance of these effects is their potential for altering the natural selection process in favour of antibiotic-resistant organisms. Subsequent effects on higher trophic levels could theoretically occur as a result of an altered microbial ecosystem. However, the effects of antibiotics on local ecological systems via changes in microbial selection pressures have not been investigated.

Consequently, the overall relevance of the few studies showing some level of toxicity in some non-target bacteria has not been explicitly investigated (Carlsson et al., 2005). Natural selection pressures on microorganisms and potential local ecosystem changes notwithstanding, how do we value some species of soil bacteria and fungi against the benefits of antibiotic use for human and economic health? Currently, there is no study investigating environmental antibiotic levels to changes in local ecology via increased selection pressures on microbial communities. However, some studies do show that increased selection pressures on non-target bacteria as a result of ambient antibiotic levels do have an effect in sewage treatment plants. For example, studies on model sewage treatment systems show that alteration in microbial populations occurred in response to the addition of antibiotics in concentrations similar to those expected in hospital effluents (Kummerer et al., 2000; Al-Ahmad et al., 1999). Within this new frame of reference, environmental antibiotic contaminants can be evaluated against the benefits to human and economic health.

The presence of antibiotics in effluent may act as a selection pressure to incrase the growth and spread of antibiotic-resistant strains. Antibiotic-resistant microorganisms have been detected in a variety of media, ranging from surface water to soil to sediments. For example, resistance to kanamycin and neomycin was reported in bacterial species in some South Carolina streams (Leff et al., 1993). Resistance to erythromycin, tobramycin, chloramphenicol and tetracycline was detected near slaughterhouse effluent (Attrassi et al, 1993). Resistance to antibiotics used in fish farms was commonly reported (Samuelsen et al., 1992; Sandaa et al., 1992; Nygaard et al., 1992). These findings are consistent with reports of high environmental persistence (virtual, as defined by continued introduction into the environment (Daughton, 2004) or traditional, via high octanol:water partitioning coefficients) for many antibiotics. For example, oxytetracycline, an antibiotic used in fish farms, has been found in concentrations of 0.1 to 4.9 mg/kg dry sediment (Jacobsen and Berglind, 1988), with an estimated half-life of 300 days (Hektoen et al, 1995). Although bacterial resistance to anti-microbial agents is a natural phenomenon (Kümmerer, 2004), it remains an issue of concern for both environmental protection and public health officials.

Lipid regulators, psychoactive and anti-inflammatory drugs

Lipid regulators

Main concerns: environmental resilience and potential bio-concentration

The most widely reported pharmaceutical contaminant of surface and ground water is clofibric acid (Dautghton and Ternes, 1999). Clofibric acid is a major metabolite of etofibrate, etofyllinclofibrate and clofibrate – a class of blood lipid-regulating drugs that are used extensively (Stumpf et al., 1999). Although the exact mechanism of action

has not been identified, these drugs lower serum lipid levels by reducing the triglyceride-rich very low-density lipoprotein fraction (Gilman et al., 1990). In 1992, 30 tons (combined volume of all three drugs) were dispensed in Germany (Stan and Heberer, 1997). These high rates of use are associated with high rates of environmental discharge. For example, a Kansas City, Missouri STP measured the annual clofibric acid loading at 2.1 kg (Hignite and Azarnoff, 1977). Environmental concentrations range over 4 orders of magnitude, from 0.002 – 0.550 μg/L in the North Sea and some Swiss lakes (Buser et al., 1998), to 0.01 μg/L in areas of the Mississippi River and Lake Pontchartrain (Dunne, 2000), to 0.270 μg/L in some German tap water (Heberer, 2002a; Heberer, 2002b), and to 1.6 μg/L in many German STP effluents (Boyd et al., 2003). Raw and finished potable water from an Ontario river had a median clofibric acid concentration of 0.5 and 0.4 μg/L, respectively (Servos et al., 2005).

No acute effects of clofibric acid at environmentally-relevant concentrations have been identified in studies on algae, crustaceans and fish (Emblidge and DeLorenzo, 2005 and Nunes et al, 2004). However, chronic and mixture effects cannot be ruled. Clofibric acid remains a contaminant of interest because it has shown strong environmental persistence with an environmental half life of $t_{1/2} = 21$ years (Buser et al.,1998; Daughton and Ternes, 1999).

Gemfibrozil is another widely-used fibrate lipid regulator prescribed to control plasma triglycerides and cholesterol. Its environmental concentrations are similar to those of clofibric acid, with the exception that it does not reach detectable concentrations in finished drinking water (Servos et al., 2004a). Mimeault et al. (2005) showed that 1) gemfibrozil can act as an endocrine disruptor (decreasing testosterone levels by 50%

following 14-day exposure to 1.5 μg/L gemfibrozil) in the goldfish *Carassius auratus,* and 2) gemfibrozil bio-concentrates in the plasma, with a BCF of 500. These results add further impetus for continued monitoring and research gemfibrozil, clofibric acid and fibrates in general.

Psycho-active drugs

Main concerns: Environmental persistence, low intrinsic degradability and potential endocrine synergism.

These are a group of drugs used as anticonvulsants (e.g. carbamazepine) or as mood regulators (e.g. fluoxentine (Prozac) and paroxetine (Paxil)). These drugs are used in relatively high volumes and are environmentally recalcitrant.

Carbamazepine (CBZ) is a cyclic compound metabolized primarily to the active CBZ-10,11 epoxide and various glucuronides (Ferrari, 2003; Jos et al., 2003). Carbamazepine has been measured in STP effluent at maximum concentrations of 6.3 μg/L, in surface waters at 1.1μg/L and in potable water at 0.03 μg/L (Terenes, 1998; Heberer 2002a). Only 3% of the administered dose is excreted as parent compound; nevertheless, it is consistently reported as one of the contaminant pharmaceuticals in aquatic environments (Ternes, 1998; Andreozzi et al., 2002). Some researchers suggest that its environmental persistence is due to the high rates of use, low rates of removal during sewage treatment (≈ 7%), and de-conjugation either in the treatment plant or in the environment (Terenes, 1998; Heberer, 2002a and b; Jones et al., 2002). Corroborating the high rates discharge into surface waters, a 2005 study estimated that 2.0 kg CBZ are discharged into surface water every week by Berlin-Ruhleben area sewage treatment

plants (the plants service approximately 1,000,000 residents and 12,060 hospital beds); a value which translates to annual discharges of 105 kg (Heberer and Feldmann, 2005).

At low concentrations, carbamazepine has not been shown to cause any acute effects in *Daphnia magna, Chlorella vulgaris var viridis* (algae), *Allium cepa* (bulbs of onion), *Vibrio fischeri* (bacterium), Vero monkey kidney cells (mammalian cells) and RTG-2 cells derived from *Oncohrhynchus mykiss* (rainbow trout gonad cells) (Jos et al., 2003). It has a 7-day NOEC on the crustacean *Ceriodaphnia dubia* of 25μg/L, a value that approaches those seen in some environmental samples. A risk assessment for CBZ did indicate a hazard quotient >1 which suggests that there is a possibility of aquatic harm if STP effluent is discharged without adequate dilution (Ferrari et al., 2003).

Fluoxetine and paroxetine belong to a class of pharmaceuticals known as selective serotonin re-uptake inhibitors (SSRI). They are used extensively in the treatment of affective disorders such as depression, compulsive behaviour and eating/personality disorders (Brooks et al., 2003a). They are usually metabolized by the cytochrome P450 system to the active norfluoxentine, and less than 10% is excreted as unchanged compound (Heimke and Hartter, 2000). Fluoxentine has been measured at 0.32 and 0.54 μg/L in effluent (Weston et al., 2001) and 0.012 μg/L in surface waters (Kolpin et al, 2002).

SSRIs in general, and fluoxetine in particular, have been identified as the most potent inducers of spawning in male bivalves, with effective concentrations as low as 0.318 μg/L (Fong, 1998). Other studies found increased developmental abnormalities and altered estradiol levels in female Japanese Medaka fish following chronic, 4-week exposure to 0.1 and 0.5 μg/L fluoxentine (Weston et al., 2003; Brooks et al, 2003a).

Thus, SSRIs remain a class of pharmaceuticals of interest because of their significant reproductive effects at environmentally relevant concentrations.

Non-Steroidal Anti-Inflammatory and Analgesic Drugs

Main concerns: High volumes of use.

Non-steroidal anti-inflammatory (NSAI) and analgesic drugs are used primarily as painkillers. These include acetaminophen (paracetamol), acetylsalicylic acid (Aspirin), diclofenac, ibuprofen (Advil) and naproxen.

Naproxen is a water-soluble NSAI drug but it is still considered to be environmentally persistent due to its constant input (Isidori et al., 2005). The daily loading to municipal wastewater treatment plants has been estimated in the order of grams (Tixier et al., 2003). It has been detected in surface water in Canada and Switzerland in concentrations ranging from 0.02 to 2.6 µg/L (Tixier et al., 2003; Boyd et al., 2003). Naproxen is active principally against primary consumers with acute EC_{50}'s in standard bioassays for *Brachionus calyciflorus* (a rotifer) and *Ceriodaphnia dubia* (a crustacean) of 260 and 620 µg/L respectively (Isidori et al., 2005). Furthermore, naproxen is genotoxic to the bacterium *Escherichia coli* (primary DNA damage in the SOS chromotest), and *Salmonella typhimurium* (point mutations on the Ames test) (Isidori et al., 2005). Chronic exposure to low µg/L concentrations of the drug's photo-degradation products caused greater growth inhibition in cultures of *C. dubia* than the parent compound (Issidori et al., 2005).

Combined sales of two other NSAIDs, acetaminophen and acetylsalicylic acid (ASA) in Europe are estimated at greater than 500 tonnes per year. These translate to

median surface water concentrations of 0.22 μg/L ASA and maximum effluent concentrations of 6.0 μg/L acetaminophen (Ternes et al., 1998; Jones et al., 2002). Rapid photo- and bio-degradation for both products is thought to explain the low concentrations in the environment (compared to expected amounts based on production volumes). This fact is corroborated by Ternes (1998) who detected concentrations of ASA metabolites up to 54 μg/L in effluent.

The NSAID diclofenac is regarded as the most important pharmaceutical contaminant of water (Heberer, 2002b). Based on German usage rates of 75 tonnes per year and log P values near 3.0, diclofenac is considered potentially bio-accumulative (Carlsson et al., 2005). It has been reported in average concentrations of 3.02 and 2.51 μg/L in German influent and effluent respectively (Heberer, 2002b). At concentrations of 1μg/L, diclofenac has a bioconcentration factor in liver of 2732 (Schwaiger, et al., 2004). BCF is concentration-dependent, decreasing as the ambient concentration increases, indicative of almost complete tissue saturation at higher concentrations (Schwaiger et al., 2004).

Recent evidence indicates that diclofenac has serious environmental implications. Several species of vultures in India have been killed following ingestion of livestock remains that contained significant levels of diclofenac. In this case, exposure by-passed the aquatic environment, but non-target species were impacted due to environmental (terrestrial) contamination. The route of exposure notwithstanding, diclofenac may be the first pharmaceutical to cause apparently acute ecological damage following normal usage (used as directed, not because of accidental spills). In 2004 Oaks et al. reported on the high death rate among three species of vultures in India and Pakistan. Further

investigations found that mortality was associated with renal failure and visceral gout, and that these correlated positively with plasma diclofenac residues (Oaks et al., 2004). Diclofenac had recently become an agricultural pharmaceutical in these countries. As scavenging birds whose primary subsistence is dead domestic livestock, the vultures fed on diclofenac-treated animals, thus accumulating dangerous levels of the drug in their systems (over the period of 36 - 58 hours). The authors showed that environmental concentrations of diclofenac were sufficient to induce the observed effects. Diclofenac residues were officially implicated as the cause of population decline in the three species of vultures in those countries (Oaks et al., 2004).

No other acute effects at environmental concentrations have been reported for diclofenac. However, chronic, 28-day exposure to 1 μg/L diclofenac caused cytological alterations (lesions) in the liver, kidneys and gills of rainbow trout (Triebskorn et al., 2004). With the exception of the direct toxicity to vultures, the ecological impact of environmental NSAID contamination remains unknown (Carlsson, 2005).

Endocrine disruptors

Main concerns: endocrine modulation at environmentally-relevant concentrations, and persistence in sewage-sludge and aquatic environment.

Endocrine disruptors in general, and pharmaceutical endocrine disruptors in particular, have been studied extensively due to their effects on the endocrine and reproductive systems. Comprehensive reviews on environmental endocrine disruption have been published elsewhere (see Mills and Chichester, 2005). The goal of this section is to identify the main concerns posed by endocrine disruption in the context of

pharmaceutical contaminants (often, but not exclusively in wastewater). Endocrine disruption by natural estrogens has been most well-characterized, but it can be effectively induced by both non-estrogen endocrine disrupting chemicals and xeno-estrogens. It should be noted that although pharmaceutical estrogens and environmental xeno-estrogens are comprised of very different types and classes of chemicals, their activity on biological systems is interrelated: for example, xenoestrogens at environmentally-relevant concentrations can modulate the activity of endogenous estrogens (Rajapakse et al., 2002).

An endocrine disruptor has been defined by the European Commission (1996) as "an exogenous substance or a mixture, that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny or (sub)populations". This group of chemicals includes various types of pesticides and their degradation products such as DDT and DDE, surfactants and plasticizers such as alkylphenol ethoxylates, and phytoestrogens such as genistein (Willingham and Crews, 1999; Miles-Richardson et al., 1999; Bennetau-Pelissero et al., 2001). With the exception of the naturally-occurring phytoestrogens, these compounds are sometimes referred to as xeno-estrogens and are frequently detected in sewage effluents and in some receiving environments (Sharpe et al., 1995). Pharmaceutical endocrine disruptors include the natural and synthetic estrogens estrone (E1), estradiol (E2) and 17α-ethinylestradiol (EE2). These and a number of other steroidal estrogens are used in considerable volumes as they form the active ingredients in contraceptive pills, hormone replacement treatments, some cancer treatments (tamoxifen and diethylstilbestrol) and some agricultural growth-promoters (Arcand-Hoy, 1998; Bell, 2004). The total volume

of estrogens used annually in the United States in the early 1990s was estimated at 2352 kg (Arcand-Hoy, 1998).

E1, E2 and EE2 are all steroidal estrogens with log P values ranging from 3.0 to 5.0 (Petrovic, 2001). They are excreted in urine as water-soluble conjugates, but de-conjugation to the original parent compound has been observed (Snyder, 1999; Arcand-Hoy, 1998). It is currently assumed that de-conjugation (via β-glucuronidase and arylsulfatase) occurs not only in the receiving environment, but also in the STP (Ternes et al., 1999; Nasu, 2000; Andersen, 2003). While wastewater treatment plants have shown removal rates from effluent of between 70 and 98%, the fate of the degradation products or the estrogenic content of resulting sewage sludge has not been simultaneously reported in those studies (Johnson et al, 2001; Andersen et al, 2003). Based on their log P values, sorption to organic particles and sediments would be a likely process (Kuster et al., 2004). In fact, leachate from solid waste disposal facilities has been linked to altered endocrine responses in fish inhabiting otherwise pristine environments (Noacksson, 2001; see section on leachate toxicity). Moreover, pharmaceutical estrogens are regarded as the major source of estrogenicity even in aquatic matrices such as sewage treatment plant effluents (Desbrow et al., 1998; Snyder, 2003; Aerni et al., 2004).

Environmental concentrations of estrogens are in the low ng/L level with concentrations in various matrices averaging 0.5 to 7 ng/L (Desbrow et al., 1998; Larsson et al., 1999; Ternes et al., 1999). Several studies report a potency gradient of endocrine disruption where xenoestrogens > natural estrogens > and synthetic estrogens. For example, the potency of EE2 (a synthetic estrogen) was 10 to 50 times higher than E2 and E1 (both natural estrogens) in *in-vivo* studies (Segner et al., 2003 and Thorpe et al.,

2003). Similarly, endocrine disruption (as measured by estrogen receptor-mediated gene transcription responses) occurred only at xenoestrogen levels 1000 to 100,000 times higher than the natural estradiol (E2) (Massaad and Barouki, 1999; Witorsch 2002).

Table 2 is a summary of some of the effects reported for very low concentrations of natural, synthetic and xeno-estrogens. Note that chronic, reproductive and sub-lethal effects such as increased vitellogenin production occur at concentrations well within the range reported in various environmental compartments. Vitellogenin (VTG) is an estrogen-dependent egg protein precursor, normally synthesised in the liver of female oviparous vertebrates. VTG levels increase sharply during oocyte development (Wallace, 1985; Specker and Sullivan, 1994). When detected in unusually high concentrations in male fish, VTG is considered to be a sensitive biomarker of estrogenic contamination (Folmar et al., 1996; Sumpter and Jobling, 1995). For example, VTG induction occurred at EE2 concentrations of only 0.1 ng/L (Purdom et al., 1994), normal sexual development and differentiation in fish was affected by exposures to only 0.1 to 0.15 ng/L EE2, and fertilization success and embryo viability of adults exposed to 1 – 10 ng/L EE2 was reduced (Hill and Janz, 2003). Furthermore, it has been shown that exposure to ng/L concentrations of the vastly less potent xenoestrogens can modulate the effects of the endogenous estrogens (Rajapakse et al., 2002; Wozniak et al., 2005).

Table 2 Sub-chronic effects of selected natural, synthetic and xeno-estrogens

Chemical Name or Class	Type of study Exposure duration	Species and Endpoint measured	Conc. (range) ng/L	Ecological / Biological Significance	Reference
Xeno-estrogens					
Pesticides – dieldrin Surfactants nonylphenol (also a pharmaceutical drug) Phytoestrogens - coumestrol Synthetic estrogen – DES	1) *In-vitro* 2) Comparison between the effects of E2 and several xenoestrogens	Mammalian cells Cellular Ca^{2+} traffic – eliciting, enhancing or inhibiting estrogenic signalling	pM and μM (ng - μg/L)	Established endogenous estrogen modulating ability of environmentally relevant xenoestrogen concentrations via non-genomic effects	Wozniak et al., 2005
Mixtures of upto 8 Xenoestrogens PCB, Parabenes, bisphenol A and genistein	1) *In-vitro* 2) YES (Yeast estrogen screen)		50% individual EC_{01}	Corroborate limitations of single-chemical assessments Established molecular-level effects Link to higher-level effects remains un-investigated	Silva et al., 2002
Natural and Synthetic Estrogens					
EE2	Chronic, life-long, multi-generational	*Danio rerio* (zebrafish) VTG induction Reproductive success Behaviour	Environmentally relevant (5 ng/L)	Suggests possible population-level effects Value of integrative assessment approach	Nash et al., 2005
EE2	Chronic, life-long	*Gasterosteus aculeatus* (threespined stickleback) growth rate behaviour mortality	100 ng/L (may reflect total estrogenic load)	Suggested deferred costs of exposure Sex and population specific effects Non-reproductive effects Supports integrative assessment approach	Bell, 2004

Chemical Name or Class	Type of study Exposure duration	Species and Endpoint measured	Conc. (range) ng/L	Ecological / Biological Significance	Reference
17β-estradiol (E2)	3-week, various life-stages	*Danio rerio* (zebrafish) VTG induction Gonadal index and histology Secondary sexual characteristics	5, 25 and 100 ng/L	VTG induction at all stages Altered sex ratio; disruption of sexual differentiation Partial feminization Timing of exposure is critical to the type of effects Early life-stages very sensitive	Brion et al., 2004
Municipal/Domestic Effluent					
Tertiary effluent	112 days during gametogenesis	*Dreissena polymorpha* (freshwater zebra mussel) ↑ vitellin-like proteins and cholesterol gonad histological changes	100% tertiary effluent	Effects are not necessarily responses to single estrogens, but rather reactions to a combination of estrogenic contaminants. Possible population level effects	Quinn et al., 2004
Field study – stream receiving secondary effluent	Fish collected in September	*Lepomis megalotis* (longear sunfish) Fish enumeration Plasma testosterone and VTG		↑ plasma VTG altered community assemblage (top predators, tolerant vs. non-tolerant species) Impairment at multiple levels of biological organization	Porter and Janz, 2003

The ecological relevance of some indicators of endocrine activity is still under investigation. For example, it is not clear whether vitellogenin induction is an indicator of exposure (van der Oost, 2003) or of effect (Daughton and Ternes, 1998). The physiological relevance of elevated levels of VTG has also been called into question, with some authors suggesting that increased levels may lead to liver, kidney and gill damage (Folmar et al., 1996). Others, believe that since VTG levels return to normal following depuration, and that no further consequences can be attributed to the initial VTG elevation. In order to address these questions, some studies attempted to establish a link between low-level estrogen exposure and community or population effects. For example, life-long exposure of zebrafish (*Danio rerio*) to 5 ng/L EE2 caused a 56% reduction in fecundity and complete population failure in the F_1 generation (Nash et al., 2004). Interestingly, in the same study, a 40-day exposure of mature adults to the same concentration had no measurable effects. This particular study corroborated not only a potential link between estrogenic exposure and population welfare, but also the importance of exposure timing (i.e., developing organisms are more susceptible to EE2 than adults).

A recent review of evidence by Mills and Chichester (2005) examined the currently available data on endocrine disruption in fish. They concluded that while laboratory studies indicate that potential harm exists, the environmental evidence is less convincing. This may reflect the (expected) lack of acute effects and the difficulty of identifying long-term, sub-lethal, chronic effects. The actual link between routine exposure to EDC and population effects remains an "open challenge" (Mills and Chichester, 2005). It should be noted, however, that many of the studies were conducted

according to the current paradigm of risk assessment (single-chemical, acute-toxicity, conservative burden of proof) which can mask the effects of mixtures via multi-generational and multi-route exposures. This would be particularly true given the known interactions between xenoestrogens and natural or pharmaceutical estrogens.

Environmental Endocrine Disruption in Humans?

Aside from effects on the ecological viability of aquatic populations, there are several studies that have investigated the human health risk posed by continued EDC contamination of the environment (OECD 1989; CSTEE, 1999; Mendes, 2002). The intense research efforts are fuelled by observations, allegations and/or hypotheses that have correlated various human diseases with an increased exposure to environmental EDCs. These include reduced human sperm quality (Sharp and Skakkebaek, 1993; Carlesn et al., 1992; Paulsen et al., 1996), testicular, prostate and breast cancer (Mendes, 2002; Coleman et al., 1993), cryptorchidism (un-descended testes) and hypospadia (urethral meatus displacement) (Jensen et al., 1995), endometriosis (Hill, 1992), sex-ratio and thyroid/neuro-endocrine alterations (Koopman-Esseboom et al., 1994; Mendes, 2002). Furthermore, Saradha and Mathur (2006) suggest that environmental contaminants induce the formation of reactive oxygen species facilitating the development of defective sperm function and male infertility. With the exception of diethylstilbestrol (DES), all of the chemicals investigated in the above studies were of industrial origin. A recent assessment of the published literature on the *in-utero* exposure to endocrine disruptors and subsequent male reproductive health concluded in that the currently available evidence was not sufficient to either support or reject the hypothesis that "environmental estrogens contribute to an increase in male reproductive disorders"

(Vidaeff and Sever, 2005). However, as Waring and Harris conclude (2005) the best answer to the question: "Are EDs a risk to humans?" is, "...EDs affect all phyla studied to date". Hence, there is room for more research. One direction may include integrating the human health and ecological aspects of this issue as discussed in chapter 3. For example, it has been shown that chronic exposure to very low concentrations of xeno-estrogens can modulate the activities of endogenous estrogens in wildlife. Can the same relationship exist in humans? Can chronic exposure to very low concentrations of estrogen (natural or synthetic) increase or decrease the activities of endogenous estrogens in humans? If so, with what effects?

Anti-neoplastic drugs

Main concerns: environmentally persistent, frequently genotoxic

These agents include cyclophosphamide, ifosamide and 5-fluoro-uracil. Compared to other pharmaceuticals, their production volumes are relatively low. For example, annual German prescription rates of ifosamide and cyclophosphamide were estimated at only 200-400 kg (Kummerer and Al-Ahmed, 2001). Based on usage rates, the predicted concentration of anti-neoplastics in hospital effluent is 5 -50 μg/l, in municipal effluent 5 ng/L, and in surface waters less than 1 ng/L (Kummerer and Al-Ahmed, 2001). Based on estimated volumes and predicted environmental concentrations, these pharmaceuticals are unlikely to cause significant environmental harm. However, they remain chemicals of concern for two reasons: first, they often have carcinogenic, mutagenic or embryotoxic properties (Skov et al., 1990; Kummerer, 2001), and second, they are recalcitrant to conventional sewage treatment methods (Steger-Hartmann et al., 1997). For example, ifosamide is neither degraded nor eliminated (to sludge) during the

treatment process (Kummerer et al., 1997). No further studies on the ecological effects of anti-neoplastic drugs have been conducted.

Cardiac drugs (beta-blockers)

β-blockers are a group of pharmaceuticals used in the treatment of cardiovascular disease and include propranolol, bisoprolol and metoprolol (Fent et al., 2005). These drugs are prescribed fairly extensively, with annual German rates of use varying between 100 and 250 tons (Schwabe and Paffrath, 2004). Surface water concentrations (in Germany), range from 0.59μg/L for propranolol to 2.9μg/L for bisoprolol, with similar concentrations being detected in STP effluents (Ternes, 1998). Chronic exposure to relatively low doses (125 and 250 μg/L) of propranolol has been linked to cardiovascular and reproductive effects in the crustacean *Ceriodaphnia dubia* (Huggett et al., 2002). In fish, the number of released eggs was reduced following 4-week exposure to 0.5 μg/L propranolol but not to 50 or 100μg/L, indicating perhaps a bimodal dose-response curve for this endpoint (Huggett et al., 2002). When applied in combination of 3 or more, some β-blockers show considerably increased toxicity with effects elicited at doses which would not induce measurable effects following individual exposure (Cleuvers, 2005; see section on CA)

Mixtures of various pharmaceuticals

Main concerns: effects measurable at individual environmental concentrations

Very few studies investigated the effects of mixtures of pharmaceutical compounds on aquatic organisms. Cleuvers conducted two studies on the effects of a mixture of β-blockers on *D. magna*, *D. subspicatus* and *L. minor* (Cleuvers, 2005) and

NSAI drugs on *D. magna* (Cleuvers, 2004). Both studies concluded that mixture effects were higher than the sum of single effects.

A study performed by Flaherty and Dodson on *Daphnia magna* (2005), found that a 6-day exposure to 36μg/L fluoxentine and 100 μg/L clofibric acid (no effect when individually administered) resulted in 62.5% mortality compared to 10% mortality in the experimental controls. When the clofibric acid concentration was reduced to 10 μg/L, they detected a 19% increase in developmental abnormalities, whereas none were observed in the control group.

Exposure to a mixture of antibiotics – 10 μg/L each erythromycin, triclosan and trimethoprim, resulted in decreased sex ratio in *D. magna* (Flaherty and Dodson, 2004). The link between antibiotic exposure and sex determination is thought to be inheritable, sex-regulating microbes of the genus Wolbachia, present in *Daphnia* (Weeks et al., 2002, Flaherty and Dodson, 2004). Flaherty and Dodson also concluded that the effects of mixtures are not always predictable from the effects of single compounds. Furthermore, their results indicate that the timing and duration of exposure can affect the severity and type of effects.

Effects of Effluent and Solid Waste Disposal run-off

With very few exceptions, these effects have been associated with the estrogenicity of the effluent/leachate manifesting as endocrine disruption. In the early 1980s, casual observation of hermaphrodism in male fish (roach) living in two settlement lagoons in the United Kingdom prompted further investigations on the safety of sewage effluent to the aquatic environment. In 1994, a landmark paper was published, which

stated that "estrogenic substances are present in the effluent of STWs. ...{*the data*} indisputably demonstrates the nationwide distribution of estrogens in the STW effluents..." (Purdom et al., 1994). Further field surveys of 8 British rivers, sampled above and below sewage treatment facilities corroborated these earlier results (Jobling et al., 1998): a large proportion of the fish sampled were intersex as defined by the simultaneous presence of both male and female gonadal characteristics. The incidence of hermaphrodism ranged from 4% at the control sites to 100% in two roach populations living downstream of large STPs (Stumpter, 1998). Correspondingly, high VTG levels were associated with hermaphrodism suggesting that perhaps both effects were caused by the same factor, i.e., STP effluent (Stumpter, 1998). Jobling and her colleagues showed that wild fish populations in many UK rivers are exposed to high levels of estrogenic chemicals. Subsequent research in England and the United States confirmed their findings (Harries et al., 1999; USEPA, 2002). Furthermore, field studies with trout (*Oncorhynchus mykiss*), wild cyprinid roach (*Putilus rutilus*) and flounder (*Platichthys flessus*) show that estrogenicity was only marginally affected by dilution and that deleterious reproductive consequences persisted downstream from STP discharge sites (Jobling et al., 1998; Auriol, et al., 2005). Effects similar to those detected in fish have been observed in the mollusc *Potamopyrgus antipodarum*, suggesting that this organism may be suitable as a bio-indicator of and model for monitoring environmental estrogenicity (Jobling et al., 2004).

There are continuing reports of endocrine disruption associated with chronic exposure to waste water treatment effluent. For example, chronic exposure (112 days) of the freshwater zebra mussel *Dreissena polymorpha* to tertiary treatment effluent during

gametogenesis resulted in 1) increases in vitellin-like proteins, 2) cholesterol (parent compound for sex hormones) doubling and 3) large increases in the intestinal tissue between the seminiferous tubules of gonad (Quinn et al., 2004). Similarly, another recent study established cytotoxic and oxidative properties of primary treated effluent to fish hepatocytes (Gagne et al., 2005).

Leachate toxicity

One of the most common methods for disposing of solid waste is to land-fill it in designated sites (McKerlie et al., 2005). Some of these sites are designed with protective barriers and leachate collection systems but in many cases these measures are not implemented (Noacksson et al, 2001). Consequently, materials leach directly into the soil and eventually make their way to the aquatic environments. Aside from receiving residential and commercial solid waste, these facilities also collect sewage sludge from local sewage treatment plants (Velagaleti, 1997).

Leachate toxicity has been minimally investigated (Noaksson et al., 2001). One exception is a series of studies conducted in Sweden starting in the late 1990s. Casual observations of excessive illness and open lesions in roach living in Lake Molnbyggen, Sweden prompted a number of investigations into possible causes. The lake is located in a pristine area, free of contaminating industrial or residential activities, but receives an average of 18000m^3 of domestic land-fill leachate annually (Noacksson et al., 2001). Studies published in subsequent years confirmed serious deleterious effects not only on the resident roach (*Rutilus rutilus*), but also on local perch (*Perca fluviatilis*), pike and burot (Noacksson et al., 2001; Noacksson et al., 2003a; Noacksson et al., 2004). The most dramatic effects were fin erosion and open sores, increased hepatic enzyme

activities, severely decreased female gonado-somatic index (GSI - by 80%; male GSI decrease by 36%), and high incidence of sexual immaturity in females (Noacksson, 2001; Noacksson, 2003a). Further investigation into the causes and mechanisms of the observed effects led to the following conclusions: 1) the effects could not be correlated with either the presence of traditional contaminants (PAH, PCBs, etc) in the effluent or to confounding factors such as lake temperature, pH or food availability, 2) there was a substantial energy cost and impaired hormonal control associated with chronic exposure to leachate in these fish, and 3) the majority of female perch were in severe reproductive failure, thereby impacting population levels (Noacksson et al., 2001; Noacksson etal., 2005).

Table 3 shows some of the effects reported for selected pharmaceutical compounds. With the exception of hormone endocrine disruptors (detailed in table 2), most of the pharmaceuticals listed induce acute effects at concentrations well in excess of environmental concentrations

Table 3: Effects of selected pharmaceutical compounds

Chemical Name	Species	Endpoint measured	Concentration (experimental) (µg/L)	Reference
Antibiotics				
Penicillin – Amoxycillin	*Microcystis aeruginosa* (cyanobacteria)	Growth EC_{50}	3.7	Holten Lotzhoft et al., 1999
	Oncorhynchus mykiss (rainbow trout)	Hepatocyte cytotoxicity 24 hr. EC_{50}	> 182700	Laville et al., 2004
	Lemna gibba (duckweed)	Wet weight, chlorophyll a/b 7d LOEC	> 1000	Brain et al., 2004
Bacitracin	*Daphnia magna* (water flea)	48 hr. LC_{50}	30500	Brambilla etal., 1994
Chlorotetracycline	*Selenastrum capricornutum* (cyanobacteria)	72 h EC_{50}	3100	Halling-Sorensen, 2000
	Microcystis aeruginosa (cyanobacteria)	7d EC_{50}	50	Halling-Sorensen, 2000
Ciprofloxacin	Activated sludge bacteria	EC_{50}	610	Halling-Sorensen, 2000
	Daphnia magna (water flea)	48h NOEC	60000	Halling-Sorensen, 2000
	Lemna gibba (duckweed)	Wet weight 7d EC_{50}	698	Brain et al., 2004
Erythromycin	*Salvelinus namaycush* (lake trout)	96h LC_{50}	410000	Marking et al., 1998
	Lemna gibba (duckweed)	Wet weight 7d LOEC	>1000	Brain et al., 2004
Metronidazole	*Daphnia magna* (water flea)	48h LOEC	>1000mg/L	Wollenberger et al., 2000
	Selenastrum capricornutum (cyanobacteria)	72h EC_{10}	19900	Lanzky and Halling-Sorensen, 1997
	Daphnia magna (water flea)	Reproduction 21d NOEC	250000	Wollenberger et al., 2000
Oxytetracycline	*Microcystis aeruginosa* (cyanobacteria)	Growth EC_{50}	207	Holten Lutzhoft et al., 1999
	Panaeus vannamei (white shrimp)	24h LC_{50}	160	US EPA (2001)
	Selenastrum capricornutum (cyanobacteria)	Growth EC_{50}	4500	Holten Luzhoft et al., 1999
	Daphnia magna (water flea)	Reproduction 21d EC_{50}	46200	Wollenberger et al., 2000
Streptomycin	*Microcystis aeruginos* (cyanobacteria)	MIC	300	Harrass et al., 1985

Chemical Name	Species	Endpoint measured	Concentration (experimental) (µg/L)	Reference
	Microcystis aeruginosa (cyanobacteria)	7d EC_{50}	7	Halling-Sorensen, 2000
Lipid Regulators				
Clofibrate	*Daphnia magna* (water flea)	24h EC_{50}	28200	Kopf, 1995
	Daphnia magna (water flea)	Reproduction 21d EC_{10}	8.4	Koph, 1995
Clofibric acid	*Brachydanio rerio* – embryos (zebrafish)	48h LC_{50}	8600	Henschel, et al., 1997
	Danio rerio – embryos (zebrafish)	Mortality 10d NOEC	7000	Ferrari et al., 2004
Gemfibrozil	*Carassius auratus* (goldfish)	1) Plasma parameters and 2) Plasma testosterone level ↓ by 50% - 14d exposure	1500	Mimeault et al., 2005
Psycho-active drugs				
Carbamazepine (antiepileptic)	*Brachionus calyciflorus* (rotifer)	Reproduction 48h NOEC	377	Ferrari et al., 2004
	Daphnia magna (water flea)	48h LC_{50}	> 1380	Ferrari etal., 2003
	Oncorhynchus mykiss (rainbow trout)	Cell function 48 h EC_{50}	111790	Jos et al., 2003
	Synechococcus leopolensis (cyanobacteria)	Growth 96h EC_{50}	33600	Ferrari et al., 2003
	Ceriodaphnia dubia (water flea)	Reproduction 7d NOEC	25	Ferrari et al., 2004
	Danio rerio – embryo (zebrafish)	Mortality 10d NOEC	25000	Farrari et al., 2004
Fluoxetine (SSRI)	*Ceriodaphnia dubia* (water flea)	48h LC_{50}	234	Brooks et al., 2003
	Oncorhynchus mykiss (rainbow trout)	Hepatocyte cytotoxicity 24h EC_{50}	1545	Laville et al., 2004
	Pimephales promelas – eggs (fathead minnow)	48h LC_{50}	705	Brooks et al., 2003
	Sphaerium striatinum (fingernail clam)	4h LOEC	1550	Fong et al., 1998
Analgesic and Non-Steroidal Anti-Inflammatory Drugs				
Acetaminophen	*Daphnia magna* (water flea)	Swimming ability 48h EC_{50}	9200	Kuhn et al., 1989
	Streptocephalus probiscideus (fairy shrimp)	24h LC_{50}	29600	

Chemical Name	Species	Endpoint measured	Concentration (experimental) (µg/L)	Reference
Acetylsalicylic acid	*Desmodesmus subspicatus* (green algae)	NOEC	3200	Cleuvers, 2003
Diclofenac	*Oncorhynchus mykiss* (rainbow trout)	Hepatocyte cytotoxicity 24h EC_{50}	6042	Laville et al., 2004
	Daphnia magna (water flea)	Immobility 48h EC_{50}	68000	Cleuvers 2004
	Danio rerio – embryo (zebrafish)	Mortality 10d NOEC	4000	Ferrari et al., 2004
Ibuprofen	*Lepomis macrochirus* (bluegill sunfish)	96h LC_{50}	7100	Reported in Webb, 2001
Naproxen	*Oncorhynchus mykiss* (rainbow trout)	96h LC_{50}	690000	Rodriquez et al., 1992
Estrogens and Hormone Regulators				
17α-ethinylestradiol	*Cyprinodon variegatua* (sheephead minnow)	NOEC and LOEC fibrosis of the testis	0.0002 and 0.002	Zillioux etal., 2001
	Cyprinodon variegatua (sheephead minnow)	NOEC – hatching success	0.02	Zillioux etal., 2001
	Marisa cornuarietis (prosobranch snail)	NOEC and LOEC Superfemales	0.001and 0.01	Schulte-Oehlmann etal., 2004
	Marisa cornuarietis (prosobranch snail)	LOEC Reduced fecundity	0.001	Schulte-Oehlmann etal., 2004
Estradiol	*Oryzias latipes* Medaka (post-hatch stage)	LOEC – all female fish 30d exposure	0.01	Nimrod and Benson 1998
Estradiol	*Oryzias latipes* Medaka (reproducing adults)	LOEC – males with testis-ova, reduce male GSI, reduced egg production 21d esposure	0.0293	Kang et al., 2002
Estradiol	*Oryzias latipes* Medaka (post-hatch stage)	LOEC 10% males and 100% males with testis-ova	0.01 0.1	Metcalfe et al., 2001

Chemical Name	Species	Endpoint measured	Concentration (experimental) (µg/L)	Reference
Ethinylestradiol	*Pimephales promelas* Fathead minnow (egg to adult)	LOEC all fish with female gonads (50% fertile) and LOEC severe physical deformities 305d exposure	0.004 0.016	Lange et al., 2001
	Pimephales promelas Fathead minnow (developing females	LOEC increased egg production	0.0001	Jobling et al., 2003
Ethinylestradiol	*Oncorhynchus mykiss* Rainbow trout (developing males)	LOEC reduced testicular growth (↓GSI) 3wk exposure	0.002	1996
	Danio rerio Zebrafish (egg to adult)	LOEC delayed maturation, altered mating behaviour, ↓ egg production and fertilization 75d exposure	0.00167	Segner et al., 2003
	Danio rerio Zebrafish 20-60 days post-hatch	LOEC altered sex ration 40d exposure	0.001	Orn et al., 2003
	Sand goby (juvenile to reproducing adults)	LOEC delayed male maturation, inhibited male sex behaviour, reduced egg production and fertilization	0.006	Robinson et al., 2003
Estriol	*Oryzias latipes* Medaka (post-hatch)	LOEC altered sex ratio	0.01	Metcalfe etal, 2001

Risk Management Strategies

Source Control – manufacturing and distributing

Drug design and use

In a 2003 monograph entitled, *Cradle-to-Cradle Stewardship of Drugs for Minimizing Their Environmental Disposition While Promoting Human Health,* Daughton suggested several means of improving drug design, delivery, marketing and dispensing

with the aim of reducing the overall loading of pharmaceutical products in the environment. Daughton suggests that "new drug design and formulations should factor in new considerations for 'environmental friendliness' or 'environmental proclivity'" (Daughton, 2003b). Such considerations would improve the drug's biodegradability and photolysis abilities, while increasing its physiologic sorption, metabolism and excretion characteristics. Further, the author advocates the introduction of a 'greener' treatment approach, including using 'nature emulating' drugs such as piscidins, and bacteriophages as well as a more extensive use of alternative medicines and medical practices. Lastly, Daughton suggested lowering the prescription rates, use "lower-than-established dosing", individualize both drug therapy (compounders) and drug delivery methods, extend expiry dates and implement stricter controls on current drug dispensing systems (Daughton, 2003a and 2003b). These approaches mirror similar movements to 'greener' alternatives advanced in other industries e.g., Green Technology, Responsible Care Initiatives (developed in Canada and later exported to the EU (Brandt, 2002)), Green Chemistry (US EPA, 1991) and Eco-Efficiency and Cleaner Production (Larsen et al., 2004). However, none of these have the potential to directly (and perhaps negatively) affect individual human health status.

Daughton suggests some very interesting and valid solutions to the issue of pharmaceutical contamination of the aquatic environment. However, he does not address the social, economic and health (both private and public) implications of this approach. For instance,

- the suggested changes in the drug manufacturing process come with a hefty financial price tag. In the face of continual charitable drives to raise money for research on various diseases, justifying increased monetary inputs for the

development of drugs that are not only efficacious but also environmentally-friendly may not be an easy task. In addition, improvements in the degradability of some drugs may not be possible without reductions in clinical efficacy.

- altering the delivery, dosage or type of treatment requires an initial financial input and a continued potential health risk.
- waste reduction efforts occur already as a design feature of a number of physiologically and economically viable medicines. For instance, most drug manufacturers aim to develop a product that will maximize the desired effect while minimizing side effects, using a most economical route of production and delivery.

Labouring towards a 'greener' pharmacy is commendable, but in the face of financial and ethical constraints, it remains a challenge. Nevertheless, some researchers anticipate its benefits. For example, a system for the environmental classification of pharmaceutical compounds has already been proposed in Sweden. It is intended to offer patients and physicians environmentally-friendly alternatives for various medications Larsen et al., 2004; Wennmalm, 2003).

Distribution

Distributors such as pharmacies could also be involved in the environmental control of PhACs. For instance, outlet stores may carry leaflets or pamphlets detailing responsible stewardship of the products they sell. This is one of the precautionary measures suggested in the EU guidance documents for new pharmaceuticals (EMEA, 2005 http://www.emea.eu.int/htms/human/qrd/qrdplt/24530905en.pdf). However, such a program has not yet been implemented either in Europe or in North America. Environmental information may also be included in technical documents such as the

Physician's Desk Reference. For example, in 2002, the Canadian Pharmacists Association released a pamphlet offering tips on appropriate drug disposal strategies, stating that disposing of drugs in the sewage system is "not good for the environment".

British Columbia instituted the voluntary (1996) and than mandatory (1997) EnviRx package as part of the Post-Consumer Residual Stewardship Program Regulation. In 2001, the program was re-named the Medications Return Program (MRP) (Government of British Columbia, 1997). Most British Columbia pharmacies (85% - 90%) belong to the MRP. The program is administered by the Post-Consumer Pharmaceutical Stewardship Association (PCPSA). According to the PCPSA, in 2004, 15,503 kg of medicines were diverted from municipal landfill/sewage treatment plants (PCPSA, 2006). The company contracted by the British Columbia MRP, Residuals Management Group of Surrey, ships the collected pharmaceuticals to Cristallo Engineering Technologies in Alberta for incineration (http://www.medicationsreturn.ca/british_columbia_en.php).

In addition to environmental protection, Daughton (2003b) noted the benefits of implementing a medicine return program. These include to ability to identify patient non-compliance and primary users of this system, isolate factors contributing to patient non-compliance and design a better drug dispensing system to avoid non-compliance. For example, the Alberta Pharmaceutical Society has used the data collected under its drug return project to find that the geriatric population was most likely to use the service. This observation led to the prescription of smaller, 'trial' doses which eliminated a large percent of drug returns. This reduced not only the volume of medications slated for disposal, but also reduced a general waste of medication and packaging materials

(Daughton, 2003b). Data on the effect of this approach on the health and comfort of the affected patients was not forthcoming in the article.

Precautionary Measures

Attempts at controlling pharmaceutical environmental contamination are actually legislated in some jurisdictions under the title, 'Precautionary Measures'. These are a direct result of the 'precedence-for-patient-benefit' principle which states that the goal of environmental risk assessments for pharmaceuticals is never to deny product licensing/registration, but to identify the need for precautionary measures. "Clearly, when evaluating pharmaceuticals, the health benefits to humans must take precedence over any environmental problems... instead it might be better to try to regulate the pathways by which pharmaceuticals enter the environment..." (Jones et al., 2002). To this end, the new EU directives for pharmaceutical products include restricted use, additional environmental monitoring and/or product labelling (Straub, 2002). For example one such label suggested in the European Union guidelines reads: "*Unused preparations or old preparations should be returned to pharmacies. Old preparations should not be disposed of via wastewater or the municipal drainage system. These measures will reduce pollution of the environment*" (EMEA 2005).

Another precautionary measure suggested in the EU is some variation of use restrictions, for example, a drug that is available only by administration in the hospital. This would entail differential collection and in-house pre-treatment of the excreted drug and its metabolites in order to ensure that the amount and version of the drug reaching the environment poses a minimum of risk to its inhabitants. Factors other than environmental concerns should also be considered such that neither the health system nor

the patients incur an undue financial burden as a consequence of measures to restrict PhAC release into the environment. For example, who will support the immediate consequences (financial and social) associated with a hospital stay based only on the need to restrict the distribution of PhACs? Moreover, can such a hospital-use-only measure be reconciled with sewer-use by-laws (e.g., such as the ones in Toronto, discussed in the section on Pollution Prevention Programmes) which limit the amount of chemicals that can be discharged by hospitals.

Source Control – post-patient

Effluent design and volume reduction

The premise of these approaches is that advanced treatment options may actually be financially viable if applied to a low volume of effluent. Considering that less than 1% of household wastewater (urine) contains most of the nutrients and PhAC residues, specifically designed effluent can be evaluated as a solution to environmental contamination with PhACs (Larsen et al., 2004). Designed effluent would have a composition that is optimal for specific treatment/disposal, and would primarily be achieved via source separation (i.e., urine separation involving consumers' cooperation) or source control (manufacturers and distributors) (Larsen et al., 2004). Proponents of effluent design point out that this approach offers a double benefit: first, specialized and often more expensive treatments could be applied. Second, if this technique were implemented in several large institutions (e.g., hospitals, retirement communities, etc.), a measurable percentage of recalcitrant PhACs would be diverted from sewage treatment plants (Henze, 1997; Giger et al., 2003). However, the wide implementation of these systems is challenging, because of the necessity to overcome initial capital investment,

public acceptance and compliance and technological hurdles (Larsen et al., 2004). The public would need to accept the need for and adapt to the in-house urine separation systems. This requires both a financial (replacing household appliances/infrastructures such as toilets and pipes) and a lifestyle commitment. Although the intention was to treat the resulting concentrated effluent with specialized wastewater treatment systems, this is not always the case. For example, in a pilot study performed in Lund, Sweden, the collected, untreated human urine was applied as liquid fertilizer for wheat crops for human consumption (Berndtsson, 2006). This was a good example of a policy which did not integrate the entire spectrum of environmental effects, concentrating instead only on reduction of nutrient inputs (ammonia N) to the sewage treatment plant.

Assuming that the differential collection toilets already in use, such as 'NoMix' or Novaquatis (Novaquatis, 2005 http://www.novaquatis.ch/english/NOVA8_e.html) encounter no further technical hurdles, and assuming that the concentrated resulting effluent is actually treated prior to its environmental release, public and regulatory acceptance, compliance, and support for such systems must be developed.

Effluent recycling

In most municipalities, wastewater and wastewater effluent are used for a) meeting non-potable water demand, b) irrigation of various crops, and c) injection into local aquifers. These applications for treated effluent can have consequences similar to those of direct discharge into waterways, as PhAC residues in the effluent are not removed. For this reason, the practice of effluent recycling needs to evaluated in a manner similar to the evaluation of direct discharges.

Table 4 shows a number of such projects operating around the world. In most instances, reclaimed wastewater effluent is used for projects requiring non-potable water. In the UK, the local authorities used sewage effluent for irrigating golf courses, parks, road verges and for commercial purposes such as car washes, cooling and fish farming (Angelakis and Bontaux, 2001). These applications ensure that no direct human exposure to PhACs occurs via the food chain, but they do not mitigate introduction of PhACs into the environment.

Table 4: Type and location of Water Reuse around the World

Type of Re-use	Location	Comments	Reference
Irrigation	France	Various crops, used for > 100 yrs. – various treatment stds.	Angelakis, 2001
Irrigation	Italy	Used for >100 yrs. – various treatment stds., some untreated.	Angelakis, 2001
Irrigation	Canada – SK, MB and AB	Treated effluent used in > 60 projects to irrigate agricultural land.	Agriculture and Agri-Food Canada[1]
Maintain ecosystems	England	Also indirect potable water reuse via river abstractions	Angelakis, 2001
Commercial reuse in carwashes, etc.	France, England	Treated to various standards.	Angelakis, 2001
Groundwater recharge to maintain seawater intrusion barrier	US - California	Reverse osmosis project entitled Water Factory 21 – advanced treatment, good results	Canada Mortgage and Housing Corporation[2]
Indirect potable water reuse	Florida	A number of similar projects are listed here.	Canada Mortgage and Housing Corporation[2]
Indirect potable water reuse	California	Tertiary treated and diluted effluent injected into aquifer	Orange County Project Coordinators[3]

SK = Saskatchewan; MB = Manitoba; AB = Alberta
1: http://www.agr.gc.ca/pfra/csidc/csidpub5_e.htm
2: http://www.cmhc-schl.gc.ca/en/inpr/su/waco/inpoware/index.cfm
3: http://www.gwrsystem.com/

Irrigation with treated and sometimes untreated effluent is an old practice. In areas around Paris and in some rural Italian fields, it has been practiced for more than 100 years (Angelakis, 2001). In Canada, Manitoba, Saskatchewan and Alberta operate more than 60 projects which irrigate a total of 5700 ha with treated effluent (Agriculture and Agri-food Canada, 2004, http://www.agr.gc.ca/pfra/csidc/csidpub5_e.htm).

In certain parts of the world, treated effluent is used to maintain a fresh water barrier against saltwater intrusion into coastal aquifers (Orange County, CA), or to replenish aquifer resources (Colorado, California) (Groundwater Replenishment System, 2004 http://www.gwrsystem.com/). There are currently no national or international guidelines addressing the standards to which such effluent should be treated. However, local governing bodies such as the State of California Department of Health Services (DHS) do set some very stringent limits on what can be injected into their aquifers. Since testing for each biological and chemical contaminant is not a feasible option, the DHS used a multi-barrier approach. That is, the effluent undergoes a series of advanced treatment, none of which on its own would be sufficient, but which together are considered to offer a sufficient level of safety (Asano and Cotruvo, 2004).

Although water/effluent recycling has been used successfully as discussed above, some issues remain yet unaddressed. First, water recycling is a highly emotionally charged issue (Schafer and Bedder, 2006). It must challenge the inherent fear of 'toilet-to-tap' recycling, particularly exposure to pathogens or potentially toxic chemicals present in the effluent, including PhACs (Daughton, 2004). Reports (founded or unfounded) of endocrine disruption and chronic exposure to carcinogenic chemicals via drinking water have become more common and heighten the public's awareness of this

issue (Schafer and Bedder, 2006). Second, legal and ethical issues such as the request for due diligence in the justice system and/or who the clients of recycled water would be have not been addressed. Because effluent re-cycling means re-cycling of PhAC residues, uncertainties relating to the evaluation of harm must be addressed and estimated. This will only come about as a result of further scientific research. Ultimately, the level of protection desired and the relative importance of possible impacts are political decisions founded on value judgements (Schafer and Bedder, 2006). Currently, there is no common basis or platform provided in the literature to support the simultaneous assessment of both scientific and political issues. Uncertainties, values, and policy with regard to pharmaceutical contamination of the environment will be further discussed in Chapter 3.

End-of-pipe Control

Infrastructure up-grades

Large-scale infrastructure upgrades are usually costly projects whose benefits are weighed against the priorities set by the community they serve (Daughton and Ternes, 1999). Where a community deems it feasible to implement such projects, these could include primary, secondary, tertiary, advanced intermediary or membrane filtration approaches. How each of these affects the level of PhACs will be considered in the following sections.

Advanced intermediary systems

Figure 11 is a diagram of the newly upgraded advanced primary treatment process employed in the Harbour Solutions Project in Halifax, NS. This system combines the

addition of a flocculant and UV disinfection to the existing primary system, yielding a "cleaner effluent" to be discharged into the harbour.

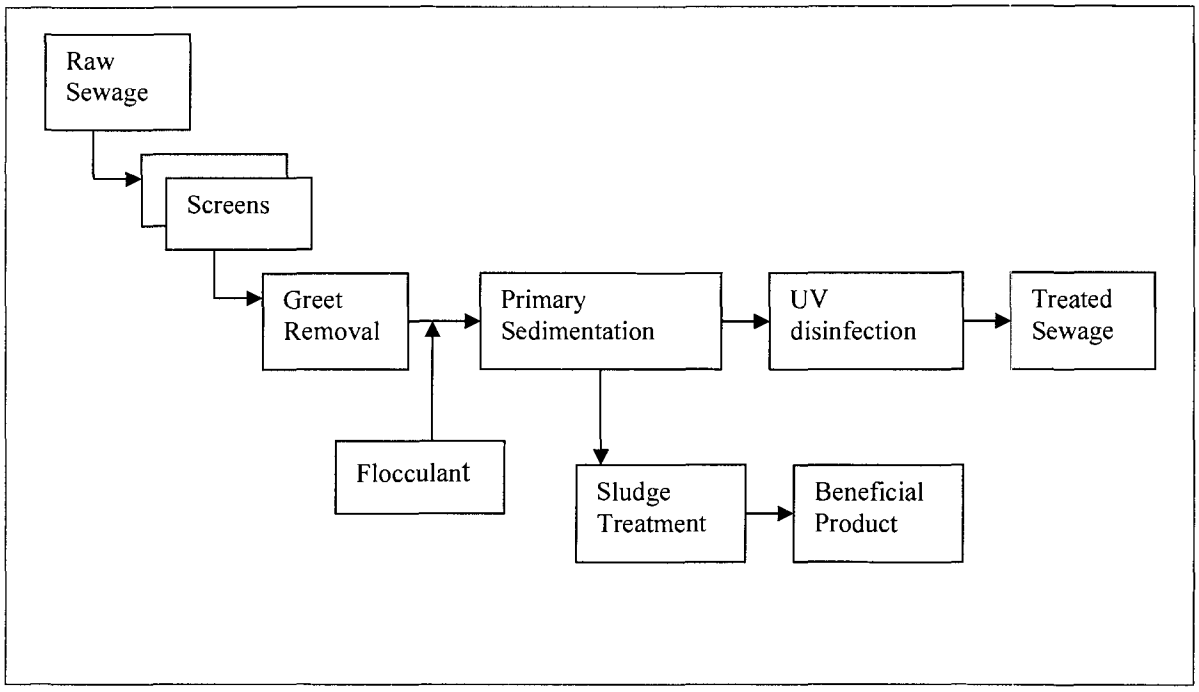


Figure 10: Advanced primary treatment system used in the Halifax Harbour Project. Adapted from (http://www.region.halifax.ns.ca/harboursol/primary_treatment.html)

This advanced primary treatment system is an improvement for Halifax's highly contaminated harbour. However, it should be noted that the addition of a flocculant will increase the removal of PhACs to the sludge component. Furthermore, while the ultraviolet radiation decreases the amounts of disinfection chemicals required, it may increase oxidation by-products as discussed in a previous section.

Ultimately, implementation of such advanced, intermediary treatment systems would have to be evaluated against the specific needs of the community they serve.

Ozonation and advanced oxidation

Ozonation is a method of water disinfection where the effluent is treated with 10 to 20 mg/L ozone. Oxidation is a method of water treatment where recalcitrant organics in the effluent such as PhACs are oxidized, usually with some combination of iron or manganese oxides, hydrogen peroxide, ozone and ultraviolet radiation (Balcioglu, 2003). Ozonation increases the number of functional groups and the molecule's polarity, while oxidation increases the molecule's reactivity by enhancing the formation of hydroxyl groups (Ternes et al., 2003). This induces a structural change in the molecule, thereby altering its intended mode of action. For example, hydroxylated estrogens lose their affinity for the estrogen receptors, while hydroxylated antibiotics are deemed unlikely to promote the development of antibiotic resistant strains (Ternes et al., 2003). However, as discussed in an earlier section on the fate of PhACs, oxidation can lead to the formation of toxic by-products. For example, carbamazepine, a persistent but comparatively innocuous contaminant is oxidized via a series of acridine intermediates (Vogna et al., 2004). Acridine is part of a class of established air and water pollutants with proven mutagenic and carcinogenic activity (Vogna et al., 2004). In this case, oxidation without complete mineralization of carbamazepine may enhance its toxicity. This situation is not unlike that encountered with chemical disinfection by-products of non-PhAC organics such as trihalomethanes.

Table 5 is a summary of some published treatment efficiencies of PhACs. It is noteworthy that the terms 'eliminated' and 'removed' are not explicitly defined, because as stated above, disappearance of the parent compound does not necessarily mean that the hazard is eliminated.

Table 5: Removal efficiency of selected PhACs by various oxidation treatments.

Treatment	Substance studied	Results	Reference
Ozonation 5-10mg/L	Antibiotics CBZ HHCB Lipid regulators Natural estrogens X-ray contrast media	<LOQ <LOQ 93% elimination* 50-62% elimination* <LOQ 13% removal*	Ternes, 2003
$O_3/H_2O_2/UV$	Paracetamol (analgesic)	30-40% mineralization	Andreozzi et al., 2003
O_3/UV	X-ray contrast media	36% removal*	Ternes, 2003
H_2O_2/UV	Carbamazepine	"effective removal via acridine intermediates"	Vogna et al., 2004
MnO_3	17 α-ethynylestradiol	81.7 % elimination* (at very high [EE2])	Rudder et al., 2004

<LOQ = below limit of quantification
* authors did not define 'elimination' and 'removal' in terms of removal of the parent compound or elimination of the environmental hazard.

Advanced oxidative treatments are generally effective in reducing the level of several PhACs as shown in Table 5. In particular, antibiotics, estrogens and musk fragrances are successfully removed by oxidation (Ternes et al., 2003; Balcioglu, 2003). Although some of these treatments display high potential for removing PhACs from effluent their large scale implementation may still need to be scientifically and financially evaluated. For example, the estimated cost for ozone treatment in a large installation is approximately 0.04 €/m^3 and that for ozone/UV treatment is 0.05 €/m^3 (approximately 0.06 and 0.07 Canadian dollars respectively) (Ternes et al., 2003). Applying this to hospital effluent may be feasible, depending on who is paying, but to a plant servicing some half a million or more residents may be too high. For example, the cost to the Anacis Island WTP in Vancouver's Lower Mainland, servicing some 740,000 residents would be the cost of treatment, 0.07 \$/m^3 applied to average daily throughput of 600,000

m^3/day. This equates to $42,000/day or an annual cost of approximately $15.3 million (Monitoring Results for Operating Certificate http://www.gvrd.bc.ca/sewerage/WWTP-reports/Annacis%20OC%20Data_February2006.pdf; Anacis Island WWTP http://www.gvrd.bc.ca/sewerage/annacis.htm).

Membrane filtration processes

Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) are all examples of treatment options for conventionally treated effluents prior to their reintroduction into the environment. These are post-disinfection treatments, often used where a more cost-effective conventional approach is not feasible. Usually, applications of these techniques include desalination for the production of potable water or finishing effluent treatment for aquifer recharge or other forms of environmental release. Depending on set-up parameters, MF removes particulate matter including bacteria, but not dissolved contaminants whereas UF removes some organics and viruses. NF successfully rejects organics and hardness (inorganic salts) entirely, and RO rejects almost all particulate and dissolved organics, inorganics and microbes, including PhACs (Schafer et al., 2001; Drewes et al., 2003). For example, Nghiem et al. (2004) found that NF and RO both effectively rejected ↓ the natural hormones estrone and estradiol. In general, membrane filtration systems (NF, UF and RO) reject substances based initially on hydrophobic adsorption and once equilibrium has been achieved, on molecular size and electrostatic charge (Nghiem and Schafer, 2006; Kimura et al., 2004). For example, a study of 52 PhACs and personal care products concluded that more polar compounds pass through NF and UF membranes more frequently than less polar compounds, indicating the importance of hydrophobic adsorption (Yoon et al., 2006). However,

under operational equilibrium conditions, the distinction between substance polarity fades. At this point the type of membrane (NF having smaller pores than UF) becomes relevant, indicating that molecular size becomes the dominant rejection criterion (Yoon et al., 2006). Finally, the price associated with the NF and RO is considered by many to be uneconomic due to capital, energy and maintenance costs (Schafer, 2001).

In affluent areas experiencing water shortages such as California, membrane filtration followed by reverse osmosis has been used for some time (Water Factory 21 – California http://www.ocwd.com/_html/wf21.htm). In other parts of the world, RO has been used to desalinate seawater. For instance, all of Kuwait's fresh water needs are met by desalinating saltwater. Consequently, there is a fair amount of published information pertaining to the efficiency and cost/energy benefit of these treatments (Kimura, et al., 2003; Parameshwaran et al., 2001; Drewes et al., 2003 and Schafer et al., 2001). The resulting quality of effluent is comparable to that of fresh water, but costs are elevated.

Pollution Prevention Programmes

The concept of pollution prevention (P2) is consistent with the new environmental management paradigm, which advocates prevention, precaution and reduction of potentially harmful environmental behaviours. P2 programmes have been successfully implemented in Australia, the United Kingdom, the United States and Canada (Allen, 2005; Tudor et al., 2005). In Canada, they are defined as "the use of processes, practices, materials, products or energy, which avoid or minimize the creation of pollutants and wastes, at the source" (Ratnaparkhe and Sertic, 2005). P2 programmes are required under some local waste-management or sewer system use by-laws, for example, Toronto's Sewer Use By-law 457-2000. Submission of a P2 plan in accordance with

local regulation is mandatory, but its implementation cannot be enforced under current municipal laws (Ratnaparkhe and Sertic, 2005). However, voluntary compliance has been reported, especially in the health-care sector. Specific examples are presented below.

Because P2 planning requirements are included into local sewer use by-laws, discharge limits can be set and enforced directly at the point-of-discharge into municipal systems. This approach applies greater compliance pressure to 'producers' of contaminating waste (Allen, 2005). None of the current literature addresses the potential conflict between such policies and prevailing social values when the waste 'producers' are hospitals. For example, what happens when the producers are mandated to handle contaminating products as would be the case when restricted use precautionary measures are applied to pharmaceutical products? How would a hospital reconcile its commitment to provide care and medicines to its patients and its requirements under sewer-use-bylaws to reduce discharge levels of the very same medicines? This situation has not yet occurred but the policies for its implementation are in place.

In the health care sector, P2 compliance options include process or equipment modification, product and/or chemical substitution or elimination, loss prevention and housekeeping, waste segregation and effluent design and closed-loop recycling (Allen, 2005). Expected benefits of P2 implementation are reduced operating costs and reduced risk of liability, enhanced public image and potential contributions to public and environmental health (Ratnaparkhe and Sertic, 2005).

Pollution prevention is aimed specifically at institutional care facilities or directed living communities such as retirement subdivisions. Healthcare waste management

(including P2) has been investigated around the world, in the USA (Lee et al., 2004), The Netherlands (Dijkema et al., 2000), Finland (Ponka et al., 1996) and India (Patil and Shekdar, 2001), and is primarily driven by high costs associated with waste disposal. Some estimates place the saving attributed to waste reduction programmes at 40 to 70% (Health Care Without Harm, 2001). Medical waste is complex, potentially posing pathogenic, chemical and physical risks to the environment. Typical hospital waste generation occurs not only in clinical patient-care areas (tissue, fluids, pathogenic human waste products, etc.) but also in dietary, laboratory, pharmacy, imaging and administrative departments (Tudor et al., 2005). In the context of this thesis, pharmaceutical, disinfectant and plastics emissions are the major chemicals of concern – with disinfectants and plastics identified as 'subject pollutants' in the Canadian approach to P2 programmes (Ratnaparkhe and Sertic, 2005; Allen, 2005; McKerlie et al., 2005). It should be noted that the 'subject pollutant' designation is dynamic. It can (and did) change in order to reflect current societal concerns or new scientific evidence (Allen, 2005; Ratnaparkhe and Sertic, 2005). Specifically, nonylphenol and its ethoxylates have been designated as toxic under the Canadian Environmental Protection Act and placed in the Schedule 1 – Toxic Substances List (CEPA – Toxic Substances, 2005 http://www.ec.gc.ca/CEPARegistry/subs_list/Toxicupdate.cfm). However, no pharmaceutical compounds have yet been placed on either the CEPA Toxic Substances List or the "subject pollutant" lists discussed above.

Several institutions in Ontario have been cited as "P2 success stories" by the Canadian government (Government of Canada, 2005 www.ec.gc.ca/nopp/doc/fact/en/health.cfm). Toronto's Hospital for Sick Children (HSC)

saved $0.6 million from its waste management budget by reducing its biomedical waste in laboratory and patient care areas by 35%. Reductions in biomedical waste of 21% were recorded at the Cambridge Memorial Hospital, ON, presumably with accompanying financial benefits for the institution. Published, peer-reviewed literature on the exact methods for the achievements of these results is not available. However, one approach has been to implement a re-definition of key terms such as 'biomedical waste'. For example, in 1993, by redefining 'biomedical waste' as newly mandated by the Ministry of the Environment, the HSC achieved a 35% reduction in biomedical volumes in one of their new patient care centres (CCPPa, 2006 http://www.c2p2online.com/documents/SickChildrenHospital.pdf).

HSC and the Centre for Addiction and Mental Health (CAMH) in Toronto, and York Central Hospital, in Richmond Hill applied P2 programs to the reduction of the subject pollutants nonylphenols and nonylphenol ethoxylates (NP and NPE) found in detergents and disinfectants (Allen, 2005). A P2 plan was implemented and the Centre achieved a 98% reduction in the use of NP and NPE within 2 years. This was 4 years and 23% ahead of the target 75% reduction within 6 years (Allen, 2005), thereby prompting Environment Canada's 'success story' designation. These remarkable P2 achievements were accomplished primarily via product substitution and/or elimination. For detergents and disinfectants used in patient-care areas including floors, furniture, washrooms and bathtubs, some facilities have developed a substitution protocol. This was a four-step approach implemented at HSC and it was based on: 1) identifying and inventorying products containing toxic compounds, 2) implementing a Green Procurement Policy (adding this clause to the hospital policies and requesting a list of alternatives from

suppliers), 3) evaluating product alternatives based on information provided by the product supplier/distributor, and 4) conducting trials on the recommended products prior to full implementation (CCPP, 2006b http://www.c2p2online.com/documents/SickKidsCaseStudy.pdf). The HSC used this approach to substitute their routine housekeeping detergent with a hydrogen peroxide one (personal communication, Director of Hospital Support Services, 2006). Similarly, the CAMH employed the efforts of the chemical suppliers in identifying "environmentally safer and less toxic alternatives for immediate substitution". The implementation of this option was facilitated by cross-departmental communication and "for the most part, the identified products were capable of offering equivalent or greater performance and only minor cost differences were noted" (Allen, 2005). York Central Hospital in Richmond Hill replaced their conventional housekeeping detergents/disinfectants with a water-based Advanced Cleaning Techniques (ACT) system in 1999 (Scarlatto, 2002). The replacement protocol resembled the four-step approach applied at Toronto's HSC. The 'scientific information' provided by the distributor in support of equivalent efficiency of the ACT system (and cited by the hospital as the scientific basis for the substitution) is not publicly available. Following personal communication with the ACT system distributor, I obtained a copy of the document used to support the efficiency of the ACT (Ekholm, 1998). It is a report of work conducted in 1998 by a group of largely hospital employees at the University Hospital in Lund, Sweden. The report "accounts for methods of establishing a quality level for the daily cleaning of wards and clinics at University Hospital in Lund Conventional cleaning methods are described and compared with a new low chemical cleaning method" (Ekholm, 1998). However, its conclusions do

not seem to be supported by the actual experiments performed. Nor do the conclusions (such as they are) unequivocally support the cleaning efficiency of the ACT system. In addition, 'efficiency' is not actually defined at any point during the study. Similarly, the quality of the 'bacteria audit' follow-up studies was below that required for a scientific evaluation of the results. I obtained a copy of the 'bacteria audits' performed by the ACT distributor but I could not draw any conclusions, as the document was incomplete and poorly labelled. Nevertheless, the success of this story is related in Sanitation Canada (Scarlatto, 2002), and on the Canadian Centre for Pollution Prevention's website (http://www.c2p2online.com/main.php3?session=&section=88&doc_id=174).

Unfortunately, there is currently no published data documenting effects (if any) on patient care outcomes as a result of substituting cleaning and disinfecting products (e.g., the incidence of nosocomial or post-operative infections, and their causes). Nor is there an abundance of data objectively comparing the performance of various detergents. The lack of data may arise because, at least in this case, success was defined in terms of cost-savings to the institution, (and/or possibly reductions in the institutional discharge in particular waste streams) rather than the length-of-stay outcomes, incidence of nosocomial infections or other considerations (for a detailed discussion of this concept see Stein, 2001). The importance of framing the research question is highlighted by this study, as is the necessity to clearly define the goals and ensure stakeholders agreement with them (see discussions on values, goals and stakeholders in Chapter 3).

To establish best practices to reduce PhAC levels in the waste streams of health care and other institutions, not only should rigorous scientific practices be employed, but the benefit to one sector (the environment, health) must be measured against benefit to

another sector (public health, public values, economics). All of the options considered must be evaluated by an independent body from this multi-factorial standpoint, perhaps using techniques such as integrated risk assessments (see Chapter 3).

CHAPTER 3:
ASSESSING THE ASSESSMENTS

The information contained in formal risk assessments forms the basis of a regulatory framework aimed at protecting the environment, including humans, from the potentially harmful effects of the chemical studied. The first part of this chapter will discuss the principles and practices of environmental risk assessments as applied to pharmaceuticals. The second part will examine some of the issues faced by researchers and regulators in managing information supplied by risk assessors. The chapter will conclude with an evaluation of the science, the concerns, and the management of pharmaceutical contamination of the environment.

Risk assessment: principles and practices

Ecological risk assessment (ERA) is "the practice of determining the nature and likelihood of the effects of human actions on animals, plants, and the environment" (Jones, 2004). It consists of two crucial elements: the data specific to the particular assessment and the underlying principles/assumptions used to interpret and analyze the data (Ruden, 2005).

Data Gathering

Chapter 2 summarized the type of information required for a risk assessment (i.e., the occurrence and concentrations of pharmaceuticals in water, dose-response and exposure measurements of various compounds on laboratory and wild animals, effects of

environmental pharmaceuticals on animals, etc.). This information is compiled into the four ERA phases listed below. Their exact definition varies according to the type of assessment, but the overall representation is retained. Comprehensive discussions of the assessment process, theory and practice are available in most toxicology texts (for example, see Ballantyne et al., 2000).

The first phase is hazard identification or problem formulation. During this stage substance-specific information such as chemical and/or toxicological properties is gathered and an investigative plan is determined. The second stage is the exposure-effects assessment or dose-response assessment. This stage demonstrates the relationship between administered dose and resulting effects (Ruden, 2005). The third stage, exposure assessment, forms a link between the actual or expected exposure rates and potential effects on the target species (humans, fishes, ecosystems). The final stage is risk characterization, where the information from the previous three stages is assimilated and integrated (Jones et al., 2004) into some likelihood (expressed as numerical probabilities) that an effect will occur at a given exposure rate. For example, in the European system, this likelihood of effects is determined by the ratio of predicted environmental concentration (PEC) to the predicted no effect concentration (PNEC), where the ERA continues to the next tier if the ratio is greater than 1. Similarly, in the U.S. system, the expected introductory concentration (EIC), or the maximum expected environmental concentration (MEEC, which is either the EIC or the expected environmental concentration (EEC), whichever is greater) is compared to acute or chronic LC_{50} figures depending on the tier level. For example, if the LC_{50} to MEEC ratio is greater than or equal to 10 and no other effects are observed, the assessment is ended.

This information is then presented to risk managers and incorporated into an overall risk management strategy aimed at reducing the identified risk to levels acceptable to the society (Stern and Fineberg, 1996).

Examples of Risk Assessment Systems

The requirement for a detailed risk assessment for all new pharmaceuticals entering the market was introduced in the U.S. risk assessment system in 1998. The European Union followed suit in 2001. The lack of information associated with the environmental risk of pharmaceuticals has delayed the PhACs ERA process in Canada. Regulatory efforts in this country have focussed on specific groups of substances such as mercury, nonylphenol and its ethoxylates and antibiotics (CEPA – Toxic Substances, 2005 http://www.ec.gc.ca/CEPARegistry/subs_list/Toxicupdate.cfm; Holtz, 2006). Regulation of the pharmaceutical contamination of the environment may be considered within Environment Canada via the Canadian Environmental Protection Act in its Toxic Substances Lists, and within the Department of Fisheries and Oceans via the Fisheries Act. However, pharmaceuticals are excluded from the Environmental Protection Act (personal communication, Dr. F. Gobas, April, 2006), and regulation under the Fisheries Act would be possible only if pharmaceuticals were considered substances deleterious to fish or fish habitat (section 36b http://laws.justice.gc.ca/en/F-14/240479.html). Lastly, environmental regulation of PhAC may also occur under the auspices of Health Canada, as this department is currently developing a framework for the environmental risk assessment of new pharmaceuticals regulated under the Food and Drug Act (Olejniczak and Spindler, 2004).

Figures 11 and 12 denote the decision making process for environmental risk assessment of pharmaceuticals in the European Union and the United States, respectively. The European and U.S. ecological risk assessment protocols have been evaluated in several comprehensive reviews (Bound and Voulvoulis, 2004; Olejniczak and Spindler, 2004). Highlights of the benefits and drawback of each system for the aquatic environment will be presented below.

The European and U.S. systems share a number of characteristics. First, they are both tiered assessments in which the ERA only continues to the next tier if risk cannot be excluded. Second, they both include an assessment of the active pharmaceutical ingredient with some mention of metabolites and transformation products (an improvement over previous ERA protocols) (Bound and Voulvoulis, 2004). Third, the assessment is performed in the context of precedence for patient benefit. Even in cases of clear negative environmental impact, precautionary measures may be required but registration/licensing will not be denied at the expense of human health. The concept of precedence for patient benefit is important as it has great implications for risk management. Fourth, the two systems rely heavily on single chemical - single dose - acute response testing. The lack of attention devoted to potential mixture effects, effects related to specific mode of action, or chronic and long term subtle effects as discussed in previous chapters is also reflected at the regulatory level (Daughton, 2004; Bound and Voulvoulis, 2004). This incomplete approach is nonetheless consistent with the current risk assessment paradigm in which acute testing of a single chemical predominates (this topic will re-occur throughout the rest of the chapter). Fifth, partitioning coefficients (log P) form the basis for a decision point in both systems, e.g., if log $P > 3.0/3.5$,

proceed to tier 3. Thus, the numerical value of log P determines the type of assessment to be completed. This is another reason why so many researchers feel that the determination of log P values should be performed under conditions appropriate for pharmaceuticals. Lastly, both systems allow for "special circumstance" cases where an ERA is performed even if expected environmental concentrations are below the trigger value. For example, the expected environmental concentration for the estrogen EE2 is below that required to trigger a phase II assessment, but the log K_{ow} value for EE2 is ≥ 3 thereby pushing the assessment process into phase II (Knacker et al., 2005).

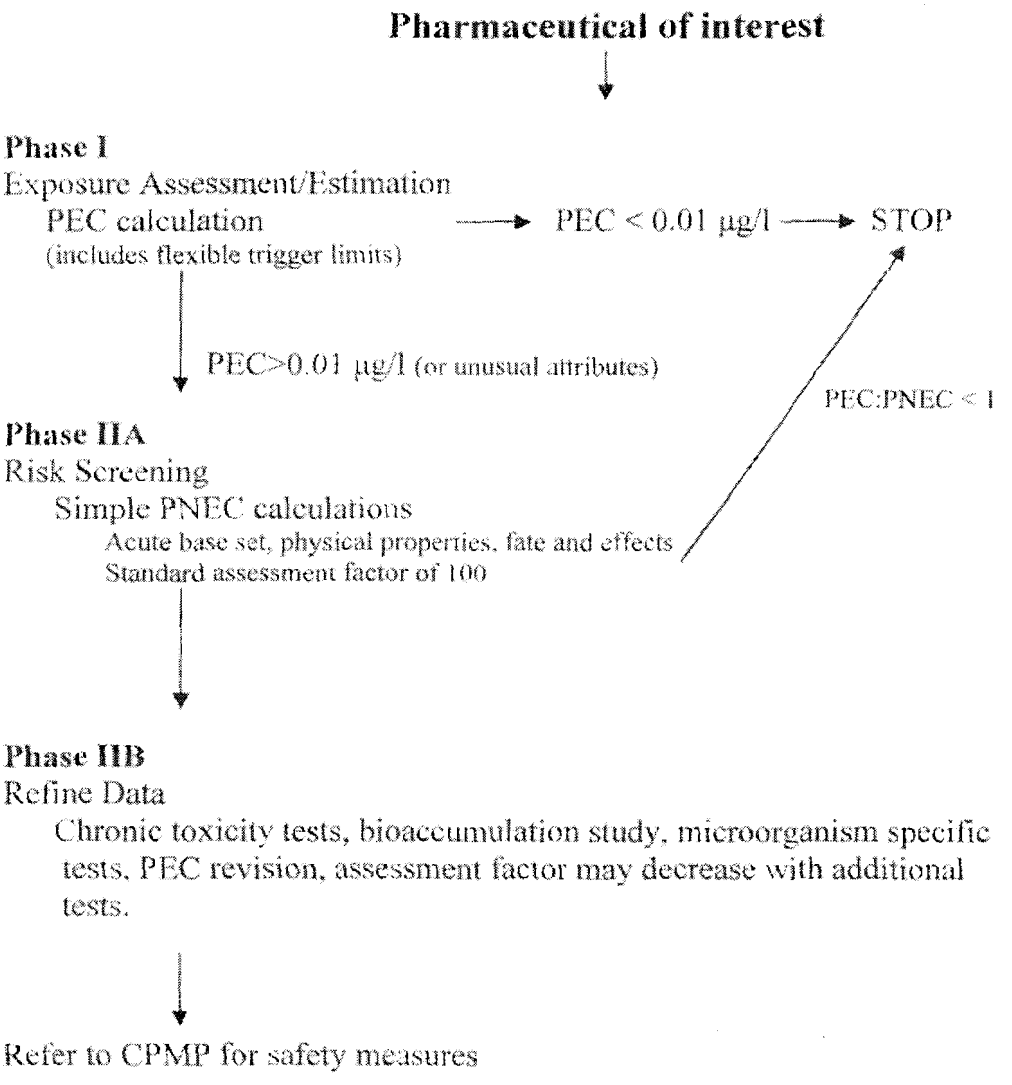


Figure 11: Decision-making process to determine the ecological risk of pharmaceutical substances under the European Union. PEC = Predicted Environmental Concentration; PNEC = Predicted No Effects Concentration; CPMP Committee for Proprietary Medicinal Products, part of the EMEA. Adapted from Bound and Voulvoulis, 2004.

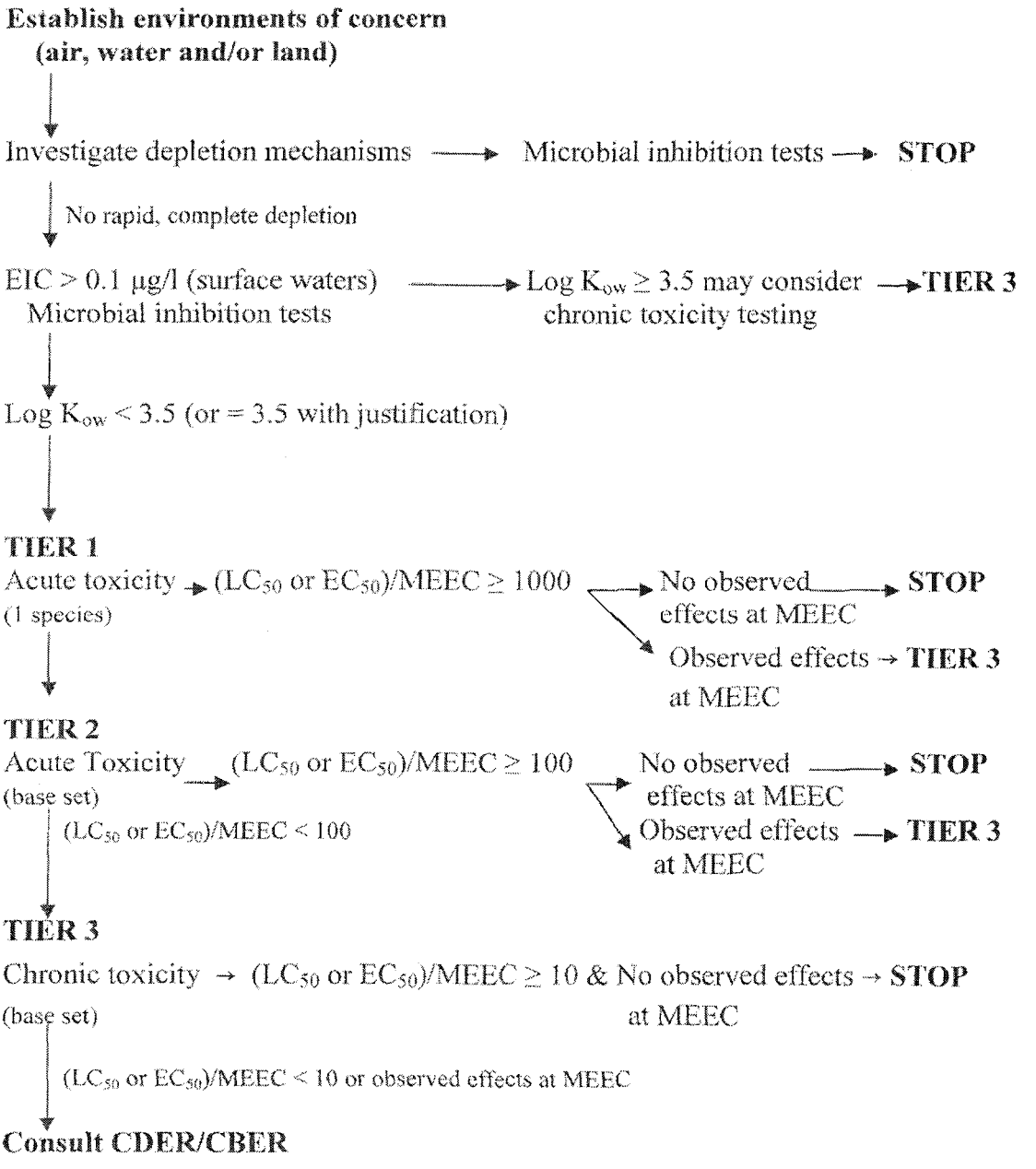


Figure 12: Decision-making process to determine the ecological risk of pharmaceutical substances in the United States system. EIC = Expected Introductory Concentration; MEEC = Maximum Expected Environmental Concentration; CDER = Centre for Drug Evaluation and Research; CBER = Centre for Biologics Evaluation and Research. Adapted from Bound and Voulvoulis, 2004

Despite the similarities, the two models vary in several important respects. First, the European model sets a trigger value for the predicted environmental concentration (PEC) which is 10 times lower than in the American model. While the European figure of 0.01 μg/L has been criticised as lacking "scientific rigour", the American cut-off value may underestimate the total burden to the environment and fail to require an ERA when predicted inputs are not in the expected ranges (Bound and Voulvoulis, 2004). This number was obtained from the U.S. Food and Drug Administration and was recommended by a the Centre for Drug Evaluation and Research (CDER). It was based on the evaluation of some 60 compounds showing "no observable adverse effects" (NOAE) at concentrations below 1μg/L at the point of entry which, assuming a 10-fold dilution results in a 0.1 μg/L value in surface water (Bound and Voulvoulis, 2004). Whether the 60 compounds are representative of the multitude of pharmaceuticals under investigation, or the NOAE levels are sufficiently protective of the environment, or the 10X dilution factor is reasonably applicable is questionable, but the process is sometimes defended as having scientific rigour (Straub, 2002; CSTEE, 2001). A 0.1 μg/L surface concentration corresponds to annual sales of individual drugs of 40 – 50 tonnes, quantities not achieved in the American market with any regularity (Kummerer, 2001). Furthermore, this 0.1 μg/L limit can be used to obtain environmental "category exclusion" status on some drugs, necessitating no further environmental assessment. These numbers imply that most drugs sold in lower quantities would be exempt from ERA requirement, and largely, this is true. The assessment protocol makes allowances for special circumstances such as known toxicity, known effects at lower than 0.1 μg/L concentrations or high K_{ow}. Moreover, there is no requirement to confirm the predicted

environmental concentration (PEC) post-marketing (Jones et al., 2004), and sometimes discrepancies can be considerable. For example, Calamari et al., (2003) showed that the difference between the predicted and the measured environmental concentrations of the lincomycin varied by up to two orders of magnitude. Furthermore, when combining similar active ingredients from several drugs (i.e. NSAIDs), the threshold value may be exceeded; but an ERA would not be triggered. These situations are not addressed in the US system.

Similar criticisms have been levelled at the European assessment protocol. For example, the published (Danish ERA) PEC for ciprofloxacin of < 0.01 µg/L would not have triggered a phase II assessment. However, an assessment was performed by Halling-Sorensen et al. (2000) and a risk quotient well above 1 was determined, indicating a potential environmental hazard. Furthermore, some pharmaceuticals are considered "acutely hazardous in any concentrations" by one agency and relatively harmless by another. For example, the U.S. Resource Conservation and Recovery Act of 1976, considers epinephrine, nicotine, nitroglycerine and warfarin acutely hazardous (P-list) and diethylstilbestrol, mitomycin and cyclophosphamide as less toxic but still of concern (U-list). The substances on both of these lists are not used in sufficiently high amounts to warrant an environmental assessment, but nevertheless, are considered as hazardous waste at the moment of disposal (Daughton 2003).

The second area of divergence between the U.S. and European ERAs is the stage at which the PEC becomes a decision point. The EMEA protocol starts by calculating the expected environmental concentration for each new pharmaceutical and the assessment continues if this concentration exceeds 0.01µg/L in surface waters. In contrast, in the

U.S. system, some pharmaceuticals are automatically exempt from the ERA process. If the drug's physical and chemical properties as well as its environmental depletion mechanisms indicate rapid disintegration, no further testing is required (Bound and Voulvoulis, 2004). The EIC is calculated as a second step, and if it exceeds 0.1 μg/l (which equates to manufacturing levels greater than 40 – 50 tonnes/year), further assessment of log P values is required. Thus, the definition and method of determination of chemical and physical properties (e.g. log P), play a critical role in triggering a risk assessment in the U.S. system.

Risk Assessment for Veterinary Medicines

Veterinary medicinal products (VMP) are of concern because they are used in large quantities especially as growth promoters and are usually excreted with raw sewage which is prone to surface leaching or directly enters receiving waters. In Germany, some veterinary drugs are used in excess of 50 tons per year (Koschorreck et al., 2002).

Perhaps because of the volumes used and the lack of sewage treatment, VMPs are more stringently controlled than human pharmaceuticals. Prior to obtaining marketing authorization, new veterinary medicines must undergo a review of their efficacy, quality, and safety to both human and environmental health (EC, 2001). Mitigation measures to minimize environmental impacts are also applied to agro-pharmaceuticals. These include limits on access to medicated livestock, limits of location of treatment and formalized collection of used dips and other medicated formulations. (Koschorreck et al., 2002).

Agro-pharmaceuticals used primarily for growth promotion are undergoing even stricter controls. In 2001, the European Union issued a total ban on antibiotic growth promoters in animal feed in response to concerns over increasing rates of antibiotic

resistance (Sanderson et al., 2004). The USFDA withdrew approval for the use of Enrofloxacin as a growth promoter in poultry in response to similar concerns (Sanderson et al., 2004). However, the US government (and presumably the Canadian government) does not support a total withdrawal or ban of antibiotic growth promoters as they do not feel that there is enough conclusive evidence to justify these actions (Sanderson et al., 2004). Health Canada's Veterinary Drugs Directorate (VDD) in conjunction with the Pest Management Regulatory Agency are responsible for approving the use of agricultural antimicrobials in Canada. Currently, these organizations are involved in conducting several data gathering and resistance surveillance projects, with the goal of using the data for subsequent policy formulation (Health Canada, 2006a http://www.hc-sc.gc.ca/dhp-mps/vet/faq/faq_amr-ram_e.html). Health Canada's VDD has stated that it "will continue its public involvement and consultation efforts to work within government, with multi-stakeholder groups, and with national and international partners to move forward on this issue" (Health Canada, 2006b http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/index_e.html).

The difference between the U.S. and European regulatory approaches to antibiotic growth promoters arises from the fact that the European agencies have enacted the precautionary principle into law, thereby allowing a contributing cause (and not exclusively the sole cause) of increasing antibiotic resistance to justify regulatory action (Sanderson, 2004). In the U.S., the regulatory agencies require that antibiotic resistance is exclusively linked to AGP in livestock. This approach to decision making (discussed in a later section) is rooted on concepts of weight-of-evidence and scientific proof and their respective definitions. The environmental assessment and management of

pharmaceutical compounds rests to a large degree on the paradigm used for analysis. For this reason, the following section will examine the current approaches to assessment and their implications for management of environmental pharmaceuticals.

The Weight of Evidence and Scientific Proof in Risk Assessment

Scientific proof is often demanded in support of policies involving change from the *status quo* because change is associated with financial or opportunity costs for some portion of society (Oreskes, 2004). As explained in a later section, information must meet a certain standard to be included in the 'scientific corpus'. Very often, multiple lines of evidence converge and a picture of the most accurate interpretation at the time emerges. This is the weight-of-evidence approach. It provides a consensus based on a process of inquiry and evaluation, and is a flexible and dynamic system allowing for constant revision (Oreskes, 2004). Indisputable 'truths' or unequivocal proofs are beyond the limits and intentions of science (Carter, 2000). They are social constructions or interpretations sometimes based on sound and credible science. However, such 'indisputable truths' can often lend credibility to policies and so are in great demand, but no amount of scientific evidence can result in policy. Policy is the result of a political decision ideally informed by credible science (Pal, 2001). But science itself cannot be objective or value-free, it cannot produce an un-arguable 'truth' (Cortner, 2000; Cullen, 1990). For further discussions of the status of scientific knowledge or the weight-of-evidence approach, see the writings of Brewer (1983) or Schneider and Ingram (1997).

In particular, the science of risk assessment is inherently subjective as it has to address conflicting needs and values (Richardson, 2005). These values are often held by opposing stakeholders, transforming the environmental assessment process in either the

stage for the mediation of competing interests, or the input for resolving value-based issues (Daniels and Walker, 1996; Healey, 1997 pp74; Forester, 2000). In other words, various stakeholders may use the process and the results of risk assessment as evidence in support of their particular, value-based point of view. A competing point of view advanced by Elling (2004) advocates the complete separation of the political process from scientific risk assessment. As it will be shown, however, values cannot be extricated from risk assessment, i.e., environmental assessment remains socially contextualized (Richardson, 2005). The question, "Do pharmaceutically active compounds have an ecological impact?" can be answered using scientific concepts and tools, but the extent and nature of subsequent regulatory action is a social policy question answerable in the context of our collective socio-economic values. In an article entitled "*Science and public policy: what's proof got to do with it?*", Naomi Oreskes (2004) examined the aims and limitations of scientifically-based information and explained the connections between scientific proof and the resulting public policy. She concluded that, in issues of environment and health, "there is no need to wait for proof, no need to demand it and no basis to expect it". For example, those who continually demand unequivocal, logically indisputable scientific evidence, become 'recalcitrant actors' on the social stage (Oreskes, 2004). Those refusing to amend farming practices to reduce the need for prophylactic antibiotic growth promoters could certainly fill that role. This line of reasoning brings into sharp focus the need to define the quantity and quality of evidence needed to provide a sound scientific basis for policy formation (see for example, Armelagos et al., 2005; Mayumi and Giampietro, 2005; Allio et al., 2005). The answer to this question may well be a socio-political one.

Principles and Assumptions

The process of assessing risk is based on the acquisition, interpretation and analysis of scientific data (Bridges and Bridges, 2004). As discussed above, this data pertains to the physical and chemical properties of substances, to their actual, potential and predicted effects at various doses and routes of exposure, and to their intrinsic toxicities. Despite concentrated efforts of researchers to provide such information, there remain large data gaps. For example, the chronic effects of substances remain largely un-investigated, as do effects on various species or effects of various mixtures of substances. To address this lack of information, a number of principles and assumptions must be employed (Wandall, 2004). For example, the view that risk assessment should be based on acute toxicology data is such a principle. The concept that lack of acute effects equates to the absence of harm to a target organism is one such assumption. The assumptions required to fill the data gaps can be influenced by values, thereby introducing biases in the scientific process of risk assessment. These principles and assumptions, in turn provide the basis for the currently acceptable but always dynamic understanding of "scientific rigour" as discussed above. When these principles and assumptions are numerical they are often referred to as uncertainty or safety factors (Beck et al., 2001). These factors are often just arbitrary conventions, such as a factor of 10 applied when high-dose to low-dose extrapolation is necessary (Beck et al., 2001).

Although this system to address data gaps is very much still in use, it is not exactly based on the 'absolute scientific proof' approach often in demand. In fact, it has been asserted that uncertainty assessment (and decision making) is entirely outside the scientific field (Aven and Kristensen, 2005). Others have taken this suggestion even

farther, stating that "all environmental science is inherently biased by subjective opinions and values" and therefore outside the realm of the natural sciences (Huesemann, 2002). The extent to which environmental risk assessment should be considered a science is not the focus of this thesis. However, it is important to note that risk assessment does contain an element of subjectivity driven by non-epistemic social values. This social dimension may be reflected in defining and framing hypotheses, choosing methodology or selecting analysis criteria (Brewer, 1983). For example, the issue of pharmaceutical contamination of the environment could be framed as an environmental problem because various ecosystems may sustain damage. However, it could also be framed as a human health problem because the public is concerned with antibiotic resistance, endocrine disruption or ingestion of carcinogenic anti-neoplastics via drinking water. The choice of reference frames is socially constructed and directly influences the subsequent development of policy. The converse is also true. Similar arguments apply to the choice of assessment methodology or analysis criteria. If there are no observable acute effects in toxicity tests of environmental concentrations of tetracycline, why incur the costs of changing farming practices? As long as the current sewage treatment facilities remove 80 to 98% of the target endocrine disruptors, why investigate their concentration in sewage sludge, or introduce any mitigating measures? Hence, both the derivation and acquisition of data and its interpretation and analysis are necessarily value and policy driven (Ruden, 2005).

Socrates, Kuhn and Post-Normal Science

Why concern ourselves with the views of philosophers on the problem of pharmaceuticals in the environment? Because knowing that pharmaceuticals occur in our environment is just an abstract piece of information. We need to critically examine this

information and for that we need some of the tools developed by philosophers. Further, we need to assess the relevance of environmental pharmaceutical contamination to the human-ecological system, and for that, we need to know the context in which we place this issue. This knowledge cannot be acquired exclusively by quantitative analysis. For example, what does it mean if the risk from pharmaceutical contamination of the environment has been quantified to (hypothetically) 1 in 2500?

The purpose of the data presented in the preceding two chapters was to highlight successes and deficiencies of the current approach to the issue of pharmaceutical contamination of the environment. Understanding these issues will facilitate a rational approach to decision making, and perhaps to the introduction of rationally-founded mitigation strategies. In the words of one author, "quantitative analysis is essentially worthless if it is not translated into effective policy" (Anderson, 1988). However, effective policy cannot be carried out in the absence of a clear understanding of the stakes, stakeholders, goals and underlying assumptions involved (Pal, 2001). For this reason, a comprehensive assessment of the risk posed by environmental pharmaceuticals necessitates a clear understanding of the epistemological foundations of risk assessment and science. Socrates stated that "scientists are those that know about their own ignorance" (Giampietro, 2006). In the context of risk assessment, a clear understanding of the extent to which risk assessment is a scientific endeavour is required. What is meant by 'scientific', is itself under debate.

Current literature distinguishes between Normal and Post-Normal Science. The concept of Normal Science was introduced by Kuhn in 1962 in response to a fierce debate over the goals and stakes in science. He defined Normal Science as "research

firmly based upon one or more past scientific achievements, achievements that some particular scientific community acknowledges for a time as supplying the foundation for its further practice" (Kuhn, 1962). The 'science' in science-based risk assessment usually refers to this understanding of science. Its connotations include objectivity, repeatability and accuracy. Many proponents of normal science believe that it is possible to define, in absolute terms 'good' and 'bad' for consumers, citizens and society. Furthermore, they believe that they can know such a definition and that its attributes will not change later in time. Lastly, they believe that issues of uncertainty and/or ignorance can be resolved by more and/or better research. For example, this view predominated in the design of the 'consensual style' environmental risk assessment process practiced in Europe until the end of the 20th century (see the section on environmental risk assessments and value judgements below). This view is still apparent in the delineation of absolute limits of exposure to various chemicals set either by Health Canada or Environment Canada (http://www.hc-sc.gc.ca/ewh-semt/pubs/air/exposure-exposition/index_e.html). Lastly, the strong demand by some groups for incontestable evidence in support of one point of view or another, may be rooted in this view of science.

The concept of Post-Normal Science describes fields of science in which "uncontested legitimization of a substantive problem structuring" cannot be achieved (Giampietro et al., 2006). It emphasizes system uncertainty and decision stakes within the scientific enterprise, thereby challenging "centuries of conventional wisdom for science, in which uncertainty was tamed, ignorance suppressed, and the supposedly value-free character of science proclaimed as a great value" (Ravetz and Funtowicz, 1999). This concept introduces the notion that "ignorance about the future is

unavoidable" and that concepts such as health, quality of life or sustainability cannot be substantively defined (Giampietro et al., 2006). More precisely, Post-Normal Science applies to situations where "typically facts are uncertain, values in dispute, stakes high, and decisions urgent" (Ravetz, 1999). The following pages will show that risk assessment meets all of the above four criteria and can thus be categorized as a post-normal science.

Risk assessment as a Post-Normal Science

Facts are uncertain

The occurrence, concentration and effects of pharmaceutical contaminants in the environment are poorly understood. This lack of information is often referred to as data gaps. Data gaps occur for several reasons, but the most relevant one is that it is impossible to provide unequivocal data for every chemical, every chemical combination and every specific situation (Jones et al., 2004). Furthermore, it is comparatively easier to prove that some effect exists than that it does not (Vainio and Tomatis, 1985; Ruden, 2005). This phenomenon is related to the factors involved in achieving statistical relevance such as the magnitude of the effect sought, the level of biological variability in the system, the size of the study group and the statistical significance that is desired. For example, Weinberg (1972) estimated that a study would need 8,000,000,000 mice in order to detect an increased mutation rate of 0.5% (at the 95% confidence level) following a particular X-ray dosage. In epidemiology, a rule of thumb is that only effects greater than 10% can be reliably detected (Ruden, 2005). Chronic and sub-chronic studies on the evolutionary fitness of organisms and/or ecosystems are clearly investigating effects below the 10% limit. Hormetic and bimodal effects at very low

doses such as those associated with estrone (E1) and 17α-ethinylestradiol (EE2) are also below that limit. In fact, the effects of pharmaceutical contaminants in the environment are generally believed to be below the 10% limit and therefore are difficult to interpret by statistical analysis. The uncertainty factors used to handle the information gaps further decrease the reliability of the data.

Values are in dispute

Environmental Risk Assessment and Value Judgements

To some researchers, the introduction of value judgements into the arena of environmental risk assessment may seem inappropriate. However, to some philosophers of science the suggestion that risk assessment and even science itself is value-free is inappropriate (Hokstad and Steiro, 2006; Aven and Kristensen, 2005; Wandall, 2004). For example, decades of debate over the nature of risk has resulted in the advancement of at least 26 definitions of 'risk', almost all of which include value-based wording (loss, adverse effects, threat, opportunity, something-different-to-different people) (Macgill and Siu, 2005). In environmental science, Huesemann (2002) identifies at least three types of biases or values: personal, institutional and socio-cultural. The selection of chemicals, methodology for assessment in terms of chronic or acute testing and definition of effects are all subjective choices based, in his opinion, on socio-cultural biases.

The concept of value judgements in the science of environmental toxicology has been recognised in law in the European Community's guidance documents on risk assessment (EMEA, 2005). The recognition of value judgements was initially linked to the need for greater transparency in ERA procedures. These documents not only offer a greater transparency of the assessment process but also lay out the qualifications of the

'scientific experts' whose opinions form the basis of management policies (Petry et al., 2005). The traditional risk assessment protocol introduced in Europe in 1842 is described by Lofstedt (2004) as the 'consensual style'. It provided a stage for regulators, industry and trade unions to formulate regulatory decisions in complete secrecy (Petry et al, 2005). Following a number of regulatory scandals in the early 1990 (tainted blood in France, BSE in England and dioxins in Belgium), a new 'participatory-transparent' system was introduced (Lofstedt, 2004; Petry et al., 2005). This new system features greater public and stakeholder participation, transparency in terms of regulatory strategies, focus on regulatory impact analysis, and greater use of the precautionary principle (EMEA, 2005; Petry et al., 2005). These features are highlighted in the newly-released guidelines for environmental risk assessment of pharmaceuticals (EMEA, 2005). Furthermore, in an effort to address issues of trust (or lack thereof) associated with scientific evidence, the guidelines also delineate exactly what qualifies as expertise, and who qualifies as an expert (Dietrich et al., 2005). This attempt to move the scientist to an unbiased position could be enhanced by a deliberate disclosure of the assumptions and values applicable to each environmental risk assessment. Workers in this field have identified two types of values which often influence the scientific sphere.

Types of values

A value is "a characteristic in virtue of which something is considered valuable" and we attach value to things we consider "positive and desirable; that it is worth making an effort to realise or to achieve [...] usually considered as opposed to 'fact'" (Wandall, 2004). Following this definition, Wandall (2004) has identified two types of values:

epistemic, highly dependent on the aim to be achieved, and non-epistemic, highly dependent on personal preferences.

Epistemic values can be universally agreed upon, as they rely heavily on the ultimate goal to be reached. As long as the interested parties can agree on the goal to be achieved, they can usually agree on the system of values to be used (Wandall, 2004). For example, the choice of acute toxicity testing of a given compound can be seen as an epistemic value if the explicit and sole goal is to detect acute effects (Wandall, 2004). It could also be seen as a non-epistemic value, a value driven by socio-cultural preferences (Huesemann, 2002), when the results are used to indicate the risk from sub-chronic exposure. In other words, epistemic values can become non-epistemic when the goal changes. Moreover, the goal can change depending on how it is framed.

Examples of epistemic values in science include coherence with accepted hypotheses, simplicity, explanatory power, fruitfulness (ability to expand the scientific knowledge base), conservative burden of proof and the decision to include or reject a test hypothesis in the 'scientific corpus' (set of statements considered true) (Wandall, 2004). Values can be injected into the factual basis of risk assessment. For example, risk assessment incorporates high levels of uncertainty arising from data gaps. We really do not know what effect will a water concentration of 4 ng/L E1 have on the resident fish populations when the ambient water temperature is 1.5°C above seasonal average. We know equally little about the sub-chronic effect of 0.4 ng/L CBZ on that population of fish. To address these problems, risk assessors rely on established assumptions; the choice to use a particular assumption and incorporate it into an assessment is a value judgement (Wandall, 2004), a judgement similar to the one other scientists make in

incorporating a new piece of evidence into the 'scientific corpus' (Ruden, 2005). Alternatively, non-epistemic values can be attached to the type and topic of research and to the type of standard methods to be applied. The choice of research topic may be restricted based on personal or social values, and the type of methods may be restricted based on practical or ethical values. For example, it is hardly justifiable to conduct an experiment requiring 8,000,000,000 mice (Weinberg, 1972), and hardly practical to conduct a chronic toxicity and carcinogenicity experiment costing an average of 1,000,000 € (Ruden, 2005). It is equally impractical to experimentally investigate every combination of pharmaceuticals in the aquatic environment (Huesemann, 2005). Furthermore, the drive to finding alternatives to *in-vivo* testing systems discussed in chapter 2 is fuelled in part by the ethical considerations of using mammals and fish for toxicity testing (Garthoff, 2005).

In general, risk assessment shares the values suggested above. That is, epistemic values are typically applied in the science-based field of risk evaluation and assessment. For example, new information is still evaluated for coherence with previously accepted hypotheses, for simplicity and for predictive power. The detailed protocol for performing toxicity studies (regardless of the subsequent use of the results) is based on the scientific method and is consistent with GLP (Good Laboratory Practice) guidelines (Ballantyne et al., 2000 ch. 1,2, 15, 21). Lastly, the conservative burden of proof is still applied in most areas. However, those epistemic values may need revision in order to reflect the divergent goals of science and risk assessment. Furthermore, risk assessment necessarily incorporates non-epistemic values as well, so far as it is defined as an expression of "uncertainty about the world seen through the eyes of the assessor and based on some

background information and knowledge" (Aven and Kristensen, 2005) or a "concept referring to human actions or events leading to consequences affecting what we humans value" (Hokstad and Steiro, 2006).

If the conclusion can be drawn that science, including that of risk assessment is not value-free, than the moral may be that scientists can and should choose (or at least state) those values which most strongly influence their work very carefully (Wandall, 2004). It is suggested that those epistemic values explained above should continue to influence the scientific approach to risk assessment. And it is reasonable to expect that scientists demand those values which influence their work to be rationally justifiable (Wandall, 2004). For risk assessors, these values may still be in dispute.

The decisions are urgent

Risk assessment decisions must be delivered in a timely manner. While other scientists may defer a decision until sufficient evidence accumulates to form a 'scientifically sound' conclusion, risk assessors must decide within weeks to months. That means that even in the absence of conclusive evidence, a substance must be treated *as if* it were toxic or non-toxic, depending, ultimately on assumptions, values and policy (Ruden, 2005).

A central condition in the definition of epistemic values is that they serve a well-defined, unanimously agreed-upon goal. If risk assessment is science-based, should it not follow that its values would be similar to those of other branches of science? Yes, to a large degree, but not entirely. The one difference identifiable in this context is the fact that science and risk assessment have somewhat divergent goals (Wandall, 2004). In

order to avoid accepting a false hypothesis, most scientists value a conservative burden of proof. However, for risk assessors, it is equally important to avoid rejecting a true hypothesis – that is, to avoid concluding that a substance is harmless when in fact it is not (Tallacchini, 2005; Wandall, 2004). A conservative burden of proof, while avoiding false positives, necessarily increases the frequency of false negatives (Wandall, 2004). The statistical tests used in scientific risk assessment reflect the conservative burden of proof most often in the confidence intervals. While a high confidence limit is an assurance that probably accidental correlations will not be accepted (type I or false positives), it is also an indication that probably real correlation may have been rejected (type II error or false negatives) (Ravetz, 2004). Both the manufacturers of new chemicals and the proponents of minimal environmental damage must follow the same scientific approaches discussed above. However, under a conservative burden of proof, it is easy to show that there are no immediate, observable effects, and much more difficult to show that minor, delayed and obscure effects may occur. In some cases, demanding incontestable proof in a system designed to protect against over-sensitivity, may give "aid and assistance" to polluters until it is unequivocally proven that their actions are harmful (Ravetz, 2004). For example, until recently, it was considered safe to treat livestock with antibiotic growth promoters (AGP). I believe that the development of antibiotic resistance associated with the indiscriminate use of these agents was overlooked partly because the conservative burden of proof approach required more data. So the practice of feeding antibiotics to animals continues until either scientists accumulate sufficient proof to show it is harmful or regulators reverse the conservative burden of proof. (Currently, there is not sufficient hard evidence of the harms posed by antibiotic growth promoters to justify

their withdrawal from the market, but some jurisdictions (the EU) have reversed the burden of proof so antibiotic growth promoters have been banned) Sanderson et al., (2004) may be thinking along similar lines when they assert that the EU ban on antibiotic growth promoters is a reflection of legalized precaution enshrined in the European environmental system.

This analysis leads to the conclusion that in risk assessment, the aims of reliability and accuracy could be achieved in a timely fashion by reducing the burden-of-proof requirement. However, this necessitates the introduction of the precautionary principle.

Precautionary Principle

The precautionary principle (PP) was introduced in the 1992 Rio Declaration on Environment and Development. It states that to "protect the environment; the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (Rio Declaration, 1992 http://www.un.org/cyberschoolbus/peace/earthsummit.htm). The PP has two major components: 1) policy should be initiated when the scientific evidence indicating "likely and substantial" harm is "limited but plausible and credible", and 2) the burden of proof should be reversed, to indicate the absence of risk rather than its presence (Grandjean et al., 2004). On the basis of these tenets, the precautionary principle has been defined in a number of ways (see Sandin, 1999) but all definitions reflect the concepts of anticipatory action and acknowledgment of uncertainty (Tickner and Geiser, 2004). For example, the US refusal to ban AGPs is based on a regulatory framework that aims to identify the

quantifiable cause (scientifically challenging), not merely a cause of bacterial resistance (Sanderson et al., 2004). Thus, the conservative burden of proof approach used in the US system makes is difficult to either acknowledge uncertainty or take anticipatory action, hence, AGPs are still in production. In Canada, the Precautionary Principle is acknowledged in the 1999 Canadian Environmental Protection Act (CEPA) and the 1995 Oceans Act, but there are no set criteria for triggering or implementing it (http://www.ceass-acee.gc.ca/015/0002/0007/7_e.htm).

The debate on the Precautionary Principle continues roughly along geographic boundaries, with the PPs virtues being espoused in the European Union, and its limitations being highlighted in the United States. Proponents of the PP suggest that it provides a framework to address persistent issues in toxicology. These issues include a) the definition and applicability of the threshold concept to carcinogenicity, b) the uncertainties related to the mode of action, variable and long term exposures, and effects methodologies (acute vs. chronic toxicity testing) and c) the effects of new classes of pollutants singly or in combination (Vineis, 2005; Grandjean, 2005). Opponents of PP decry what they see as lack of scientific rigour. They point out that the implementation of the principle can be paralyzing, as it sets no guidelines for the minimum level of suspicion required to implement it. This, in itself, is a value-driven criticism as it rests on the assumption that a minimum level can/should exist. In addition, the PP is criticized because risks are often evaluated independent of associated benefits (Vineis, 2005). Further, critics claim that the PP responds to and nourishes the public's irrational fears. This direction of argument rests on Hans Jonas' view of the 'heuristics of fear' which states that in the face of scientific uncertainty, the "prophecy of doom" supersedes the

"prediction of hope" (Hans Jonas, 1985; Tallacchini, 2005). This is an interesting approach as it shifts the value system in environmental science from an epistemic to a cultural/psychological one (Tallacchini, 2005).

Evaluation of risk and benefits has been incorporated into new approaches to risk assessment discussed earlier. This issue is expanded upon in the section on integrated risk assessment below. In an article entitled "*The precautionary principle stimulus for solutions- and alternatives-based environmental policy*", Tickner and Geiser (2004) discuss the advent of alternatives assessment as part of a new paradigm of environmental policy rooted in solutions rather than in problems. This is the foundation of the pollution prevention approach to the management of pharmaceutical contamination discussed in chapter 2.

The precautionary principle continues to be the focus of intense debate involving arguments such as the ones listed above. However, the PP is also a political principle which "considers certain risks inconsistent with the high level of protection chosen for the [European] Community...an eminently political responsibility" (CEC, 2000). This political principle has not been fully adopted in the United States and Canada. Consequently, the debate on the merits and drawbacks of this approach, of the danger posed by each contaminant drug, and of the methodology used to study it are consistent with the prevailing view of environmental management. The disagreement may not necessarily be about the specific risk posed by pharmaceuticals to the system, but rather about the political and social meaning attached to that risk.

On an even more fundamental level, these views address the contract between science and governance. This topic has been treated in elaborate detail elsewhere (see for

example, Nowotny et al., 2001, and Liberatore and Funtowicz, 2003). It has also been a recurring theme throughout this chapter – what are we willing to do with the information, even if we do not understand it completely? In essence, the contract attempts to reconcile the intrinsic incompleteness of science with the need to make immediate policy decisions (Tallacchini 2005).

Assuming that the PP approach is implemented in some form, there is disagreement about the stage at which it should be applied during an ERA (Wandall, 2004). Does it belong in initial stages (problem formulation/data gathering), the later stages (analysis and risk characterization), or does it belong entirely in the field of risk management and policy? If policies are informed by the results of risk assessments, those assessments must be performed from the same platform. That is, they must have the same common denominator so policies acceptable to all stakeholders can be formulated. In other words, the value systems that underlie risk management must be reflected to some degree in the risk assessment phase in order to allow the transfer of scientific information between the two areas (Suter II et al., 2005). This suggests that precaution must be applied early in the risk assessment process as the outcomes are often irreversible (Wandall, 2004). For example, if a substance is assessed as non-toxic, but further use/research proves it to be toxic, the effects cannot be reversed. Some of the effects may be mitigated, but the damage to the affected individuals cannot easily be eliminated. The false negatives (type II errors), cannot be reversed at the later stages (the substance either is or is not toxic; further research can only affect our future decisions, not those made in the past). Precaution in the risk management phase is simply too late (Wandall, 2004). Consider the example of diclofenac use in India (Oaks et al., 2004).

Although efforts could be mounted to restock the vulture populations to pre-diclofenac use levels, the individual vultures that died cannot be brought back. Those efforts do not negate the occurrence of a type II error.

In conclusion, the PP promotes the application of scientific knowledge while acknowledging the value-laden dimension of science. It does so by increasing the legitimacy and objectivity of shared decisions (Tallacchini, 2005).

The relationship between risk assessment and risk management

Examining the environmental risk assessments available in the published literature, one finds that many of these assessments do not include a discussion of values, costs, benefits or contextual settings associated with their results. Indeed, there is a traditional divide between the fields of risk assessment and risk management, and questions of values or contextual settings usually fall on the side of the risk managers or policy makers (Pal, 2001). However, there is another view which finds common ground between the two fields. This view is expressed pictorially in the diagram below, and places the last stage of risk assessment within both the risk manager's and risk assessor's domain.

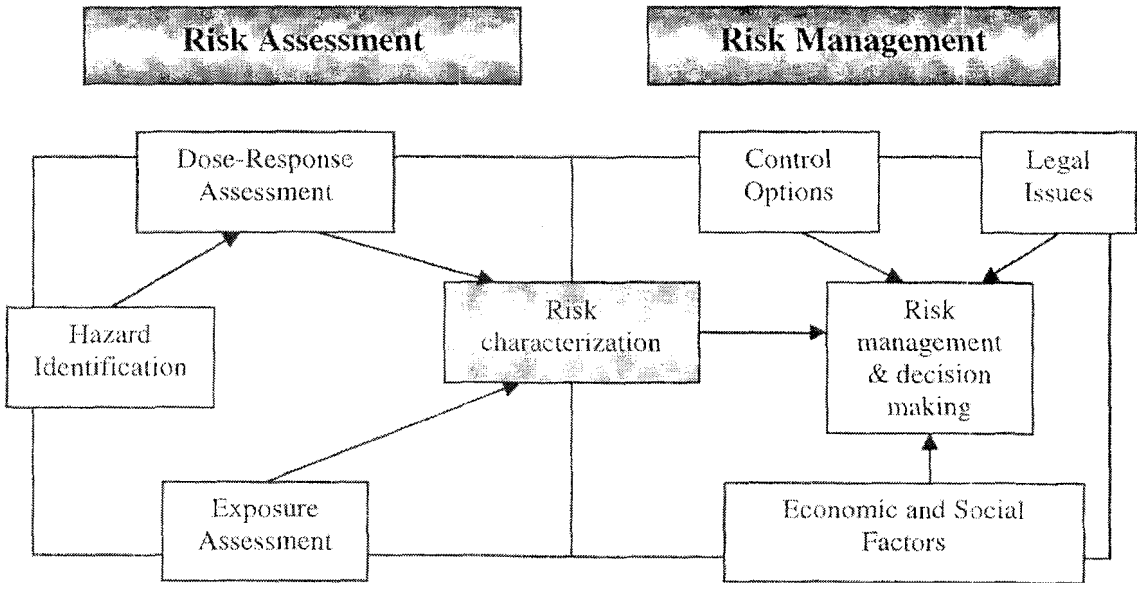


Figure 13: Overlap of risk assessment and risk management via the fourth step in the assessment process, risk characterization. Revised from Montague, 2005

How much do risk assessors directly influence risk managers? The current protocol for conducting risk assessments offers useful data that informs regulators and decision makers. However, this same system can, at times, offer conflicting and incomplete information. Montague (2004) observed that risk assessors, in the normal course of their work, often:

- Evaluate one single option in the absence of alternatives
- Evaluate effects borne by others, often without their informed consent
- Do not assess the benefits/or lack of thereof to those bearing the cost
- Constrain decisions to science-based knowledge, excluding other non-epistemic information (historical, ethical or personal preferences), thereby reducing public participation
- May facilitate the development of a false sense of security by a) providing a numerical result which often has the connotation of "sound science", and b) producing a statement originating from 'professional, expert' investigation.

Montague may be thinking along the same lines as the proponents of the new integrated framework for health and ecological risk assessment in raising concerns about the lack of alternatives or the cost and benefits to those who bear the potential hazard (i.e. Tickner and Geiser, 2004; Suter II et al., 3003; Suter II et al, 2005). These concepts will be discussed in the next section.

Montague's contention is that risk assessors bear responsibility for the policy implications of their work (Montague, 2005). This view is reminiscent of Grandjean's assertion that it is the responsibility of toxicologists to accurately translate science into policy protective of human health (Grandjean, 2005). Even in the absence of complete data, should risk assessors still be expected to take responsibility for their results? Montague and others would argue that the answer is yes, in the same way that a public health official would have the ethical responsibility to protect the public from harm (Montague, 2005; Grandjean, 2003). This responsibility is even more difficult to accept when the products of risk assessors are misused or used out of context (for further discussion on this topic see Grandjean, 2005; Melnick, 2001; Crump, 1996 and Gori, 1996; Ong and Glantz, 2001 and Chalmers, 1990; Chan et al, 2004 and Markowitz and Rosner, 2002 and Greenberg, 2003; Axelson et al., 2003). A possible solution to this issue is to apply the 'doctrine of the duty-to-warn' (Montague, 2005), which requires a clear warning conveying the nature of potential harm and steps required for minimizing it (Falkof, 1995). This refers to the duty to warn about the risk assessment document (i.e. no observable acute effects does not mean no harm), not about the difficulties encountered by assessors in developing it.

Implicit in the preceding discussion is the fact that toxicology in general, and environmental toxicology in particular, are non-precautionary. The first two chapters of this thesis address the approach to toxicological information gathering and processing. Table 6 summarizes the factors that bias toxicology towards Type II errors. Many authors assess their studies for protection against type I errors (false positives), not against type II errors (false negatives) which would be an approach more protective of consumers (Sanderson and Petersen, 2002; Hornbaker and Cullen, 2003; Sanderson et al., 2004). This is why the argument has been made that precaution (i.e. minimizing type II errors) should be incorporated in the risk-assessment stage and not be reserved for the risk-management phase (Wandall, 2004). The existence of these problems is seen by Grandjean (2005) as evidence that "toxicology has failed the purpose of science in society by striving to reach only the limited goals of solving simplified riddles and in recognizing, rather than exploring uncertainty". This is a rather strong criticism, but it highlights one of the themes of this work: continued scientific investigation of pharmaceutical compounds as environmental contaminants will not necessarily result in clear and incontestable policies. Such policies are informed and supported by that evaluation, but require a broader approach where pharmaceutical contamination is placed in a human - ecological context (Doll, 2002; Sanderson et al., 2004). That context is the subject of the next section.

Table 6: Scientific features that may bias toxicology toward missing a true association (false negative) (revised from Grandjean, 2004)

Toxicological studies in general
Low statistical power
Use of 5% probability level (conservative burden of proof)
Use of 20% probability level to minimize risk of type II error
Vigilance against false alarms (from both researchers and outside interests)
Epidemiological studies of toxicants
Inappropriate control group (difficult to replicate exact experimental conditions)
Exposure misclassification
Inadequate follow-up of exposed subjects (cases lost to follow-up, follow-up too short)
Experimental toxicology
Exposure to single substances (no mixtures of stressors or of substances)
Limited number of dose levels
Exposure duration less than lifetime
Standard effect measures (may overlook some effects, particularly sub-acute ones)
Inbred strains to limit genetic variability (extrapolation creates additional uncertainty)

Integrated human health and ecological risk assessments

Integrated risk assessment (IRA) has its roots in the traditional approach to human health and ecological risk assessment strategies (HHRA and ERA, respectively). The previous approach maintained a clear separation between the two types of risk assessments, thereby perpetuating distinct terminology, methodology and data analysis (Bridges, 2003; Bridges and Bridges, 2004). It made it difficult for individuals from outside the field to understand and use the assessment information. Consequently, a new

framework for integrated human health and ecological risk assessment has been developed, jointly by the World Health Organization's International Program on Chemical Safety, the United States Environmental Protection Agency and the Organization for Economic Cooperation and Development (WHO 2001; Suter et al., 2003). These agencies have defined IRA as the "assessment of risks to human and non-human receptors from chemical, physical and biological agents. It includes assessments that predict the effects of proposed actions and those that estimate the ongoing effects of past actions at particular places and assessments of risks from hazardous agents independent of location. Finally, integration extends across all phases of the assessment process from the planning of the assessment to the decision making" (WHO, 2001). IRAs aim specifically at 1) improving the efficiency of information exchange between risk assessors and risk managers, and 2) providing a complete and coherent basis for the decision making process (Sutter et al., 2005). These aims should ultimately be met by 1) decreasing reliance on single-chemical/single-dose assessments, 2) increasing analysis of multi-chemical, multimedia, multi-route and multi-species exposures (Sekizawa and Tanabe, 2005), and 3) defining and identifying the commonalities between risk assessment and risk management. Further, the integration paradigm must be assimilated into all phases of risk assessment, starting with the problem formulation stage and continuing through the dose and exposure assessment, data analysis and risk characterization stages (Sekizawa and Tanabe, 2005). For example, an integrated risk assessment for endocrine disrupting compounds (EDC) would acknowledge that sewage effluent is just one of the potential sources of EDC in the environment (other sources could include industrial/agricultural contamination and natural phytoestrogens).

Subsequent calculations of concentrations, effects and/or interactions would be performed within this new paradigm. This approach would address the multi-chemical, multi-exposure and multi-media conditions. Duplication of time, effort and resources would be avoided by cross-reading of information from both human and ecological studies. This type of information sharing may be particularly relevant to decipher the mode of action of various EDCs (Bridges and Bridges, 2004). For example, in the early years of DDT use, it was widely thought that its main effects on humans were on the developing nervous system (IPCS, 1979). Later, ecological studies noted hormone-base effects in seagulls, particularly egg-shell thinning (IPCS, 1989). It is now thought that both humans and wildlife may show effects arising from common mechanisms of action and common routes of exposure (Sekizawa and Tanabe, 2005). Further benefits of the IRA approach include the development of better testing systems and of a common basis for comparing and therefore valuing human and animal risks (Bridges and Bridges, 2004). For example, in a study evaluating endocrine disrupting effects of some organochlorine compounds, comparing body burdens and tissue distribution during the exposure assessment stage presents a more integrated result. These measurements account for multiple routes of exposure (Sekizawa and Tanabe, 2005). Similarly, Gurjar and Mohan (2003) conducted an integrated risk analysis of the acute risks associated with accidental release of a hazardous substance. Their case study includes variables not usually associated with ERAs, such as background risk and individual risk factors (Gurjar and Mohan, 2003). The analysis is intentionally simplified as the intention of the study was to show how IRA can be applied. However, IRAs will face some difficult technical challenges (e.g. harmonization of terminology and assessment methodology, cross-

species interpolation, etc) as well as financial challenges (Bridges and Bridges, 2004). Several extensive reviews of the IRA process have been published (see for example Suter II et al., 2003a; Munns et al., 2003; Sutter II et al., 2003b and Suter II et al., 2003c).

The concept of integration between human health and ecological data is particularly relevant to the issue of pharmaceutical compounds in the environment, because the data available are predominantly related to human health effects. However, by the same token, the ecological data now being acquired can expand the limits of knowledge on the effects of pharmaceuticals both on humans and other animal species. This approach would complement but not replace classical research.

It is also notable that the trend towards an integrated risk assessment paradigm is consistent with a larger movement in the field of risk assessment theory aimed at integrating the conventional risk assessment process with social determinants of ecological health (Rapport and Singh, 2005). The contemporary EcoHealth framework for conducting State of the Environment reports (SOER) employed in Canada, exemplifies the wider applications of integrated environmental evaluation. This framework rests on the original Pressure-Stress-Response model, first introduced in 1979 by Statistics Canada and later widely adopted by the OECD (Forget and Lebel, 2001). This model acknowledges the insufficiency of market-based environmental management strategies and allows for a greater interdependence between the human and ecological systems (Rapport and Singh, 2005). It advances the concept of integrated environment and human health evaluation of environmental issues and promotes the accommodation of both anthropocentric and ecocentric needs (Rapport and Sing, 2005). Lastly, it provides a platform from which policies may be evaluated for the unintended

consequences of 'sub-optimal' decisions, for example, the introduction of restricted-use risk management measures for environmentally hazardous human pharmaceuticals. I believe that such policies could have negative consequences not only on human health and the health care system, but also on the prevailing social system of beliefs which values equity of access to health opportunities (Health Canada Act, 1985). The two social values – access to health care and environmental protection are in obvious conflict in this case. The means to resolving this conflict is a social question, but the governing value system must be acknowledged. An IRA may provide a platform or basis upon which such conflicts may be resolved.

Integration is part of a larger scientific movement aimed at converging independent lines of inquiry (Kerkhoff, 2005). This movement was analyzed by Gibbons et al. in 1994 who proposed the distinctions between Mode-1 (traditional, independent) and Mode-2 (integrative) sciences. Echoes of this line of reasoning appear in a book by Nowotny and colleagues dealing with 'segregated' and 'integrated' means of scientific discovery (Notowtny et al., 2001) and in Ziman's work on 'post-academic' science (Ziman, 2000). All of these authors contend that the integrated approach is better suited to address a field of study which is necessarily value-driven.

Integrated risk assessment: framework and example

Several recent articles explain in some detail the technical aspects of IRAs (see for example, Vethaak et al., 2005; Bridges and Bridges, 2004; Munns et al., 2003). The need for integration of information is consistent with the issues advanced in the first two chapters relating to the heavy reliance on single chemical testing, lack of sub-chronic and chronic toxicity studies and outdated definitions of hydrophobicity and environmental

persistence. This implies that those assessments, valuable as they are in providing sets of reproducible data, do not extend to analysing risk management options or to contextualizing those options. The second aim of an IRA may fill this gap; that of devising a common framework upon which the interests of various stakeholders could be balanced. Figure 14 provides an example of such an assessment proposed by Sekizawa and colleagues in 2003. This IRA approach addresses some of the issues discussed in this thesis: 1) it examines multiple sources and multiple media, 2) it considers different levels on the food web 3) it identifies effects on multiple levels of biological organization following acute, chronic and repeated exposure, and 4) it accounts for varying social and economic values. However, the cost of implementing this system is considerable, and cost is in fact, the main problem associated with the implementation of the IRA (Bridges and Bridges, 2004). Others include others include agreement on common exposure models and identification of causality and a common weight-of-evidence framework.

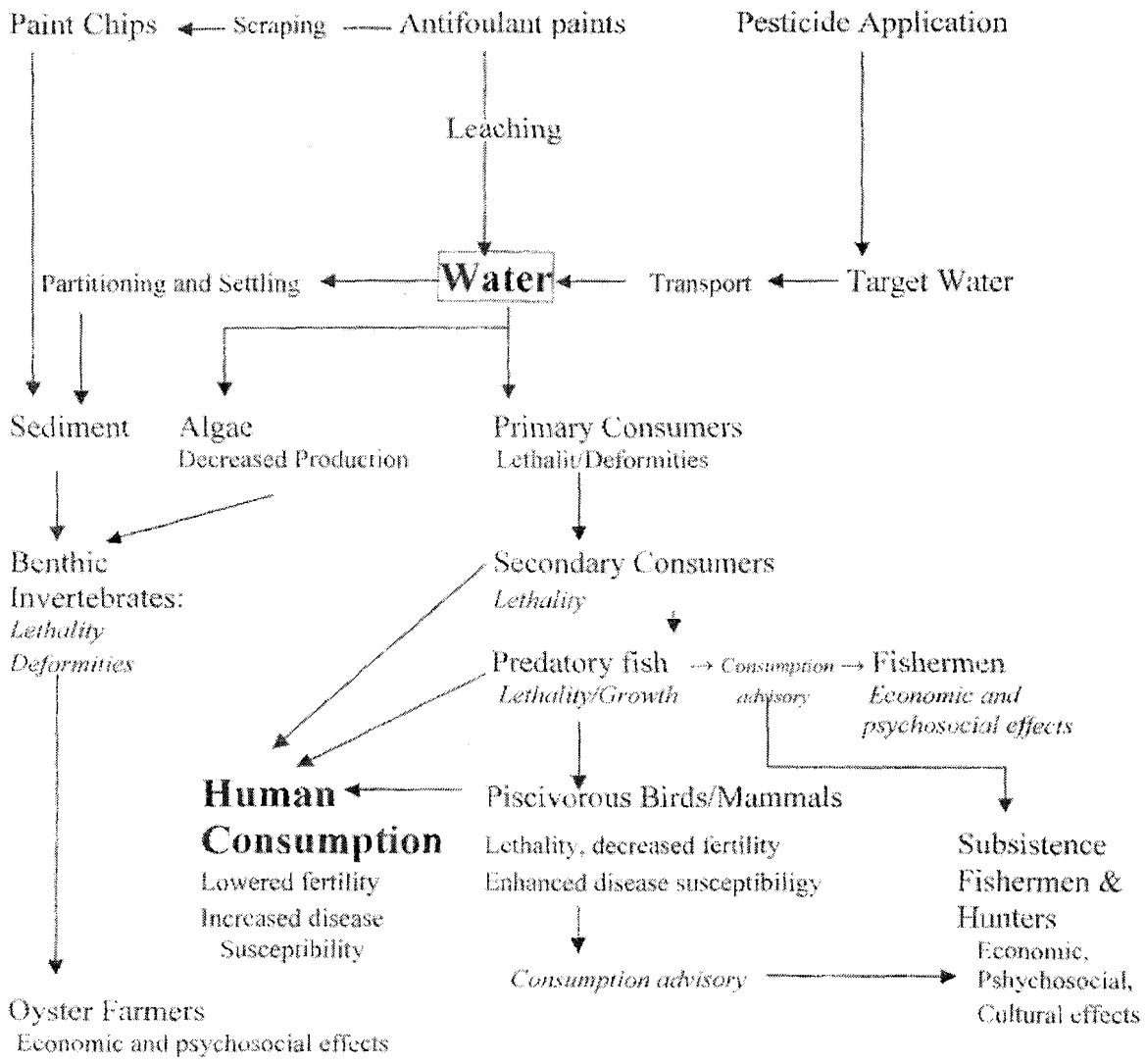


Figure 14: Conceptual integrated risk assessment approach for exposure/effects of humans and some marine animals to tributyltin and triphenyltin compounds. Revised from Sekizawa et al., 2003

An inclusive framework for the analysis of risk in environmental toxicology confers some benefits to the resulting analysis. These include:

- better information exchange between involved parties (scientists, legislators, public) leading to more comprehensive understanding of factual information pertaining to concentrations, doses, receptors, effects, mechanisms of action, etc.

- appropriate inputs to decision making bodies by providing common scenarios and assumptions (the same value-driven assumptions from an earlier section) so that risks and benefits can be compared without the need for arbitrary extrapolation. This would allow assessors and managers to agree on the extent/size of the system to be evaluated and would prevent the introduction of short-sighted policies (Suter II et al., 2005).
- assessment of environmentally-mediated effects not exclusively on human health, but on human welfare. These include psychological, social and/or economic costs/benefits associated with the pollution of a given area. This concepts are not addressed by human health risk assessors because they have only indirect impacts on clinical aspects of human health, and are also not addressed by environmental risk assessors as they do not impact directly on the environment (for a more detailed discussion of this point see Stephen and Ribble, 2001; DiGiulio and Benson, 2002 and Suter II et al., 2005).
- assessment of the interactive nature of many environmental risks therefore allowing for simultaneous evaluation of alternatives (Bridges and Bridges, 2005). Particularly, variance and uncertainty can be presented in the same terms for all endpoints studied, and spatial, temporal and biological organization scales can be standardized to suit the particular study system (Sutter et al., 2005). (For example, one would study chronic effects at the organism level for EE2 exposure, not just acute, enzymatic or hormonal concentration effects. The acute (or enzymatic) observations would be performed as part of the study, but could not necessarily stand alone. This addresses the temporal and biological organization scales).
- understanding the needs of decision makers and stakeholders simultaneously, and identify modes of interaction between the risk assessors, managers and stakeholders appropriate to the social context.
- decreased duplication of research thereby reducing waste of time, money, effort, etc.

- opportunity for risk assessors to move freely across the entire spectrum of biological organization, from the molecular effects to the effects on community survival and even beyond to the effects on human welfare (Suter et al., 2005).

Contextual placing of pharmaceutical contaminants

Despite the forgoing, there is virtually no literature placing the problem of pharmaceutical contamination of the environment in a larger, social and economic context. Consider the following:

- all of the numerical risk factors and potential hazard evaluations to date include large levels of uncertainty.
- where is the transition between the environmental impact and the impact on human health? On human welfare? How are those impacts measured? What kinds of values do we attach to each of those impacts?
- if we decide to manage this problem so we avert a (real or perceived) crisis (assuming we can define what constitutes a crisis), what will out guiding principles be? Precedence for patient benefit? Precedence for all patients and for all benefits? Will our management plan consider economic benefits? For whom – the care facilities? The farming industry? The pharmaceutical industry? The building developers and municipalities?

These questions only marginally qualify as issues in formal risk management; they have more in common with the socio-economic sciences. Yet, it appears that toxicologists have a responsibility to ensure that their work is translated into appropriate policy (Grandjean, 2005) or at least to facilitate that transition. In that case, it may be within the realm of environmental risk assessors to initiate cross-discipline collaboration (with other scientists, risk managers, public) in presenting their results. That collaboration can occur within framework for IRA presented above, or it can take the

form of charts and tables such as the ones presented below. It must, however, include the efforts of scientists other than environmental risk assessors, especially in the areas of economic analysis and numerical harmonization of the risk factors. This may facilitate comparisons between varying and often conflicting cost, benefits and/or values. (Note that these tables are presented as the end result of detailed risk analysis so the derivation of the costs and benefits is based on available scientific, social and economic data.

Table 7: Sample evaluation of proposed mitigation strategies to address the use of antibiotics as growth promoters in the animal farming industry[1].

GOALS Both the goals and their priority must be determined separately	CRITERIA Evaluate each alternative based on these considerations	Alternatives considered (Any number of these may be considered)		
		MAINTAIN CURRENT LEVEL/TYPE OF USE	Restricted use **	Ban & educate
Protect Human Health	Impact on human health	-	Mild +	+
Protect Environmental Health	Impact on the health of populations of aquatic organisms	-	Mild +	+
Efficient Use of Fiscal Resources	Impacts on Multi-ministries*	-	-	+
Equitable distribution of cost	Impacts on Farmers	+ but anticipated -	+/-	- initially but + over long term***
	Impacts on the Pharmaceutical Industry	+	-	-
	Impacts on Tax Payers	-	+/-	+
	Impacts on Consumers	-	-	+****

[1] This table is based qualitative analysis which can draw on quantitative data (this particular table is not based on quantitative data). The purpose of this table is to illustrate some of the variables which should be considered in conducting IRA. It can incorporate the results of modelling and of quantitative research.

* refers to the financial impact on all affected ministries including the Ministry of Health, Ministry of the Environment, Ministry of Agriculture and Agri-Food, Ministry of Economic Development and the Ministry of Finance.

** for example, may not use within a certain distance of streams, creeks or rivers, or of the water table. There are many options for restricting the use of AGP; each of which would have to be stated and evaluated separately.

*** as the costs become externalized across society, with integrated changes not only within the animal husbandry industry but also within society (e.g., integrating legal and consumer policies). This approach would work in a manner similar to the ban on the use of DDT in North America (the farming industry is still viable, even under the ban).

**** this is a + in the same way that DDT-free products are positives for consumers. As the industry and society adjusts to AGP-free practices, there will be a net positive effect. (However, it is likely that AGPs will be replaced with another product. The safety of the replacement product should also be evaluated).

+ benefit

- cost or drawback

+/- refers to the overall neutral effect as benefits would be equal to drawbacks.

Table 8: Sample evaluation of proposed mitigation strategies to address the use of hormones for birth control and hormone replacement therapies[1].

GOALS	CRITERIA	Alternatives Considered			
		MAINTAIN CURRENT USE	**Label as env. tox & educate**	**Restrict to hospital use**	**p2 with low limits**
Protect/promote Human Health	Impacts on human health	+++	+++	_ _d	_ _e
	Impacts on social values	++	++	_ _d	_e
Protect Environmental Health	Impacts on aquatic organisms	_ _ _	_ _ _a	++	Mild+
	Subsequent impacts on human health/economics	?b	?	_	_f
Efficient Use of Fiscal Resources	Impacts on Multi-ministry* finances	+++	+	_ _ _	_
Equitable* distribution of cost	Impacts on various facilities (hospital, nursing home)	+g	+g	_	_ _ _
	Impacts on the Drug Industry	+++	+c	_ _	_
	Impacts on Tax Payers/ STP operators	++	++	_	+
	Impacts on Consumers/ Patients	+++	++	_ _ _d	_ _

[1] This table is based qualitative analysis which can draw on quantitative data (this particular table is not based on quantitative data). The purpose of this table is to illustrate some of the variables which should be considered in conducting IRA. It can incorporate the results of modelling and of quantitative research

a This alternative would lead to only minimal reductions in overall input volumes.

b currently there is no conclusive evidence suggesting negative impacts on human health connected to the use of estrogens for birth control and hormone replacement therapy. Future research may show otherwise.

c Drug companies would shoulder the financial responsibility for their products under life cycle stewardship initiatives, thus mild+.

d Unequal access. What about residential patients? Private financing of hospital stay.

e Care facilities may restrict use to meet targets.

f Benefit of clean water may be outweighed by health limitations due to lack of these medications.

g These facilities may be required to respect hormone discharge limits potentially imposed on them under pollution prevention legislation.

* refers to the financial impact on all affected ministries including the Ministry of Health, Ministry of the Environment, Ministry of Agriculture and Agri-Food, Ministry of Economic Development and the Ministry of Finance. +++ strong benefits + mild benefits --- strong drawbacks or opposition - mild drawbacks or opposition.

Summarizing issues related to pharmaceuticals in the environment

Ample research has been conducted to establish the fact that pharmaceutical residues enter the environment on a continuous basis following normal use. Further, more research indicates that effects at various levels of biological organization can be attributed to many of the medicinal substances isolated in environmental waters. Many other effects are circumstantially connected to those contaminants. Workers in this field studied the fate of pharmaceutical products in the sewage system and in the environment, modelled their distribution in the aqueous, terrestrial and bio-spheres and evaluated the strengths and weaknesses of the outputs. Lastly, scientists have identified knowledge gaps and needs for yet further research. Current consensus suggests that:

- the likelihood of imminent acute negative impacts on the aquatic environment from pharmaceutical residues is low to negligible
- the likelihood of chronic negative impacts on the aquatic environment from pharmaceutical residues is higher compared to the acute impacts
- the likelihood of negative impacts on aquatic populations from pharmaceutical contaminants is under investigation, with preliminary results indicating that population impacts are possible
- the likelihood and extent of negative impacts on population and ecosystem stress resilience associated with environmental pharmaceutical contamination remains un-investigated.
- current sewage treatment systems are largely inadequate for removing the environmental hazard associated with pharmaceutical substances in the environment. Advanced systems fare better but are vastly more expensive.
- alternative mitigation strategies exist, but no clear approach has yet emerged

- Data gaps will be addressed by changes in research approaches to reflect the fact that:
    - assessment of the physical and chemical properties of drug residues must be adjusted to account for chemicals diverging from the character of those on which the models are based
    - environmental persistence needs to account for continuous introduction resulting in a *de-facto* increased persistence
    - mixtures of chemicals and mixtures of stressors should be assessed as opposed to individual chemicals and/or stressors
    - toxicity testing should be chosen so as to reflect the actual and expected mode of action, timing of effects and range of doses.
    - the 'omics' approach can be further refined and applied to the analysis and modelling of environmental pharmaceuticals.

The foundation of risk assessment is currently under revision. The complexity of issues occurring at the intersection between environmental and human welfare is recognized with more and more regularity. This acknowledgement has prompted a re-definition of the goals of risk assessment in such a way as to include:

- a precautionary approach based on anticipatory and preventative action. This is why pharmaceutical contamination of the environment is studied with such intensity. There is no crisis, yet But there is no reason to wait for a crisis in order to implement remedial measures.
- an integrative approach based on alternative assessment, information sharing and commonality of action platforms. Pharmaceuticals are products designed specifically for immediate human health benefits (agro-pharmaceuticals notwithstanding). A cost associated with those benefits is potential harm to the environment which in turn threatens the long-term human welfare. A

comprehensive cost-benefit analysis must be integrative across scientific and socio-economic disciplines.

REFERENCES

Agriculture and Agri-food Canada (2004) http://www.agr.gc.ca/pfra/csidc/csidpub5_e.htm, accessed July, 2005

Ahel, M; Jelicic, A (1999) Occurrence of analgesics in a municipal solid waste landfill and adjacent groundwater aquifer. In Proceedings on the Third IWA Specialized Conference on Hazard Assessment and Control of Environmental Contaminants, Otsu, Japan

Al-Ahmed, A and Kümmerer, K (2001) Biodegradation of the antineoplastics vindesine, vincristine and vinblastine and toxicity against bacteria in the aquatic environment. *Cancer Detection and Prevention 25:102-107*

Al-Ahmed, A; Daschner, FD; Kummerer, K (1999) Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G and sulfamethoxazole and inhibition of waste water bacteria. *Archives of Environmental Contamination and Toxicology 37:158-163*

Al-Jayyousi, O (2003) Greywater reuse: towards sustainable water management *Desalination 156:181-192*

Allen, MR (2005) Effective pollution prevention in healthcare environments. *Journal of Cleaner Production – in press*

Andersen, L; Holbech. H; Gessbo, A; Norrgren, L; Petersen, GI (2003) Effects of exposure to 17α-ethinylestradiol during early development on sexual differentiation and induction of vitellogenin in zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology part C: Toxicology and Pharmacology 134:365-374*

Anderson, RH; Hansen, M; Kjølholt, J; Stuer-Lauridsen, F; Ternes, T; Halling-Sorensen, B (2005) Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. *Chemosphere 61:139-146*

Andreozzi, R; Caprio, V; Ciniglia, C; de Champdore, M; Giudice, R; Marotta, R; Zuccato, E (2004) Antibiotics in the environment: Occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin. *Environmental Science and Technology 38:6832-6838*

Andreozzi, R; Marotta, R; Pinto, G and Pollio, A (2002) Carbamazepine in water: persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity *Water Research 36:2869-2877*

Andreozzi, R; Raffaele, M; Nicklas, P (2003) Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environments. *Chemosphere 50(10):1319-1330*

Angelakis, A and Bontoux, L (2001) Wastewater reclamation and reuse in Eureau countries *Water Policy 3:47-59*

Anonymous (2002) The cost of antibiotic resistance: Effect of resistance among *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni* and *Pseudomonas aeruginosa* on length of hospital stay *Infection Control and Hospital Epidemiology* 25(2):106-108

Arcand-Hoy, LD; Nimron, AC; Benson, WH (1998) Endocrine-modulating substances in the environment: estrogenic effects of pharmaceutical products. *International Journal of Toxicology 17:139-158*

Arifin, Z; Bendell-Young, LI (1997) Feeding behaviour of the blue mussel exposed to environmentally relevant seston matrices. *Marine Ecology Progress Series 160:241-253*

Asano, T and Cotruvo, J (2004) Groundwater recharge with reclaimed municipal wastewater: health and regulatory considerations. *Water Research 38(8):1941-1951*

Attarassi, B, Saghi, M and Flatau, G (1993) Multiple antibiotic resistance of bacteria in Atlantic coast (Morocco). *Environmental Technology 14:186-193*

Auriol, M; Filali-Mekanassi, Y; Tyagi, RD; Adams, CD; Surampalli, RY (2005) Endocrine disrupting compounds removal from wastewater, a new challenge. *Process Biochemistry 41(3):525-539*

Aven, T; Kristensen, V (2005) Perspectives on risk: review and discussion of the basis for establishing a unified and holistic approach. *Reliability Engineering and System Safety 90:1-14*

Axelson, O; Balbus, JM; Cohen,G; Davis, D; Donnay, A; Doolittle, R; Duran, BM; Egilman, D; Epstein, SS; Goldman, L; Grandjean, P; Hansen, ES; Heltne, P; Huff, J; Infante, P; Jacobson, MF; Joshi, TK; LaDou, J; Landrigan, PJ; Lee, PR; Lockwood, AH; MacGregor, G; Melnick, R; Messing, K; Needleman, H; Ozonoff, D; Ravanesi, B; Ricter, ED; Sass, J; Schubert, D; Suzuki, D; Teitelbaum, D; Temple, NJ; Terracini, B; Thompson, A; Tickner, J; Tomatis, L; Upton, AC; Whyatt, Rm; Wigmore, D; Wilson, T; Wing, SB; Sharpe, VA (2003) Re: regulatory toxicology and pharmacology. *International Jounal of Occupational and Environmental Health 9:386-390*

Ayscough, NJ; Fawell, J; Franklin, G; Young, W (2000) Review of human pharmaceuticals in the environment. Technical Report P390, Bristol, UK: Environment Agency of England and Wales

Balcioglu, I and Otker, M (2003) Treatment of pharmaceutical wastewater containing antibiotics by O_3 and O_3/H_2O_2 processes *Chemosphere 50:85-95*

Ball, DJ (2002) Environmental risk assessment and the intrusion of bias. *Environment International 28:529-544*

Ballantyne, B (2000) Repeated Exposure Toxicity In General and Applied Toxicology, 2nd Ed. Ballantyne, B; Marrs, T; Syversen, T (Eds.), Macmillan Reference LTD, London

Ballantyne, B; Marrs, T and Syversen, T (2000) Fundamentals of Toxicology In General and Applied Toxicology, 2nd Ed. Ballantyne, B; Marrs, T; Syversen, T (Eds.), Macmillan Reference LTD, London

Ballantyne, B; Marrs, TC; Turner, P (1995) Fundamentals of Toxicology In General and Applied Toxicology. College Ed. Ballantyne, B; Marrs, T; and Turner, P (Eds.) Stockton Press, New York

Ballie, TA; Cayen, MN; Fouda, H; Gerson, RJ; Green,m JD (2002) Drug metabolites in safety testing. *Toxicology and Applied Pharmacology 182:188-196*

Bard, S M.(2000) Multixenobiotic resistance as a cellular defence mechanism in aquatic organisms. *Aquatic Toxicology 49:357-389*

Barza, M (2002) Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals *Clinical Infectious Disease 34(Suppl. 3):S123-S125*

Bayley, M; Nielsen, JR and Baatrup, E (1999) Guppy sexual behaviour as an effect biomarker for estrogen mimics. *Ecotoxicol Environmental Safety 43:68-73*

Beausse, J (2004) Selected drugs in solid matrices: a review of environmental determination, occurrence and properties of principal substances. *Trends in Analytical Chemistry 23 (10-11):753-761*

Beck, B; Slayton, TM; Ccalabrese, EJ; Baldwin, LA; Rudel, R (2001) The use of toxicology in the regulatory process. In Principles and Methods of Toxicology. 4th Ed. A Hayes (Ed.) Taylor and Francis, Philadelphia

Belfroid, AC; Sijm, DTHM, van Gestel, CAM (1996) Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. *Environmental Review 4:276-299*

Bell, A (2004) An endocrine disruptor increases growth and risky behavior in threespined stickleback (*Gasterosteus aculeatus*). *Hormones and Behavior 45(2):108-114*

Bendell-Young, LI; Arifin, Z (2004) Application of a kinetic model to demonstrate how selective feeding could alter the amount of cadmium accumulated by the blue mussel (*Mytilus trossolus*). *Journal of Experimental Marine Biology and Ecology 298(1):21-33*

Bendz, D; Paxeus, NA; Ginn, TR; Loge, FJ (2005) Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. *Journal of Hazardous Materials – 122(3):195-204*

Benneatau-Pelissero, C; Breton, BB; Bennetau, B; Corraze, G; Helou, C (2001) Effect of genistein-enriched diets on the endocrine process of gemetogenesis and on reproduction efficiency of the rainbow trout (Oncorhynchus mykiss). *General and Comparative Endocrinology 121(2):173-187*

Bentel, JM; Lebwohl, DE; Cullen, KJ; Rubin, N; Mendelsohn, J; Miller, WH (1995) Insulin-like factors modulate the growth inhibitory effects of retinoic acid on MCF-7 breast cancer cells. *Journal of Cellular Physiology165:212-221*

Bernet, D; Schmidt, H; Wahli, T; Burkhardt-Holm, P (2001) Effluent from a sewage treatment works causes changes in serum chemistry of brown trout. *Ecotoxicology and Environmental Safety 48:140-147*

Better Buildings Program (2004) (http://www.betterbuildings.ca/ accessed April 2004

Bhogal, N; Grindon, C; Combes, R; Balls, M (2005) Toxicity testing: creating a revolution based on new technologies. *Trends in Biotechnology 23(6):299-307*

Birnbaum, LS (1994) Endocrine effects of prenatal exposure to PCBs, dioxins, and other xenobiotics; implications for policy and future research. *Environmental Health Perspectives 102:676-679*

Bliss, CI (1935) The calculation of the dosage mortality curve. *Analytical and Applied Biology 22:134-167*

Bliss, CI (1940) The relation between exposure time, concentration and toxicity in experiments on insecticides. *Annals of the Entomological Society of America*

Borgert, CJ; Quill, TF; McCarty, LS; Mason, AM (2004) Can mode of action predict mixture toxicity for risk assessment? *Toxicology and Applied Pharmacology 201:85-96*

Bortone, SA and Davis, WP (1994) Fish intersexuality as an indicator of environmental stress. *Bioscience 44:165-172*

Bortone, SA; Davis, WP (1994) Fish intersexuality as indicator of environmental stress. BioScience 44(3):165-172

Bosch, X (1998) Household antibiotic storage [Letter]. *Science281:785*

Bound, JP; Voulvoulis, N (2004) Pharmaceuticals in the aquatic environment – a comparison of risk assessment strategies. *Chemosphere 56:1143-1155*

Boyd, GR; Reemtsma, H; Grimm, DA; Mitra, S (2003) Pharmaceuticals and personal care products in surface and treated waters of Louisiana, USA and Ontario, Canada. *The Science of the Total Environment 311:135-149*

Brain, RA; Wilson, CJ; Johnson, DJ; Sanderson, H; Bestari, K; Hanson, ML; Sibley, PK; Solomon, KR (2005) Effects of a mixture of tetracyclines to *Lemna gibba* and *Myriophyllum sibiricum* evaluated in aquatic microcosms. *Environmental Pollution 138(3):425-442*

Brambilla, G; Civitarcale, C and Migliore, L (1994) Experimental toxicity and analysis of bacitracin, flumequine and sulphadimethoxine in terrestrial and aquatic organisms as a predictive model for ecosystem damage. *Quimica analitica 13(sup.1):114-118*

Brandt, C (2002) Sustainable development and responsible care. *Chemie in unserer Zeit 36(4) 214-224 in German, as cited in Larsen et al., 2004*

Brevik, K; Wania, F (2003) Expanding the applicability of multimedia fate models to polar organic chemicals. *Environmental Science and Technology 37:4934-4943*

Brewer, GD (1983) Some costs and consequences of large-scale social systems modelling. *Behavioural Science 28(2):166-185*

Bridges, J (2003) Human health and environmental risk assessment: the need for a more harmonized and integrated approach. *Chemosphere 52:1347-1351*

Bridges, JW; Bridges, O (2004) Integrated risk assessment and endocrine disrupters. *Toxicology 205:11-15*

Brion, F; Tyler, CR; Palazzi, X; Laillet, B; Porscher, JM; Garric, J; Fammarion, P (2004) Impacts of 17β-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (*Danio rerio*). *Aquatic Toxicology 68:193-217*

Brooks, BW; Foran, CM; Richards, SM; Weston, J; Turner, PK, Stanley, JK; Solomon, KR; Slattery, M; La Point, TW (2003) Aquatic ecotoxicology of fluoxetine. *Toxicology Letters 142:169-183*

Brooks, BW; Turner, PK, Stantley, JK; Weston, JJ; Glidewell, EA; Foran, CM (2003) Waterborne and sediment toxicity of fluoxetine to select organisms. *Chemosphere 52:135-142*

Build Smart Program (2004) http://www.buildsmart.ca/ accessed April 2004

Buser, HR; Muller, MD; Theobald, N (1998) Occurrence of the pharmaceutical drug clofibric acid and the herbicide mecoprop in various Swiss lakes and in the North Sea. *Environmental Science and Technology 32(1)188-192*

Byrns, G. (2001) The fate of xenobiotic organic compounds in wastewater treatment plants. *Water Research 35:2523-2533*

Cahen, F; Dubreuil, E; Pons, JC (1996) Prenatal exposure to diethylstilbestrol and the mother-daughter relationship. *European Journal of Obstetrics, Gynecology and Reproductive Biology 65(2) :181-187*

Calabrese, EJ (2005) Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. *Environmental Pollution 138(3):378-411*

Calabrese, EJ (2005) Toxicological awakenings: the rebirth of hormesis as a central pillar of toxicology. *Toxicology and Applied Pharmacology 204:1-8*

Calabrese, EJ; Baldwin, LA (2001) Hormesis: U-shaped dose responses and their centrality in toxicology. *Trends in Pharmacological Sciences 22 (6):285-291*

Calabrese, EJ; Baldwin, LA (2002) Application of hormesis in toxicology, risk assessment and chemotherapeutics. *TRENDS in Pharmacological Sciences 23(7):331-337*

Calabrese, EJ; Blain, R (2005) The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview. *Toxicology and Applied Pharmacology 202:289-301*

Calamari, D; Zuccato, E; Castiglioni, S; Bagnati, R; Faneli, R (2003) Strategic survey of therapeutic drugs in the rivers Po and Lambro in Northern Italy. *Environmental Science and Technology 37:1241-1248*

California Ground Water Replenishment (2004) http://www.gwrsystem.com/ accessed April 2004

Canadian Centre for Pollution Prevention (CCPP) (2006a) *The Benefits of Environmental Awareness At The Hospital For Sick Children* http://www.c2p2online.com/documents/SickChildrenHospital.pdf, accessed March, 2006

Canadian Centre for Pollution Prevention (CCPP) (2006b) http://www.c2p2online.com/documents/SickKidsCaseStudy.pdf, accessed March 2006

Canadian Environmental Protection Act (CEPA) (1999) Toxic Substances 2005 update, http://www.ec.gc.ca/CEPARegistry/subs_list/Toxicupdate.cfm, accessed April, 2006

Canadian Fisheries Act (1995) http://laws.justice.gc.ca/en/F-14/240479.html, accessed April, 2006

Canadian Pharmacists Association (2004) http://www.pharmacists.ca/flash.cfm Accessed April, 2006

Cantor, RS (1998) The lateral pressure profile in membranes: a physical mechanism of general anesthesia. *Toxicology Letters, 100-101:451-458*

Carballa, M; Omil, F; Lema, JM (2005) Removal of cosmetic ingredients and pharmaceuticals in sewage primary treatment. *Water Research 39:4790-4796*

Carballo, M; Aguayo, S; de la Torre, A; Munoz, J (2004) Plasma vitellogenin levels and gonadal morphology of wild carp in receiving rivers downstream of sewage treatment plants. *Science of the Total Environment 341(1-3):71-79*

Cargouet, M; Perdiz, D; Mouatassim-Souali, A; Tamisier-Karolak, S; Levi, Y (2004) Assessment of river contamination by estrogenic compounds in Paris area (France). *Science of the Total Environment 324:55-66*

Carlsen, E; Giwercman, A; Keiding, N; Skakkebaek, N (1992) Evidence for decreasing quality of semen during past 50 years. *BMJ 305:609-613*

Carlsson, C; Johansson, A; Alvan, G; Bergman, K; Kuhler, T (2005) Are pharmaceuticals potent environmental pollutants? Part I: Environmental risk assessments of selected active pharmaceutical ingredients. *Science of the Total Environment – in press*

Castiglioni, S; Fanelli, R; Calamari, D; Bagnati, R; Zuccato, E (2004) Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy. *Regulatory Toxicology and Pharmacology 39:25-32*

Cavas, T; Ergene-Gozukara, S (2005) Genotoxicity evaluation of metronidazole using the piscine micronucleus test by acridine orange fluorescent staining. *Environmental Toxicology and Pharmacology 19:107-111*

Chalmers, I (1990) Underreporting research is scientific misconduct. *Journal of the American Medical Association 263:1405-1408*

Chan, AW; Hrobjartsson, A; Haahr, MT; Altman, DG (2004) Empirical evidence for selective reporting of outcomes in randomized trials: comparison of protocols to published articles. *Journal of the American Medical Association 291:2457*

Chapman, PM (2002) Ecological risk assessment (ERA) and hormesis. *The Science of the Total Environment 288:131-140*

Chapman, PM; Cardwell, RS; Chapman, PF (1996) A warning: NOECs are inappropriate for regulatory use. *Environmental Toxiclogy and Chemistry 15:77-79*

Chapman, PM; Ho, KT; Munns Jr., WR; Solomon, K; Weinstein, MP (2002) Issues in sediment toxicity and ecological risk assessment. *Marine Pollution Bulletin 44:271-278*

Chee, YE (2004) An ecological perspective on the valuation of ecosystem services. *Biological Conservation 120:549-565*

Chen, Z; Pavelic, P; Dillon P and Naidu, R (2002) Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection *Water Research 36:4830-4838*

Christensen, FM (1998) Pharmaceuticals in the environment – a human risk? *Regulatory Toxicology and Pharmacology 28:212-221*

City of Toronto Wastewater Treatment (2004) http://www.city.toronto.on.ca/water/wastewater_treatment/process.htm accessed April 2004

Clara, M; Strenn, B and Kreuzinger, N (2004) Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration *Water Research 38(4):947-954*

Clark, T; Clark, K; Paterson, S; Mackay, C; Nostrom, RJ (1988) Wildlife monitoring, modelling and fugacity. *Environmental Science and Technology 22:120-127*

Cleuvers, M (2003) Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicology Letters 142:185-194*

Cleuvers, M (2004) Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. *Ecotoxicology and Environmental Safety – in press*

Cleuvers, M (2005) Initial risk assessment for three β-blockers found in the aquatic environment. *Chemosphere 59:199-205*

Clewell, H (2005) Use of mode of action in risk assessment: Past, present and future. *Regulatory Toxicology and Pharmacology 42:3-14*

Cohern, J; Jao, J and Jusko, W (1971) Pharmacokinetics of cyclophosphamide in man. *Br. J. Pharmacol. 43:677-680*

Cole, M; Kenig, MD and Hewitt, VA (1973) Metabolism of penicillins to penicillonic acids and 6-animopenicillanic acid in man and its significance in assessing penicillin absorption. *Antimicrobial Agents and Chemotherapy 3(4):436-468*

Coleman, MP; Esteve, J; Damiecki, P; Arslan, A; Renard, H (1993) Trends in Cancer Incidence and Mortality, IARC Scientific Publications no. 121. International Agency for Research on Cancer, Lyon, France.

Combes, RD (2005) Assessing risk to humans from chemical exposure by using non-animal test data. *Toxicology in Vitro 19(7):921-924*

Commission of the European Communities (2000) Communication from the Commission on the Precautionary Principle. Brussels 2.2.2000,COM(2000)1

Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) (1999) Opinion on Human and Wildlife Health Effects of Endocrine Disrupting Chemicals, with Emphasis on Wildlife and on Ecotoxicology Test Methods expressed at the 8th CSTEE plenary meeting, Brussels, 4 March, 1999. DGXXIV, Consumer Policy and Consumer Health Protection

Comoretto, L; Chiron, S (2005) Comparing pharmaceutical and pesticide loads into a small Mediterranean river. *Science of the Total Environment 349(1-3):201-210*

Conly, J (2002) Antimicrobial resistance in Canada. *Canadian Medical Association Journal 167:885-891*

Cook, JC, Johnson, L et al. (1998) Effects of dietary 17β-estradiol exposure on serum hormone concentrations and testicular parameters in male Crl:CD BR rats. *Toxicological Sciences 44:155-168*

Cortner, HJ (2000) Making science relevant to environmental policy. *Environmental Science and Policy 3:21-30*

Cronin, MTD (2004) Predicting chemical toxicity and fate in humans and the environment – an introduction. In Predicting Chemical Toxicity and Fate, Cronin, M; Livingstone, DJ (Eds.), CRC Press, Boca Raton, USA

Cronin, MTD; Livingstone, DJ (2004) Predicting Chemical Toxicity and Fate. Cronin, MTD; Livingstone, DJ (Eds.), CRC Press, Boca Raton

Crump, KS (1996) The linearized multistage model and the future of quantitative risk assessment. *Human and Experimental Toxicology 15:787-798*

Cullen, P (1990) The turbulent boundary between water science and water management. *Freshwater Biology 24:201-209*

Cunningham, V; Constable, DJC; Hannah, RE (2004) Environmental risk assessment of paroxetine. *Environmental Science and Technology 38:3351-3359*

Cunningham, VL (2004) Special Characteristics of Pharmaceuticals Related to Environmental Fate. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin.

Daniels, SE; Walker, GB (1996) Collaborative learning: improving public deliberation in ecosystem-based management. *Environmental Impact Assessment Review 19:71-102*

Daughton, C (2003a) Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rationale for and avenues toward a green pharmacy *Environmental Health Perspectives 111 No.5:757-774*

Daughton, C (2003b) Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health II. Drug Disposal, Waste Reduction, and Future Directions *Environmental Health Perspectives 111 No.5:775-785*

Daughton, CG (2004) Non-regulated water contaminants: emerging research. *Environmental Impact Assessment Review 24:711-732*

Daughton, CG, and Ternes, TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives 107 suppl.6:907-938*

Dearfield, KL; Cimino, MC; McCarroll, NE; Mauer, I; Valcovic, LR (2002) Genotoxicity risk assessment: a proposed classification strategy. *Mutation Research 521:121-135*

Deneer, JW; Sinnige, TL, Seinen, W; Hermens,JLM (1998) The joint acute toxicity to *Daphnia magna* of industrial organic chemicals at low concentrations. *Aquatic Toxicology 12:33-38*

Desbrow, C; Routledge, EJ; Brighty, GC; Sumpter, JP; Waldock, M (1998) Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation an in-vitro biological screening. *Environmental Science and Technology, 32:1549-1561*

Di Giulio, R; Benson, W (2002) Interconnections Between Human Health and Ecological Integrity, SETAC Press, Pansacola, FL

Diaz-Cruz, M; de Alda, M and Barcelo D (2003) Environmental behaviour and analysis of veterinary and human drugs in soils, sediments and sludge *Trends in Analytical Chemistry 22:340-351*

Diaz-Cruz, MS; de Alda, MJL; Barcelo, D (2003) Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *Trends in Analytical Chemistry 22:340-351*

Dietrich, DR; Hitzfeld, BC; O'Brien, E (2005) Toxicology and riskassessment of pharmaceuticals. In Organic Pollutants in the Water Cycle Reemtsma, T (Ed.) Wiley-VCH, Weinheim

Dijkema, GPJ; Reuter, MA; Verhief, EV (2000) A new paradigm for waste management. *Waste Management 20:633-638*

DiPasquale, LC; Hayes, AW (2001) Acute toxicity and eye irritancy. In Principles and Methods of Toxicology. 4th Ed. A Hayes (Ed.) Taylor and Francis, Philadelphia

Doll, TE; Frimmel, FH (2003) Fate of pharmaceuticals – photodegradation by simulated solar UV-light. *Chemosphere, 52:1757-1769*

Donald, D; Sverko, E (2005) Persistence of pharmaceuticals under ice. Environment Canada Workshop on PPCPs in the Environment. Canada Centre for Inland Waters, Burlington, Ontario

Dorado, J; Almendros, G (2001) Assessment of molecular descriptors involved in the sorptive interactions of phenoxy-carboxylic acid. *Toxicology and Environmental Chemistry 84:21-32*

Dorato, MA; Engelhardt, JA (2005) The no-observed-adverse-effect-level in drug safety evaluations: Use, issues, and definition(s). *Regulatory Toxicology and Pharmacology 42:265-274*

Drewes, J; Reinhard, M and Fox, P (2003) Comparing microfiltration-reverse osmosis and soil-aquifer treatment for indirect potable reuse of water *Water Research, 37:3612-3621*

Drillia, P; Stamatelatou, K; Lyberatos, G (2005) Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere 60:1034-1044*

Dunne, M (2000) Traces of medicines found in New Orleans area water. *The Advocate, July 9, 2000*

Eertmans, F; Dhooge, W; Stuyvaer, S; Comhaire, F (2003) Endocrine disruptors: effects on male fertility and screening tools for their assessment. *Toxicology in Vitro 17:515-524*

Ekholm, Ann-Kristin (1998) Cleaning methods with low chemical use. Acomparison of cleaning methods at University Hospital in Lund. Not published. Author has made a copy of the report available to me. Author's e-mail address: ann-kristin.ekholm@skane.se

Elling, B (2004) Modernity and communicative reflection in environmental assessment. In Planning for sustainable development – the practice and potential of environmental assessment. Hilding-Rydevik, T; Theodorsdottir, A (Eds.) Proceedings from the 5th Nordic Environmental Assessment Conference, Reykjavik, Iceland

Emblidge, JP; DeLorenzo, ME (2005-2006) Preliminary risk assessment of the lipid-regulating pharmaceutical clofibric acid, for three estuarine species. *Environmental Research – in press*

Emmanuel, E; Perrodin, Y; Keck, G; Blanchard, JM; Vernande, P (2005) Ecotoxicological risk assessment of hospital wastewater: a proposed framework for raw effluents discharging into urban sewer network. *Journal of Hazardous Materials A117:1-11*

Eufemia, N and Epel, D (1999) Induction of the multixenobiotic defence mechanism (MXR), P-glycoprotein, in the mussel *Mytilus californianus* as a general cellular response to environmental stresses. *Aquatic Toxicology 49:89-100*

European Community (EC) (2001) Directive 2001/82/EC of the European Parliament and of the Council of 6th November 2001 on the Community code relating to veterinary medicinal products.

European Federation of Animal Health (FEDESA) (1997) Press Release, Brussels.

European Medicines Agency (EMEA) 2005 Draft: Guideline on environmental risk assessment of medicinal products for human use. CPMP/SWP/4447/00draft http://www.emea.eu.int/htms/human/qrd/qrdplt/24530905en.pdf, accessed February, 2006

Falkof, BB (1995) Updating the duty to warn. *Design News Feb. 20, 1995*

Farrington, JW (1991) Biogeochemical processes governing exposure and uptake of organic pollutant compounds in aquatic organisms. *Environmental Health Perspectives 90:75-84*

Faust, M; Altenburger, R; Backhaus, T; Blanck, H; Boedeker, W; Gramatica, P; Hamer, V; Scholze, M (2001) Predicting the joint algal toxicity of multicomponent s-triazine mixtures at low-effect concentrations of individual toxicants. *Aquatic Toxicology 56:13-32*

Fent, K; Weston, A; Caminada, D (2005) Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology 76(2):122-159*

Ferrari, B; Paxeus, N; Giudice, RL; Pollio, A; Garric, J (2003) Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac *Ecotoxicology and Environmental Safety 55:359-370*

Flaherty, CM; Dodson, SI (2005) Effects of pharmaceuticals on *Daphnia* survival, growth and reproduction. *Chemosphere 6(2):200-207.*

Folmar, LC; Denslow, N et al. (1996) Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio)* captured near a major metropolitan sewage treatment plant. *Environmental Health Perspectives 104:1096-1101*

Fong, P (1998) Zebra mussel spawning is induced in low concentrations of putative serotonin reuptake inhibitors. *Biological Bulletin 194:143-148*

Forester, J (2000) The deliberative practitioner: encouraging participatory planning processes. MIT Press, Cambridge, MA

Forget-Leray, J; Landriau, I; Minier, C; Leboulenger, F (2005) Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod *Eurytemora affinies* (Poppe) *Ecotoxicology and Environmental Safety 60:288-294*

Fromme, H; Otto, T and Pilz, K (2000) Levels of synthetic musks: bromocyclene and PCBs in eel and PCBs in sediment samples from some waters of Berlin, Germany *Chemosphere 39:1723-1735*

Fromme, H; Otto, T and Pilz, K (2001) Polycyclic musk fragrances in different environmental compartments in Berlin, Germany *Water Resources* 35:121-128

Gad, SC (2000) Alternatives to in-vivo studies in toxicology. In General and Applied Toxicology (2nd Ed.) Ballantyne, B; Marrs, T; Syversen, T (Eds.), Macmillan Reference, London, UK

Gagne F; Blaise, C; Andre, C (2006) Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Ecotoxicology and Environmental Safety – in press*

Garthoff, B (2005) Alternatives to animal experimentation: The regulatory background. *Toxicology and Applied Pharmacology 207(2) Suppl.1:388-392*

Giampietro, M; Mayumi, K; Munda, G (2006) Integrated assessment and energy analysis: Quality assurance in multi-criteria analysis of sustainability. *Energy 31:59-86*

Gieringere, JH; Wenz, AF; Just, HM; Daschner, FD (1986) Effect of 5-fluorouracil, mitoxantrone, methotrexate, and vincristine on the antibacterial activity of ceftriaxone, ceftazidime, cefotiam, piperacillin and metilmicin. *Chemotherapy 32(5):418-424*

Gilman, AG; Rall, TW; Niles, AS; Taylor, P (1990) Goodman and Gilman's the Pharmacological Basis of Therapeutics 8th Ed. Pergamon, New York

Gobas, FAPC; Muir, DCG; Mackay, D (1988) Dynamics of dietary bioaccumulation and fecal elimination of hydrophobic organic chemicals in fish. *Chemosphere 17:943-962*

Gobas, FAPC; Wicockson, JB; Russell, RW; Haffner, GD (1999) Mechanism of biomagnification in fish under laboratory and field conditions. *Environmental Science and Technology 33:133-141*

Gobas, FAPC; Zhang, X; Wells, R (1993) Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. *Environmental Science and Technology 27:2855-2863*

Goeptar, AR; Scheerens, H; Vermeulen, NPE (1995) Oxygen reductase and substrate reductase activity of cytochrome P450. *Critical Reviews of Toxicology 25:25-65*

Goksoyr, A; Forlin, L (1992) The cytochrome P450 system in fish, aquatic toxicology and environmental monitoring. *Aquatic Toxicology 22:287-312*

Golet, EM; Alder, AC; Hartmann, A; Ternes, TA; Giger, W (2001) Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Analytical Chemistry 73:3632-3638*

Golet, EM; Xifra, I; Siegrist, H; Alder, AC; Giger, W (2003) Environmental exposure assessment of flouoroquinolone antibacterial agents from sewage to soil. *Environmental Science and Technology 37:3243-3249*

Gool, van S (1993) Possible environmental effects of antibiotics residues in animal manure. *Tijdschrift voor Diergeneeeskunde (Netherland) 8:10 English Summary*

Gori, GB (1996) Science, imaginable risks, and public policy: anatomy of a mirage. *Regulatory Toxicology and Pharmacology 23:304-311*

Goss, KU, Schwarzenbach, RP (2001) Linear free energy relationships used to evaluate equilibrium partitioning of organic compounds. *Environmental Science and Technology 35:1-9*

Government of British Columbia (1997) Medicines Return Program http://www.env.gov.bc.ca/epd/epdpa/ips/meds/reports/meds1998.html, accessed November, 2005

Government of Canada (2005) P2 Success Stories www.ec.gc.ca/nopp/doc/fact/en/health.cfm, accessed January, 2006.

Grandjean, P (2005) Non-precautionary aspects of toxicology. *Toxicology and Applied Pharmacology 207:S652-S657*

Greenberg, DS (2003) Conference deplores corporate influence on academic science. *Lancet 362:302-303*

Groten, JP; Feron; VJ, Suhnel, J (2001) Toxicology of simple and complex mixtures. *Trends in Pharmacological Science 22 (6):316-322*

Groundwater Replenishment System (2004) http://www.gwrsystem.com/, accessed November 2004 and March 2006-03-13

Guillette, LJ; Woodward, AR et al. (1999) Plasma steroid concentrations and male phallus size in juvenile alligators from seven Florida lakes. *General and Comparative Endocrinology 116:356-372*

Guitton, J; Burronfosse, T; Sanchez, M; Desage, M (1997) Quantitation of propofol. *Analitical Letters 30:1369-1978*

Gurjar, BR; Mohan, M (2003) Integrated risk analysis for acute and chronic exposure to toxic chemicals. *Journal of Hazardous Materials A 103:25-40*

Hahlebeck, E; Katsiadaki, I; Mayer, I, Adolfsson-Erici, M; James, J; Bengtsson, B (2004) The juvenile three-spined stickleback (*Gasterosteus aculeatus L.*) as a model organism for endocrine disruption II – kidney, hypertrophy, vitellogenin and spiggin induction. *Aquatic Toxicology 70:311-326*

Halely BA; Jacob, TA and Lu, AY (1989) The environmental impact of the use of ivermectin. Environmental effects and fate. *Chemosphere 18:1543-1563*

Hallare, AV; Kohler, HR; Triebskorn, R (2004) Developmental toxicity and stress protein responses in zebrafish embryos after exposure to diclofenac and its solvent, DMSO. *Chemosphere 56:659-666*

Halling-sorensen, B; Lutzhoft, HHC; Andersen, HR; Ingerslev, F (2000) Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. *Journal of Antimicrobial Chemotherapy 46Suppl.1:53-58*

Halling-Sorensen, B; Nielsen, S et al. (1998) Occurrence, fate and effects of pharmaceutical substances in the environment – a review. *Chemosphere 36:357-393*

Hamscher, G; Pawelzick, HT; Hoper, H; Nau, H (2004) Antibiotics in soil: routes of entry, environmental concentrations, fate and possible effects. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Hamscher, G; Sczesny, S; Hoper, H; Nau, H (2002) Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Analytical Chemistry 74(7):1509-1518*

Harbor Solutions Project, Halifax, NS (2004) http://www.region.halifax.ns.ca/harboursol/primary_treatment.html accessed April 2004

Harries, JE; Janbakhsh, A; Jobling, S; Matthiessen, P; Sumpter, JP; Tyler, C (1999) Estrogenic potency of effluent from two sewage treatment works in the United Kingdom. *Environmental Toxicolgy and Chemistry 18(5):932-937*

Harries, JE; Sheahan, DA (1996) A survey of estorgenic activity in United Kingdom inland waters. *Environmental Toxicology and Chemistry 15:1993-2002*

Harries, JE; Sheahan, DA; Jobling, S; Matthiessen, P; Neall, P; Sumpter, JP; Tylor, T; Zaman, N. (1997) Estrogenic activity in five United Kingdom rivers by measurement of vitellogenesis in caged male trout. *Environmental Toxicology and Chemistry 16:534-542*

Hayes, A (2001) Principles and Methods of Toxicology. 4th Ed. A Hayes (Ed.) Taylor and Francis, Philadelphia

Hayes, D and Jensen H (2001) Economic impact of a ban on the use of over the counter antibiotics in the US swine rations *International Food and Agribusiness Management Review 4:81-97*

Hayes, D and Jensen, H (2003) Lessons from the Danish Ban on Feed-Grade Antibiotics *Choices, Third Quarter, 2003*

Hayes, D; Jensen, H and Fabiosa, J (2002) Technology choice and the economic effects of a ban on the use of antimicrobial feed additives in swine rations *Food Control 13:97-101*

Healey, P (1997) Collaborative planning: shaping places in fragmented societies. Macmillan Press, Basingstoke

Health Canada (2004) Final Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health. http://www.hc-sc.gs.ca/vetdrugs-medsvet/amr_final_report_june27_12_e.html (accessed November 30, 2004)

Health Canada (2006a) Antibiotic Resistance http://www.hc-sc.gc.ca/dhp-mps/vet/faq/faq_amr-ram_e.html, accessed March 2006

Health Canada (2006b) Policies on Antibiotic Resistance http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/index_e.html, accessed March 2006

Health Canada Applied Research and Analysis Directorate - ARAD (2004) - Health Policy Research Bulletin *Volume 1, Issue 6*

Heberer, T (2002) Occurrence, fate and removal of pharmaceutical residues in the aquatic environment: a review of recent research data *Toxicology Letters 131:5-17*

Heberer, T (2002) Tracking persistent pharmaceutical residues from municipal sewage to drinking water *Journal of Hydrology 266:175-189*

Heberer, T; Feldmann, D (2005) Contribution of effluents from hospitals andprivate households to the total loads of diclofenac and carbamazepine in municipal sewage effluents – modelling versus measurements. *Journal of Hazardous Materials 122(3):211-218*

Hektoen, H, Berge, HA and Hormazabal, V (1995) Persistence of antibacterial agents in marine sediments. *Aquaculture 133:175-184*

Hellinger, W.C (2000) Confronting the Problem of Increasing Antibiotic Resistance *Southern Medical Journal 93-9:842-848*

Hemond, HF; Fethner, EJ (1994) Chemical Fate and Transport in the Environment. Academic Press, San Diego, USA

Herman, RL and Kincaid, HL (1988) Pathological effects of orally administered estradiol to rainbow trout. *Aquaculture 72:165-172*

Hermens, JLM; Verhaar, HJM (1996) QSARs in environmental toxicology and chemistry – Recent developments, classical and three-dimentinal QSAR in agrochemistry. ACS Symposium Series 606:130-140

Hignite, C; Azarnoff, DL (1977) Drugs and drug metabolites as environmental contaminants: chlorophenoxyidobutyrate and salicylic acid in sewage water effluent. *Life Sciences 20:337-342*

Hill, JA (1992) Immunologic factors in endometriosis and endometriosis-associated reproductive failure. *Infertility and Reproduction. Medical Clinics of North America 3:583-591*

Hirsch, R; Ternes, T; Haberer, K; Kratz, KL (1999) Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment 225:109-118*

Hokstad, P; Steiro, T (2006) Overall strategy for risk evaluation and priority setting of risk regulations. *Reliability Engineering and System Safety 91:100-111*

Holtz, S (2006) There is no 'away' Pharmaceuticals, personal care products and endocrine disrupting substances: emerging contaminants detected in water. Canadian Institute for Environmental Law and Policy

Hornbaker, MH; Cullen, A (2003) The precautionary principle in practice: applying the Ashford Framework to technological risk. *Human and Ecological Risk Assessment 9(3):789-810*

Huang, CH; Cwiertney, D; Sedlak, DL (2001) Estrogenic hormones in effluents from conventional and advanced wastewater treatment plants. *Environmental Toxicology and Chemistry 20:133-139*

Huang, WW; Yin, Y; Chiang, TC; Garner,N; Vuoristo, J; McLachlan, JA; Ma, L (2005) Developmental diethylstilbestrol exposure alters genetic pathways of uterine cytodifferentiation. *Molecular Endocrinology 19:669-682*

Huesemann, MH (2002) The inherent biases in environmental research and their effects on public policy. *Futures 34:621-633*

Huggett, DB; Brooks, BW; Peterson, B; Foran, CM; Schlenk, D (2002) Toxicity of selected beta andrenergic receptor-blocking pharmaceuticals on aquatic organism. *Archives of Environmental Contamination and Toxicology 43(2)229-235*

Hurnanen, D (1997) The protective effect of metallothionein against lipid peroxidation caused by retinoic acid in human breast cancer cells. *Journal of Pharmacology and Experimental Therapeutics 283:1520-1528*

Hutchinson, TH; Pickford, DB (2002) Ecological risk assessment and testing for endocrine disruption in the aquatic environment. *Toxicology 181:383-387*

Ignaz, B; Poiger, T; Muller, M and Buser, H (2003) Caffeine, an anthropogenic marker for wastewater contamination of surface waters *Environmental Science and Technology 37:691-700*

Ingerslev, F and Halling-Sorrensen, B (2000) Biodegradability properties of sulfonamides in activated sludge. *Environmental Toxicology and Chemistry 19:2467-2473*

International Programme on Chemical Safety (IPCS) (1979) – Environmental Health Criteria 9, DDT and Its Derivatives. World Health Organization, Geneva

International Programme on Chemical Safety (IPCS) (1989) – environmental Health Criteria 83, DDT and its Derivatives – Environmental Aspects. World Health Organization, Geneva.

Isidori, M; Lavorgna, M; Nardelli, A; Parrella, A; Previtera L; Rubino, M (2005) Ecotoxicity of naproxen and its phototransformation products. *Science of the Total Environment 348:93-101*

Isidori, M; Lavorgna, M; Nardelli, A; Pascarella, L; Parrella, A (2004) Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment 346:87-98*

Jacobsen, P; Berglind, L (1988) Persistence of oxytetracycline in sediment from fish farms. *Aquaculture 70:365-370*

Jensen, TK; Toppari, J;Keiding, N; Skakkebaek, NE (1995) Do environmental estrogens contribute to the decline in male reproductive health? *Clinical Chemistry 41/42:1896-1901*

Jha, AN (2004) Genotoxicological studies in aquatic organisms: an overview. *Mutation Research 552:1-17*

Jiang, JQ; Yin, Q; Zhou, JL; Pearce, P (2005) Occurrence and treatment trials of endocrine disrupting chemicals (EDCs) in wastewaters. *Chemosphere 61(4):544-550*

Jjemba, K (2002) The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land. *Agriculture, Ecosystems and Environment 93:267-278*

Jjemba, PK (2005) Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. *Ecotoxicology and Environmental Safety 63(1):113-130*

Jobling , S; Sheahan, D et al. (1996) Inhibition of testicular growth in rainbow trout *(Oncorhynchus mykiss)* exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry 15:194-202*

Jobling, S; Casey, D; Rodgers-Grey, T; Oehlmann, J; Schulte-Oehlmann U (2004) Comaprative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquatic Toxicology 66:207-222*

Jobling, S; Nolan, M; Tyler, CR; Brighty, G; Sumpter, JP (1998) Widespread sexual disruption in wild fish. *Environmental Science and Technology 32:2498-2506*

Johnson, AC; Belfroid, A; Di Corcia, A (2000) Estimating steroid estrogen inputs into activated sludge treatment works and observations on their removal from the effluent. *Science of the Total Environment 256:163-173*

Jonas, H (1985) The Imperative of Responsibility. Search of an Ethics for the Technological Age University of Chicago Press, Chicago, USA

Jones, O; Voulvoulis, N and Lester, J (2002) Aquatic environmental assessment of the top 25 English prescription pharmaceuticals *Water Research 36:5013-5022*

Jones, OA; Voulvoulis, N; Lester, JN (2004) Potential ecological and human health risks associated with the presence of pharmaceutically active compounds in the aquatic environment. *Critical Reviews in Toxicology 34(4):335-350*

Jos, A; Repetto, G; Rios, JC; Hazen, MJ; Molero, ML, del Peso, A; Salguero, M; Fernandez-Freire, P; Perez-Martin, JM; Camean, A (2003) Ecotoxicological evaluation of barbamazepine using six different model systems with eighteen endpoints. *Toxicology in vitro 17:525-532*

Joss, A, Keller, E; Alder, AC; Gobel, A; McArdell, CS; Ternes, T (2005) Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Research 39(14):3139-3152*

Kackay, D; Paterson, S; Shiu, WY (1992) Generic models for evaluating the regional fate of chemicals. *Chemosphere 24(6):695-717*

Kasim, NA; Whitehouse, M; Ramacharndran, C; Bermejo, M; Amidon, GL (2004) Molecular Properties of WHO essential drugs and provisional biopharmaceutical classification. *Molecular Pharmaceutics 1(1):85-96*

Keller, V (2005) Risk assessment of "down-the-drain" chemicals: search for a suitable model. *Science of the Total Environment – in press, corrected proof*

Kelly, L; Smith, D and Snary, E (2004) Animal growth promoters: to ban or not to ban? A risk assessment approach. *International Journal of Antimicrobial Agents 24:7-14*

Kerkhoff, L (2005) Integrated research: concepts of connection in environmental science and policy. *Environmental Science and Policy8:452-463*

Khan, SJ; Ongerth, JE (2004) Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations. *Chemosphere 54:355-367*

Kimura, K; Amy, G; Drewes, J and Heberer T (2003) Rejection of organic micropollutants(disinfection by-products, endocrine disrupting compounds, and pharmaceutically active compounds) by NF/RO membranes *Journal of Membrane Science 227:113-121*

Kimura, K; Toshima, S; AMY< G: Watanabe, Y (2004) Rejection of neutral endocrine disrupting compounds (EDCs) and pharmaceutically active compounds PhACs by RO membranes. *Journal of Membrane Science 245:71-78*

Klein, G; Pack, A; Reuter, G (1998) Antibiotic resistance patterns of enterococci and occurrence of vancomycin-resistant enterococci in raw minced beef and pork in Germany. *Applied and Environmental Microbiology 64(5):1825-1830*

Knacker, T; Duis, K; Ternes, T; Fenner, K; Escher, B; Schmitt, H; Boxall, AB (2005) The EU-project ERAPharm. Incentives for further development of guidance document. *Environmental Science and Pollution Research International 12(2):62-65*

Kolpin, DW, Furlong, ET, Meyer, MT, Thurman, EM, Zaugg, SD, Barber,LB (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: a national reconnaissance. *Environmental Science Technology 36:1202-1211*

Kolpin, DW; Skopec, M; Meyer, MT; Furlong,ET; Zuagg, SD (2004) Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. *Science of the Total Environment 328:119-130*

Koopman-Esseboom, C; Morse, DC; Weisglas-Kuperus, N; Tuinstra, LGMT; Brouwer, A; Sauer, PJJ (1994) Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatric Research 36:468-473*

Kortenkamp, A; Altenburger, R (1999) Approaches to assessing combination effects of oestrogenic environmental pollutants. *The Science of the Total Environment 233:131-140*

Koschorreck, J; Koch, C; Ronnefahrt, I (2002) Environmental risk assessment of veterinary medicinal products in the EU – a regulatory perspective. *Toxicology Letters 131:117-124*

Koutsouba, V; Heberer, T; Fuhrmann, B; Schmidt-Baumler, K; Tsipi, D (2003) Determination of polar pharmaceuticals in sewage water of Greece by gas Chromatography-mass spectrometry. *Chemosphere 51:69-75*

Kristensen, P; Tyle, H (1991) The assessment of bioaccumulation. In Nagel, R; Loskill, R (Eds) *Bioaccumulation in Aquatic Systems. Contributions to the Assessment. VCH Publishers, Weinheim*

Kuhn, TS (1962) The Structure of Scientific Revolutions. University of Chicago Press, Chicago.

Kümmerer, K (2001) Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere 45:957-969*

Kummerer, K (2004) Phamaceuticals in the Environment – Scope of the Book and Introduction. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin.

Kummerer, K (2004) Resistance in the environment. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Kummerer, K and Al-Ahmed, A (1997) Biodegradability of the anti-tumor agents 5-fluoro-uracil, cytarabine and gemcitabine: impact of the chemical structure and synergistic toxicity with hospital effluent. *Acta hydrochimica et Hydrobiologica 25:166-172*

Kümmerer, K; Al-Ahmad, A; Bertram, B; Wiebler, M (2000) Biodegradability of antineoplastic compounds in screening tests: influence of glucosidation and of stereochemistry. *Chemosphere 40:767-773*

Kümmerer, K; Alexy, R; Huttig, J; Scholl, A (2004) Standardized tests fail to assess the effects of antibiotics on environmental bacteria. *Water Research 38:2111-2116*

Kümmerer, K; Steger-Hartmann, T; Meyer, M (1997) Biodegradability of the anti-tumor agent ifosamide and its occurrence in hospital effluents and communal sewage. *Water Research 31:2705-2710*

Kurelec, B (1992) The multidrug resistance-like mechanism in a marine sponge *Tethya aurantium*. *Marine Environmental Research 34:249-253*

Kurelec, B (1995) Inhibition of multixenobiotic resistance mechanism in aquatic organisms: ecotoxic concequences. Science of the Total Environment 171(1-3):197-204

Kurelec, B (1997) A new type of hazardous chemical: the chemosensitizers of multixenobiotic resistance. *Environmental Health Perspectives 105 suppl.4:855-860*

Kurelec, B; Britvi, S; Pivevi, B; Smital, T (1998) Fragility of mulixenobiotic resistance in aquatic organisms enhances the complexity of risk assessment. *Marine Environmental Research, 45(1-5):415-419*

Kurelec, B; Pivevi, B (1991) Evidence for a mulitxenobiotic resistance mechanism in the mussel Mytilus galloprovincialis. *Aquatic Toxicology, 19(4):291-301*

Kuspis, D; Krenzelok, E (1996) What happens to expired medications? A survey of community medication disposal. *Veterinary and Human Toxicology 38:48-49*

Kuster, M; de Alda, MJL; Barcelo, D (2004) Analysis and distribution of estrogens and progestogens in sewage sludge, soils and sediments. *Trends in Analytical Chemistry 23 (10-11):790-798*

Lai, KM; Scrimshaw, MD; Lester, JN (2002) Prediction of thee bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems. *Science of the Total Environment 289:159-168*

Lang, R and Dietrich, D (2002) Environmental risk assessment of pharmaceutical drug substances – conceptual considerations *Toxicology Letters 131:97-104*

Lange, R and Dietrich, D (2002) Environmental risk assessment of pharmaceutical drug substances – conceptual considerations. *Toxicology Letters 131:97-104*

Lanning, CL; Ayad, HM; Aboudonia, MB (1997) P-glycoprotein involvement in cuticular penetration of C-14 tiodicarb in resistant budworms. *Toxicology Letters 85:127-133*

Lanning, CL; Fine, RL; Corcoan, HM; Ayad, RL (1996) Tobacco budworm P-glycoprotein-biochemical characterization and its involvement in pesticide resistance. *Biochemical and Biophysical Acta General Sub. 1291:155-162*

Lanzky, PF and Halling-Sorensen, B (1997) The toxic effect of the antibiotic metronidazole on aquatic organisms. *Chemosphere 35: 2553-2561*

Larsen, TA; Lienert, J; Joss, A; Siegrist, H (2004) How to avoid pharmaceuticals in the aquatic environment. *Journal of Biotechnology 113:295-304*

Larson, DGJ; Adolfsson-Erici, M; Parkkonen, J; Pettersson, M; Olson, PE (1999) Ethinylestradiol – an undesired fish contraceptive? *Aquatic Toxicology 45:91-97*

Laville, N; Ait-Aissa, S and Gomez, E (2004) Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes *Toxicology196:41-55*

Law, FCP (2001) Personal Communication, Simon Fraser University, Burnaby, BC

Lech, JJ; Vodicnik, MJ (1985) Biotransformation. In Fundamentals of Aquatic Toxicology: Methods and Applications, Rand, GM; Petrocelli, SR (Eds.) Hemisphere Publishing Corporation, New York, USA

Lee, B; Ellenbecker, MJ; Moure-Ersaso, R (2004) Alternatives for treatment and disposal cost reduction of regulated medical wastes. *Waste Management 24:143-151*

Leff, LG; Dana, JR; McArthur, JV (1993) Detection of Tn5-like sequences in kanamaycin-resistant strain bacteria and environmental DNA. *Applied and Environmental Microbiology 59(2):417-421*

Levy, SB (1998) The challenge of antibiotic resistance. *Scientific American 278:46-53*

Li, X; Zhao, H (2006) Development of a model for predicting trihalomethanes propagation in water distribution systems. *Chemosphere, 62(6):1028-1032*

Liberatore, A; Funtowicz, S (Eds.) Special Issue on Democratising Expertise, Expertising Democracy *Science and Public Policy 3, volume 30*

Lieberman, P; Wootan, M (1998) Protecting the Crown Jewels of Medicine *Center for Science in the Public Interest* http://cspinetorg/reports/abiotic.htm (accessed November 30, 2004)

Lindqvist, N; Tuhkanen, T; Kronberg, L (2005) Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Research 39:2219-2228*

Lofstedt, R (2004) Risk Communication and Management in the 21st Century. Joint Centre for Regulatory Studies, Washington, DC

Long, C; Crane, M (2002) Environmental risk assessment of veterinary pharmaceuticals in the EU: Reply to Montforts and de Knecht *Toxicology Letters 142:219-225*

Lu, W; Leung, A (2003) A preliminary study on potential of developing shower/laundry wastewater reclamation and reuse system. *Chemosphere 52:1451-1459*

Luckenback,T; Corsi, I; Epel, D (2004) Fatal attraction: synthetic musk fragrances compromise multixenobiotic defense systems in mussels. *Marine Environmental Research 56:215-219*

Luedeking, A; Koehler, A (2004) Regulation of expression of multixenobiotic resistance (MXR) genes by environmental factors in the blue mussel *Mytilus edulis. Aquatic Toxicology 69:1-10*

Lundholm, CE (1997) DDE-induced eggshell thinning in birds:Effects of p,p'-DDE on the calcium and prostaglandin metabolism of eggshell gland. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicolgy and Endocrinology 118(2):113-128*

Lyman, WJ; Reehl, WF; Rosenblatt, DH (1990) Handboog of chemical property estimation methods. American Chemical Society, Washington, DC

MacDonald, BA; Ward, JE (1994) Variation in food quality and particle selectivity in the sea scallop *Placopecten magellanicus. Marine Ecology Progress Series 108:251-264*

Macgill, SH; Siu, YL (2005) A new paradigm for risk analysis. *Futures 37:1105-1131*

Mackay, D; Paterson, S (1991) Evaluating the multimedia fate of organic chemicals: a level III fugacity model. *Environmental Science and Technology 25:427-436*

Manz, M; Weissflog, L; Kuhne, R (1999) Ecotoxicological hazard and risk assessment of heavy metal contents in agricultural soils of central Germany *Ecotoxicology and Environmental Safety 42:191-201*

Markowitx, G; Rosner, G (2002) Deceit and Denial. The Deadly Politics of Industrial Pollution. University of California Press, Berkely, CA

Massaad, C; Barouki, R (1999) An assay for the detection of xenoestrogens based on a promoter containing overlapping EREs. *Environmental Health Perspectives 107:563-566*

McEwen, S and Fedorka-Cray, P (2002) Antimicrobial use and resistance in animals *Clinical Infectious Disease 34:S93-S106*

McKerlie, K; Knight, N; Thorpe, B (2005) Advancing extended producer responsibility in Canada (2005) *Journal of Cleaner Production 14:616-628*

Melnick, RL (2001) Is peroxisome proliferation an obligatory precursor step in the carcinogenicity of di(2-ethihexyl) phthalate(DEHP)? *Environmental Health Perspectives 109:437-442*

Mendes, AJJ (2002) The endocrine disrupters: a major medical challenge. *Food and Chemical Toxicology 40:781-788*

Metcalfe, C; Miao, XS; Hua, W; Letcher, R; Servos, M (2004) Pharmaceuticals in the Canadian Environment In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Metcalfe, CD; Koening, BG; Bennie, TD; Servos, M; Ternes, TA; SHirsch, R (2003b) Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. *Environmental Toxicology and Chemistry 22:2881-2880*

Metcalfe, CD; Miao, XS; Koening, BG; Struger, J (2003a) Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower great lakes. *Canadian Environmental Toxicology and Chemistry 22:2881-2889*

Metzger, JW (2004) Drugs in municpal landfills and landfill leachates In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Miao, X; Koening, BG, Metcalfe, CD (2002) Analysis of acidic drugs in the effluents of sewage treatment plants using liquid chromatography – eletrospray ionization tandem mass spectrometry. *Journal of Chromatography A 952:139-147*

Miao, XS; Bishay, F; Chen, M; Metcalfe, CD (2004) Occurrence of antimicrobials in the final effluents form wastewater treatment plants in Canada. *Environmental Science and Technology – in press*

Migliore, L; Civittarcale, C; Bambilla, G (1997) Toxicity of several important agricultural antibiotics to *Artemia. Water Research 31(7):1801-1806*

Miles-Reichardson, SR; Pierens, SL; Nichols, KM; Kramer, VJ; Snyder, EM; Snuder, SA; Rendr, JA (1999) Effects of waterborne exposure to 4-nonylphenol and nonylphenol ethoxylate on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelas) Environmental Research 80:S122-S137*

Mills, LJ; Chichester, C (2005) Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? *Science of the Total environment 343:1-34*

Mimeault, C; woodhouse, AJ; Miao, X; Metcalfe, CD; Moon, TW; Trudeau, VL (2005) The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in goldfish *Carassius auratus. Aquatic Toxicology 73:44-54*

Moggs, JC; Tinwell, H; Spurway, T; Chang,HS; Soames, AR, Kimber, I; Orphanides, G (2004) Phenotypic anchoring of gene expression changes during estrogen-induced uterine growth. *Environmental Health Perspectives 112:1589-1606*

Moggs, JG (2005) Molecular responses to xenoestrogens: Mechanistic insights from toxicogenomics. *Toxicology 213:177-193*

Monosson, E (2005) Chemical Mixtures: Considering the evolution of toxicology and chemical assessment. *Environmental Health Perspectives 113 (4):383-390*

Montague, P (2004) Reducing the harms associated with risk assessments. *Environmental Impact assessment Review 24:733-748*

Montforts, MHMM (2005) Validation of the exposure assessment for veterinary medicinal products. *Science of the Total Environment 358:121-136*

Montforts, MHMM; van Rijswick, HFMW; Udo de Haes, HA (2004) Legal constraints in EU product labelling to mitigate the environmetal risk of veterinary medicines at use. *Regulatory Toxicology and Pharmacology 40:327-335*

Montserrat, S; Raldua, D; Barcelo, D and Porte, C (2003) Long-term exposure effects in vitellogenin, sex hormones, and biotransformation enzymes in female carp in relation to a sewage treatment works *Ecotoxicology and Environmental Safety 56:373-380*

Moore, MN; Depledge, MH; Readman, JW; Leonard, DRP (2004) An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. *Mutation Research 552:247-268*

Munns, WR; SuterII, GW; Damstra, T; Kroes, R; Reiter, W; Marafante, E (2003) Integrated risk assessment – results from an international workshop. *Human and Ecological Risk Assessment 9(1):379-386*

Nash, JP; Kime, DE; Van der Ven, TML; Wester, PW; Brion, F; Maack, G; Tyler, CR (2004) Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environmental Health Perspectives 112(17):1725-1733*

Nawaz, MS; Erickson, BD; Khan, AA; Khan, SA; Pothuluri, JV; Rafii, F; Sutherland, JB; Wagner, RD; Cerniglia, CE (2001) Human Health impact and regulatory issues involving antimicrobial resistance in the food animal production environment. *Regulatory Research Perspectives 1:10-16*

Nghiem, DL; Manis, K; Soldenhoff; A; Schafer, A (2004) Estrogenic hormone removal from wastewater using NF/RO membranes. *Journal of Membrane Science 242:37-45*

Nghiem, LD; Schafer, AI (2006) Critical risk points of nanofiltration and reverse osmosis processes in water recycling applications. *Desalination 187 (2006) 303-312*

Niederman, M (2001) Impact of antibiotic resistance on clinical outcomes and the cost of care. *Critical care medicine 29-4:N114-N120*

Noacksson, E; Gustavsson, B; Linderoth, M; Zebuhr, Y; Broman, D; Balk, L (2004) Gonad development and plasma steroid profiles by HRGC/HRMS during one reproductive cycle in reference and leachate-exposed female perch (Perca fluviatilis). *Toxicology and Applied Pharmacology 195:247-261*

Noacksson, E; Linderoth, M; Bosveld, ATC; Balk, L (2003) Altered steroid metabolism in several teleost species exposed to endocrine disrupting substances in refuse dump leachate. *General and Comparative Endocrinology 134:273-284*

Noacksson, E; Linderoth, M; Bosveld, ATC; Norrgren, L; Zebuhr, Y; Balk, K (2003) Endocrine disruption in brook trout (*Salvelinus fontianalis*) exposed to leachate from a public refuse dump. *The Science of the Total Environment 305:87-103*

Noacksson, E; Linderoth, M; Gustavsson, B; Zebuhr, Y; Balk, L (2005) Reproductive status in female perch (*Perca fluviatilis*) outside a sewage treatment plant processing leachate from a refuse dump. *Science of the Total Environment 340:97-112*

Noaksson, E; Tjarnlund, U; Bosveld, AT; Balk, L (2001) Evidence for endocrine disruption in perch and roach in a remote Swedish lake in the vicinity of a public refuse dump. *Toxicology and Applied Pharmacology 174:160-176*

Novaquatis 2005 http://www.novaquatis.ch/english/NOVA8_e.html, accessed February, 2005

Nowotny, H; Scot, P; Michael, G (2001) Rethinking Science: Knowledge and the Public in an Age of Uncertainty. Polity Press, London

Nunes, B; Carvalho, F; Guilhermino, L (2004) Acute and chronic effects of clofibrate and clofibric acid on the enzymes acetylcholinesterase, lactate dehaydrogenase, and catalase of the mosquitofish, *Gambusia holbrooki. Chemosphere 57:1581-1589*

Nygaard, K et al. (1992) Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. *Aquaculture 104:31-36*

Oaks, LJ; Gilbert, M; Virani, MZ; Watson, R; Meteyer, CU; Rideout, BA; Shivaprasad, HL; Ahmed, S; Chaudhry, MJI; Arshad, M; Mahmood, S; Ali, A; Khan, AA (2004) Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature 427:630-633*

Oberemm, A; Onyon, L; Gundert-Remy, U (2005) How can toxicogenomics inform risk assessment? *Toxicology and Applied Pharmacology 207:S592-S598*

OECD (1989) Report of the OECD Workshop on Ecological Effects Assessment, OECD Environment, Monograph no. 26

Oehlmann, J; Schulte-Oehlmann, U; Tillmann, M; Markert, B (2000) Effects of endocrine disruptors on prosobranch snails in the laboratory. Part I: bisphenol a and octylphenol as xeno-estrogens. *Ecotoxicology 9;383-397*

Olden, K (2004) Genomics in environmental health research – opportunities and challenges. *Toxicology 1998 (1-3):19-24*

Olejniczak, K; Spindler, P (2004) Environmental risk assessment of medicinal products for human use: aspects of its regulations in the Eurpean Union, Canada and United States. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Ong, EK; Glantz, SA (2001) Constructing "sound science" and "good epidemiology" tobacco, lawyers, and public relations firms. *American Journal of Public Health 91:1749-1757*

Opperhuizen, A (1991) Bioconcentration and biomagnification: is a direction necessary? In Nagel, R; Loskill, R (Eds.) *Bioaccumulation in Aquatic Systems. VCH Publishers, Weinheim*

Oreskes, N (2004) Science and public policy: what's proof got to do with it? *Environmental Science and Policy 7:369-383*

Oros, D; Jarman W. and Lowe, T (2003) Surveillance for previously unmonitored organic contaminants in the San Francisco Estuary *Marine Pollution Bulletin 46:1102-1110*

Ottinger, MA; Abdelnabi, M; Quinn, M; Golden, N; Wu, J; Thompson, N (2002) *Neurotoxicology and Teratology 24:17-28*

Pain, S; Parant, M (2003) Response of multixenobiotic defence mechanism in *Dreissena polymorpha* exposed to environmental stress. *Chemosphere 52:1105-1113*

Pal, LA (2001) Beyond Policy Analysis: public issue management in turbulent times. 2nd Ed. Nelson-Thomson Learning, Scarborough, ON

Panhho, A; Culver, D and Gavnes, R (1992) Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975 – 1991 *Infection Control and Hospital Epidemiology, 13:582-586*

Parameshwaran, K; Fane, A; Cho, B and Kim, K (2001) Analysis of microfiltration performance with constant flux processing of secondary effluent *Water Resources 35:4349-4358*

Parant, M and Pain, S. (2001) Potential use of multixenobiotic defence mechanism (MXDM) in *Dreissena polymorpha* as a biomarker for the monitoring of freshwater pollution. *Water Research 35:3743-3748*

Patil, AD; Shekdar, AV (2001) Health-care waste management in India. *Journal of Environmental Management 63:211-220*

Paulsen, CA; Bernam, NG; Wang, C (1996) Data from men in greater Seattle reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. *Fertility and Sterility 65:1015-1020*

Paxeus N; Schroder, H (1996) Screening for non-regulated organic compounds in municipal waste water in Goteberg, Sweden *Water Science and Technology 33:9-15*

Payne, J; Rajapakse, N; Wilkins, M; Kortenkamp, A (2000) Prediction and Assessment of the effects of mixtures of four xenoestrogens. *Environmental Health Perspectives 108 (10):983-987*

PCPSA (2006) Post Consumer Pharmaceutical Stewardship Association – Medicines Return Program. http://www.medicationsreturn.ca/, accessed March, 2006

Peakall, DB; Lincer, JL (1996) Do PCBs cause eggshell thinning? *Environmental Pollution 91(1):127-129*

Petrovic, M; Eljarrat, E; de Alda, L; Barcelo, D (2001) Analysis and environmental levels of endocrine-disrupting compounds in freshwater sediments. *Trends in Analytical Chemistry 20:637-648*

Petry, T; Lofstedt, RE; Dietrich, DR (2006) Editorial. *Regulatory Toxicology and Pharmacology 44:1-3*

Pomati, F; Netting, AG; Calamari, D; Neilan, BA (2004) Effects of erythromycin, tetracycline and ibuprofen on the growth of *Synechocystis* sp and *Lemna minor. Aquatic Toxicology 67:387-396*

Ponka, A; Kashi,A; Lahdevirta, J (1996) Recommendations for the management of wastes from healthcare facidities in Helsinki. *Waste Management and Research 14:145-150*

Porter, C and Janz, D (2003) Treated municipal sewage discharge affects multiple levels of biological organization in fish *Ecotoxicology and Environmental Safety 54:199-206*

Power, M; McCarty, LS (1997) Fallacies in ecological risk assessment practices. *Environmental Science and Technology 31:370A-375A*

Pratt, IS; Barron, T (2003) Regulatory recognition of indirect genotoxicity mechanisms in the European Union. *Toxicology Letters, 140-141:53-62*

Pugh, D. M (2002) The EU precautionary bans of animal feed additive antibiotics *Toxicology Letters 28:35-44*

Purdom, CE; Hardiman, PA; Bye, VJ; Eno, NC; Tyler, CR; Sumpter, JP (1994) Estrogenic effects of effluents from sewage treatment works. *Chemistry and Ecology 8:275-285*

Quinn, B; Gagne, F; Costello, M; McKenzie, C; Wilson, J; Mothersill, C (2004) The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha). Aquatic Toxicology 66:279-292*

Rajapakse, N; Silva, E; Kortenkamp, A (2002) Combining xenoestrogens at levels below individual no-observed-effect-concentrations dramatically enhances steroid hormone action. *Environmental Health Perspectives 110 (9):917-921*

Rang, HP and Dale, MM (1991) Pharmacology – Churchill Livingstone, 2nd ed. pp:955

Rapport, DJ; Singh, A (2006) An EcoHealth-based framework for State of Environment Reporting. *Ecological Indicators 61:409-428*

Ratnaparkhe, V; Sertic, D (2005) The City of Toronto's sewer use by-law and pollution prevention. *Journal of Cleaner Production – in press*

Ravetz, HR (1999) What is Post-Normal science? *Futures 31:647-653*

Ravetz, J (2004) The post-normal science of precaution. *Futures 36:347-357*

Ravetz, J; Funtowicz, S (1999) Post-Normal Science – an insight now maturing. *Futures 31:641-646*

Reddersen, K; Heberer,T; Dunnbier, U (2002) Identification and significance of phenazone drugs and their metabolites in ground adndrinking water. *Chemosphere 49:539-544*

Ren, S (2002) Predicting three narcosis mechanisms of aquatic toxicity. *Toxicology Letters 133:127-139*

Rhodes, C (2000) Principles of testing for acute toxic effects. In General and Applied Toxicology 2nd Ed. Macmillan Reference LTD.

Rhodes, C (2000) Principles of testing for acute toxic effects. In General and Applied Toxicology, 2nd Ed. Ballantyne, B; Marrs, T; Syversen, T (Eds.), Macmillan Reference LTD, London

Richardson, T (2005) Environmental assessment and planning theory: four short stories about power, multiple rationality and ethics. *Environmental Impact Assessment Review 25:341-365*

Rio Declaration, (1992) Report of the United Nations Conference on Environment and Development http://www.un.org/cyberschoolbus/peace/earthsummit.htm, accessed January 2006

Rogers, N; Birtwell, I and Krunznki, G (1986) Organic extractables in municipal waste water, Vancouver, British Columbia, Canada. *Canadian Journal of Water Pollution Research 21:187-204*

Routledge, EJ; Sheahan, D; Desbrow, C; Brightly, G; Waldock, M and Sumpter, JP (1998) Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. *Environmental Science and Technology 32:1559-1565*

Ruden, C (2005) Principles and practices of health risk assessment under current EU regulations *Regulatory Toxicology and Pharmacology 44:14-23*

Rudholm, N (2002) Economic implications of antibiotic resistance in a global economy *Journal of Health Economics 21:1071-1083*

Russell, WMS; Burch, RL (1959) The Principles of Humane Experimental Technique Methuen, London

Safe, S (1990) Polychlorinated biphenols (PCBs), dibenzo-p-dioxins (PCDDs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Toxicology 21:51-87*

Safe, SH (1995) Environmental and dietary estrogens and human health: is there a problem? *Environmental Health Perspectives 103:346-351*

Samuelsen, OB; Torsvik, V; Ervik, A (1992) Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *Science of the Total Environment 114:25-36*

Sandaa, RA; Torsvik, VL and Goksoyr, J (1992) Transferable drug resistance in bacteria from fish-farm sediment. *Canadian Journal of Microbiology 38:1061-1065*

Sanderson, H; Brain, RA; Johnosn, DJ; Wilson, CJ; Solomon, KR (2004a) Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular and sex hormones. *Toxicology, 203:27-40*

Sanderson, H; Johnson, D; Reitsma, T; Brain, R; Wilson, C and Solomon, K (2004) Ranking and prioritization of environmental risks of pharmaceuticals in surface waters *Regulatory Toxicology and Pharmacology 39:158-183*

Sanderson, H; Petersen, S (2002) Power analysis as a reflexive scientific tool for interpretation and implementation of the precautionary principle in the European Union. *Environmental Science and Pollution Research International 9(4):221-226*

Sandin, P (1999) Dimensions of the precautionary principle. *Human and Ecological Risk Assessment 5(5):889-907*

Saouter, E; Pittinger, C; Feijtel, T (2001) Aquatic environmental impact of detergents: from simple to more sophisticated models. *Ecotoxicology and Environmental Safety 50:153-159*

Saradha, B; Mathur, PP (2006) Effect of environmental contaminant on male reproduction. *Environmental Toxicology and Pharmacology 21:34-41*

Scarlatto, 1999 York Central Hospital: P2 in Healthcare Housekeeping Presentation available online at http://www.c2p2online.com/main.php3?session=&section=88&doc_id=174, accessed March 2006

Schafer, A; Fane, A and Waite, T (2001) Cost factors and chemical pretreatment effects in the membrane filtration of waters containing natural organic matter *Water Resources 35:1509-1517*

Schafer, AI; Beder, S (2006) Relevance of the precautionary principle in water recycling. *Desalination 187:241-252*

Scheytt, T; Mersmann, P; Lindstädt, R; Heberer, T (2005) Determination of sorption coefficients of pharmaceutically active substances carbamazepine, diclofenac, and ibuprofen, in sandy sediments. *Chemosphere 60:245-253*

Schneider, AL; Ingram, H; (1997) Policy Design for Democracy. University Press of Kansas, Lawrence, KS

Schulte-Oehlmann, U; Oetken, M; Bachmann, J; Oehlmann, J (2004) Effects of ethinyloestradiol and methyltestosterone in prosobranch snails. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Schultz, TW; Cronin, MTD; Walker, JD; Aptula, AO (2003) Quantitative structure – activity relationships (QSARs) in toxicology: a historical perspective. *Journal of Molecular Structure 622:1-22*

Schultz, TW; Netzeva, TI (2004) Development and evaluation of QSARs for ecotoxic endpoints: the benzene response-surface model for Tetrahymena toxicity. In Predicting Chemical Toxicity and Fate. Cronin, MTD; Livingstone, DJ (Eds.), CRC Press, Boca Raton

Schwab, BW; Hayes, EP; Fiori, JM; Mastrocco, FJ; Roden, NM; Cragin, D; Meyerhoff, RD; D'Aco, VJ; Anderson, PD (2005) Human pharmaceuticals in the US surface waters: A human health risk assessment. *Regulatory Toxicology and Pharmacology 42:296-312*

Schwabe, U; Paffrath, D (2004) Arzneiverordnungsre-port 2003, Springer Press, Berlin, Germany. Quoted in Cleuvers (2005).

Schwaiger, J; Ferling, H; Mallow, U; Wintermayr, H; Negele, RD (2004) Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquatic Toxicology 68:141-150*

Schwartz, S; Berding, V; Matthies, M (2000) Aquatic fate assessment of the polycyclic musk fragrance HHCB Scenario and variability analysis in accordance with the EU risk assessment guidelines *Chemosphere 41:671-679*

Schwartz, T; Kohnen, W; Jansen, B; Obst, U (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiology Ecology 43:325-335*

Sedlak, D; Gray, J; Pinkston, K (2000) Understanding microcontaminants in recycled water. *Environmental Science and Technology Dec.1, 2000:509A-515A*

Segner, H; Navas, JM; Schafers, C; Wenzel, A (2003) Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo. *Ecotoxicology and Environmental Safety 54:315-322*

Seiler, R; Zaugg, S and Thomas, J (1999) Caffeine and pharmaceuticals as indicators of waste water contamination in wells *Ground Water 37:405-410*

Sekizawa, J; Tanabe, S (2005) A comparison between integrated risk assessment and classical health/environmental assessment: Emerging beneficial properties. *Toxicology and Applied Pharmacology 207: S617-S622*

Servos, MR; Bennie, DT; Burnison, BK; Jurkovic, A; McInnis, R; Neheli, T; Schnell, A; Seto, P; Smyth, SA; Ternes, TA (2005) Distribution of estrogens, 17β-estradiol and estrone in Canadian municipal wastewater treatment plants. *Science of the Total Environment 336:155-170*

Servos, MR; Bennie, DT; Jurkovic, A; McInnis, R; Schnell, A; Seto P; Ternes, TA (2004b) Removal of hormone, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. *Science of the Total Environment – 336(1):155-163*

Servos, MR; Smith, M; McInnis, M; Burnison, K; Seto, P ; Smyth, SA; Backus, R (2004a) Presence and removal of acidic drugs in drinking water treatment plants in Ontario, Canada. *Water Quality Research Journal of Canada – in press*

Sharpe, RM and Skakkebaek, NE (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet 103:1136-1143*

Sharpe, RM; Fisher, JS; Millar, M; Jobling, S and Sumpter, JP (1995) Gestational and lactational exposure of rates to xenoestrogens results in reduced testicular size and sperm production. *Environmental Health Perspectives 103:1136-1143*

Sheahan, DA; Bucke, P; Matthiessen, JP; Sumpter, MF; Kirby, P; Waldock, M (1994) The effects of low levels of 17-alpha-ethynylestradiol upon plasma vitellogenin levels in male and female rainbow trout held at two acclimation temperatures. In Sublethal and Chronic Effects of Pollutants on Freshwater Fish. Muller and Lloyd Ed. Fishing News Books pp. 99-112

Shuilleabhain, S; Davoren, M; Mothersill, C; Sheehan, D; Hartl, M; Kilemade, M; O'Brien, NM; O'Halloran, J; Van Pelt, FNAM; Lyng, FM (2005) Identification of a multixenobiotic resistance mechanism in primary cultured epidermal cells from *Oncorhynchus mykiss* and the effects of environmental complex mixtures on its activity. *Aquatic Toxicology 73:115-127*

Sijm, DTHM; Opperhuizen, A (1989) Biotransformation of organic chemicals by fish: enzyme activities and reactions. In Handbook of Environmental Chemistry Reactions and Processes, Hutzinger, O (Ed.) Springer, Berlin

Sijm, DTHM; Seinen,W; Opperhuizen, A (1992) Life-cycle biomagnification study in fish. *Environmental Science and Technology 26:2162-2174*

Silva, E; Rajapakse, N; Kortenkamp, A (2002) Something from "Nothing" – Eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environmental Science and Technology 36:1751-1756*

Skov, T; Yynge,E; Maarup, B; Olsen, J; Roth, M (1990) Risks for physicians handling antineoplastic drugs. *Lancet 336:1446*

Slagot, M; Huertas, E; Weber, S; Dott, W; Hollender, J (2006) Wastewater reuse and risk: definition of key objectives. *Desalination 187:29-40*

Smital, T and Kurelec, B. (1998) The chemosensitizers of multixenobiotic resistance mechanism in aquatic invertebrates: a new class of pollutants. *Mutation Research 399:43-53*

Smital, T; Luckenback T; Sauerborn, R; Hamdoun, AM; Vega, RL; Epel, D (2004) Emerging contaminants – pesticides, PPCPs, microbial degradation products and natural substances as inhibitors of the multixenobiotic defence in aquatic organisms. *Mutation Research 552:101-117*

Snyder, SA; Keith, TL; Verbrugge, DA; Snyder, EM; Gross, TS; Gewsy, JP (1999) Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. Environmental Science and Technology 33:2814-2820

Snyder, SA; Westerhoff, P; Yoon, Y; Sedlak, DL (2003) Pharmaceuticals, personal care products and endocrine disrupters in water: implication for the water industry. *Environmental Engineering and Science 20(5):449-469*

Spacie, A; Hamelink, JL (1982) Alternative models for describing the bioconcentration of organics in fish. *Environmental Toxicology and Chemistry 1:309-320*

Specker J; Sullivan, CV (1994) Vitellogenesis in fishes: status and perspectives. In Perspectives in comparative endocrinology Davey, KG; Peter, RG; Tobe, SS (Eds.) Ottawa

Stan, HJ and Heberer, T (1997) Pharmaceuticals in the aquatic environment. *Analysis Magazine 25:M20-M23*

Stecko, JR; Bendell-Young, LI (2000) Contrasting the geochemicstry of suspended particulate matter and deposited sediments within an estuary. *Applied Geochemistry, 15(6):753-775*

Stegeman, JJ; Brouwer, M; Richard, TDG; Forlin, L; Fowler, BA; Sanders, BM van Veld, PA (1992) Molecular responses to environmental contaminants: enzyme and protein systems as indicators of chemical exposure and effect. In Biomarkers: Biochemical, Physiological nd Histological markers of Anthropogenic Stress Huggett, RJ; Kimerly, RA; Mehrle, PM (Eds.) Lewis Publishers, Chelsea, USA

Steger-Hartmann et al. (1997) Biological degradation of cyclophosphamide and its occurrence in sewage water. *Ecotoxicology and Environmental Safety 36:174-179*

Steger-Hartmann, T; Lange, R; Schweinfurth, H (1999) Environmental risk assessment for the widely used iodinated X-ray contrast agent iopromide (Ultravist). *Ecotoxicology and Environmental Safety 42:274-281*

Stein, JG (2001) The Cult of Efficiency. House of Anansi Press, Toronto, ON

Steinemann, A (2004) Human exposure, health hazards and environmental regulations. *Environmental Impact Assessment Review 24:695-710*

Stephen, C; Ribble, C (2001) Death, disease and deformity: using outbreaks in animals as sentinels for emerging health risks. *Global Change and Human Health 2:108-117*

Stern, PC; Fineberg, HV (1996) Understanding risk: informing decisions in a democratic society. National Academy Press, Washington, DC

Straub, O (2002) Environmental risk assessment for new human pharmaceuticals in the European Union according to the draft guideline/discussion paper of January 2001 *Toxicology Letters 135:231-237*

Stuer-Lauridsen, F; Birkved, M; Hansen, LP; Holten, HC; Halling-Sorensen, B (2000) Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere 40:783-793*

Stumpf, M; Ternes, TA; Wieken, RD; Rodrigues, SV; Baunamm, W (1999) Polar drug residues in sewage and natural waters in the state of Rio de Janiero, Brazil. *Science of the Total Environment 225:135-141*

Sukhai, M; Piquette-Miller, M (2000) Regulation of the multidrug resistance genes by stress signals. *Journal of Pharmacy and Pharmaceutical Sciences 3(2) 268-280*

Sumpter, JP (1998) Xenoendocrine disrupters – environmental impacts. *Toxicology Letters 102-103:337-342*

Sumpter, JP and Jobling, S (1995) Vitellogenesis as a biomarker for oestrogenic contamination of the aquatic environment. *Environmental Health Perspectives 103(sup.7):173-178*

Suter II, GW; Vermeire, T; Munns Jr., WR; Sekizawa, J (2005) An integrated framework for health and ecological risk assessment. *Toxicology and Applied Pharmacology 207:S611-S616*

Suter, GW (1996) Abuse of hypothesis testing in ecological risk assessment. *Human and Ecological Risk Assessment 2:331-349*

Sutter II, GW; Munns, WR; Sekizawa, J (2003b) Types of integration in risk assessment and management and why they are needed. *Human and Ecological Risk Assessment 9(1):273-279*

Sutter II, GW; Norton, SB; Barnthouse, LW (2003c) The evolution of frameworks for ecological risk assessment form the Red Book ancestor. *Human and Ecological Risk Assessment 9(5):1349-1360*

Sutter II, GW; Vermier, T; Munns, WR; Sekizawa, J (2003a) Framework for the integration of health and ecological risk assessment. *Human and Ecological Risk Assessment 9:281-302*

Tabak H; Bloomhuff; RN and Bunch, RL (1981) Steroid hormones as water pollutants II. Studies on the persistence and stability of natural urinary and synthetic ovulation-inhibiting hormones in treated and untreated wastewaters. *Dev. Ind. Microbiol. 22:457-519*

Tallacchini M (2005) Before and beyond the precautionary principle: Epistemology of uncertainty in science and law. *Toxicology and Applied Pharmacology 207:S645-S651*

Tauxe-Wuersh, A; De Alencastro, LF; Grandjean, D; Tarradellas, J (2005) Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. *Water Research 39:1761-1772*

Teles, M; Pacheco, M; Santos, MA (2006) Biotransformatin, stress and genotoxic effects of 17β-estradiol in juvenile sea bass (Dicentrarchus labbrax L.). *Environment International – in press.*

Ternes, T (1998) Occurrence of drugs in German sewage treatment plants and rivers *Water Research 32:3245-3260*

Ternes, T; Stuber, J; Herrmann, N; McDowell, D. (2003) Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? *Water Research 37:1976-1982*

Ternes, TA; Hirsch, R (2000) Occurrence and behaviour of X-ray contrast media in sewage facilities and the aquatic environment. *Environmental Science and Technology 34(13):2741-2748*

Ternes, TA; Kreckel, P; Mueller, J (1999) Behaviour and occurrence of estrogens in municipal sewage treatment plants Part II aerobic batch experiments with activated sludge. *Science of the Total Environment 225:91-99*

Teuber, M (1999) Spread of antibiotic resistance with food-borne pathogens *Cellular and Molecular Life Sciences 56:755-763*

Thayer, KA; Melnick, R; Burns, K; Davis, D; Huff, J (2005) Fundamental flaws of hormesis for public health decisions. *Environmental Health Perspectives 113 (10):1271-1276*

Thiele-Bruhn, S; Beck I (2005) Effects of sulphonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere 59:457-465*

Thomulka, KW; McGee, DJ; Lange, JH (1993) Detection of biohazardous materials in water by measuring bioluminescent reduction with marine organisms Vibrio harveyi. *Journal of Environmental Science and Health 28:2153-2166*

Thorpe, KL; Cummings, RI; Hutchinson, TH; Scholze, M; Brightly, G; Sumpter, JP; Tyler, CR (2003) Relative potencies and combination effects of steroidal estrogens in fish. *Environmental Science and Technology 37:1142-1149*

Tickner, JA; Geiser, K (2004) The precautionary principle stimulus for solutions- and alternatives-based environmental policy. *Environmental Impact Assessment Review 24:801-824*

Timbrell, JA (1991) Principles of Biochemical Toxicolgy, 2nd Ed. Taylor & Francis, London

Tixier, C; Singer, HP; Iellers, S; Muller, SR (2003) Occurrence and fate of carbamazepine, ibuprofen, ketoprofen and naproxen in surface waters. Environmental Science and Technology 37(6):1061-1068

Toft, G and Baatrup, E (2001) Sexual characteristics are altered by 1-tert-octylphenol and 17β-estradiol in the adult male guppy. *Ecotoxicology and Environmental Safety 48:76-84*

Tolls, J (2001) Sorption of veterinary pharmaceuticals in soils: a review. *Environmental Science and Technology 35:3397-3406*

Travis, KZ; Pate, I; Welsh, ZK (2005) The role of the benchmark dose in a regulatory context. *Regulatory Toxicology and Pharmacology 43:280-291*

Triebeskorn, R; Casper, H; Heyd, A; Eikemper, R (2004) Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part II: Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss). *Aquatic Toxicology 68:151-166*

Tudor, TL; Noonan, CL; Jenkin, LE (2005) Healthcare waste management: a case study from the National Health Service in Cornwall, United Kingdom. *Waste Management 25: 606-615*

Turner, L; Choplin, P; Dugard,P; Hermens,R; Roberts, D (1987) Sturcture-activity relationships in toxicology and Ecotoxicology: An Assessment. *Toxicology In Vitro 1:143-171*

Ueda, Y; Saito, A; Fukuoka, Y; Yamashiro, Y; Ikeda Y; Taki,H; Yasuda, T; Saikawa, I (1983) Interactions of beta-lactam antibiotics and antineoplastic agents. *Antimicrobial agents and Chemotherapy 23(3):374-378*

United States Environmental Protection Agency (US EPA) (1991) Green Chemistry. http://www.epa.gov/greenchemistry, accessed November, 2004

United States Environmental Protection Agency (US EPA) (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. EPA 630/R-00-002, Washington, DC; US Environmental Protection Agency, Risk Assessment Forum

United States Environmental Protection Agency (USEPA) (2002) Draft detailed review paper on a fish two-generation toxicity test. EPA contract number 68-W-01-023, Washington, DC, USA

van Aggelen, G; Osachoff, H; Skirrow, R; Tremblay, A; Brunom, J (2005) "Fish & Chips"- Environmental toxicogenomic tools for emerging environmental stressors. Society of Environmental Toxicology and Chemistry 26th Annual General Meeting Platform, Baltimore, 2005

van der Oost, R; Beyer, J; Vermuelen, NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology 13:57-149*

Van der Oost, R; Opperhuizen, A; satumalay, K; Heida, H; Vermeulen, NPE (1996) Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*): I. Bioaccumulation: biota-sediment ratios of PCBs, OCPs, PCdds and PCDFs. *Aquatic Toxicology 35:21-46*

van der Ven, K; van Dongen, W; Maes, BUW; Esmans, L; Blust, R; De Coen, WM (2004) Determination of diazepam in aquatic samples by capillary liquid chromatography-electospray tandem mass spectrometry. *Chemosphere 57:967-973*

van Kerkhoff, L (2005) Integrated research: concepts of connection in environmental science and policy. *Environmental Science and Policy 8:452-463*

van Loon, WMGM; Verwoerd, ME; Eijnker, FG; van Leeuwen, CJ (1997) Estimating total body residues and baseline toxicity of complex organic mixtures in effluents and surface waters. *Environmental Toxicology and Chemistry 16:1358-1365*

Velagaleti, R (1997) Behavior of pharmaceutical drugs (human and animal health) in the environment. *Drug information journal 31:715-722*

Ven, K; Dongen, WV; Maes, BUW; Esmans, EL; Blust, R; Coen, W (2004) Determination of diazepam in aquatic samples by capillary liquid chromatography – elecrospray tandem mass spectrometry. *Chemosphere 57:967-973*

Vermeulen, NPE (1996) Role of metabolism in chemical toxicity. In Cytochromes P450: Metabolic and Toxicological Aspects, Ioannides, C (Ed.) CRC Press, Boca Raton, USA

Vethaak, AD; Lahr, J; Scharp, SM; Belfroid, A; Rijs, G; Gerritsen, A; de Boer, J; Bulder, AS; Grinwis, GC; Kuiper, RV; Legler, J; Murk, TA; Peijnenburg, W; Verhaar, HJ; de Voogt, P (2005) An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. *Chemosphere 59: 511-524*

Vidaeff, AC; Sever, LE (2005) In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence. *Reproductive toxicology 20:5-20*

Vieneis, P (2005) Scientific basis for the Precautionary Principle. *Toxicology and Applied Pharmacology 207:S658-S662*

Vogna, D; Marotta R; Andreozzi, R (2004) Kinetic and chemical assessment of the UV/H_2O_2 of antiepileptic drug carbamazepine *Chemosphere 54:497-505*

Voulvoulis, N (2004) Environmental risk management for pharmaceutical compounds as novel contaminants. *CIESM Workshop Monographs No:26*

Waldmann, P; Pivecevic, B; Werner, EG; Muller, RK; Kurelec, B (1995) Increased genotoxicity of acetylaminofluorene by moduclators of multixenobiotic resistance mechanism: studies with the fres water clam corbicula fluminea. *Mutation Research/Genetic Toxicology, 342(3-4), 113-123*

Wallace, R (1985) Vitellogenesis and oocyte growth in non-mammalian vertebrates. In Developmental Biology. Browder, LW (Ed.) Plenum, New York

Walmann, P. et al. (1995) Increased genotoxicity of acetylaminofluorene by modulators of multixenobiotic resistance mechanism: studies with the fresh water clam *Cobicula fluminea. Mutation Research 342:113-123*

Wandall, B (2004) Values in science and risk assessment. *Toxicology Letters 152:265-272*

Waring, RH; Harris, RM (2005) Endocrine disrupters: A human risk? *Molecular and Cellular Endocrinology 244:2-9*

Water Factory 21, California, (2004) http://www.ocwd.com/_html/wf21.htm and http://www.cmhc-schl.gc.ca/en/imquaf/himu/wacon/wacon_019.cfm accessed April 2004

Webb, S; Ternes, T; Gibert, M; Olejniczak, K (2003) Indirect human exposure to pharmaceuticals via drinking water. *Toxicology Letters 142:157-167*

Webb, SF (2004) A data-based perspective on the environmental risk assessment fo human pharmaceuticals I – collation of available ecotoxicity data. In Pharmaceuticals in the environment: sources, fate, effects and risks Kummerer, K (Ed.), Springer, Berlin

Weber, S; Khan, S; Hollender, J (2006) Human risk assessment of organic contaminants in reclaimed wastewater used for irrigation. *Desalination 187:53-64*

Weeks, AR; Raynolds, KT; Hoffmann, AA (2002) *Wolbachia* dynamics and host effects: What has (and has not) been demonstrated? *Trends in Ecology and Evolution 17:257-262*

Weigel, S; Kuhlmann, J; Huhnerfuss, H (2002) Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. *The Science of the Total Environment 295:131-141*

Weinberg, AM (1972) Science and trans-science. *Minerva 10:208-210*

Wennmalm, A (2003) A proposed environmental classification system for medicinal products. *Envirpharma Conference, Lyon, France*

Weston, JJ; Foran, CM; Slattery, M; Brooks, BW; Huggett, DB (2003) Reproductive assessment of Japanese medaka (Oryzias latipes) following a four-week fluoxetine exposure. Unpublished results, quoted in Brooks et al., 2003.

White, PA and Rasmussen, JB (1998) The genotoxic hazards of domestic wastes in surface waters. *Mutation Research 410:223-236*

WHO (2001) Report on Integrated Risk Assessment WHO/IPCS/IRA/01/12. World Health Organization, Geneva.

Wilcock, A; Pun, M; Khanona, J and Aung, M (2004) Consumer attitudes, knowledge and behavior: a review of food safety issues *Trends in Food Science and Technology 15:56-66*

Wilken, R; Ternes, T; Heberer, T (2000) Pharmaceuticals in sewage, surface and drinking water in Germany In: Security of Public Water Supplies, Kluwer Publishers, Deiniger, R Ed. Pp:227-240

Willingham, E; Crews, D (1999) Sex reversal effects of environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination. *General and Comparative Endocrinology 113:429-435*

Wilson, CJ; Brain, RA; Sanderson, H; Johnson, DJ; Ketut, TB; Sibley, RK; Solomon, KR (2004) Structural and functional responses of plankton to a mixture of four tetracyclines in aquatic micocosms. *Environmental Science and Technology 38 (23):6430-6439*

Witorsch, RJ (2002) Endocrine disruptors: can biological effects and environmental risks be predicted? Regulatory Toxicology and Pharmacology 36:118-130

Wolff, MS; Toniolo, PG; Lee, EW; Rivera M; Dubin, N (1993) Blood levels of organochlorine residues and risk of breast cancer. *Journal of the National Cancer Institute 85:648-652*

Wollenberger, L; Halling-Sorensen, B; Kusk, KO (2000) Acute and chronic toxicity of veterinary antibiotics to Daphnia Magna. *Chemosphere 40:723-730*

Wozniak, AL; Bulayeva, NN; Watson, CS (2005) Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-α-mediated Ca^{2+} fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environmental Health Perspectives 113(4):431-439*

Yoon, Y; Westerhoff, P; Snyder, SA; Wert, EC (2006) Nanofiltration and ultrafiltration of endocrine disrupting compounds, pharmaceuticals and personal care products. *Journal of Membrane Science 270:88-100*

Zakrzewski, SF (2002) Environmental Toxicology, 3rd Ed. Oxford University Press, Oxford.

Zhang, X; Chen, P; Wu, F; Deng, N; Liu, J; Fang, T (2005) Degradation of 17α-ethinylestradiol in aqueous solution by ozonation. Journal of Hazardous Materials – In press

Ziman, JM (2000) Real Science: What It Is and What It Means Cambridge University Press, Cambridge, MA

Zukowska, B; Breivik, K; Wania, F (2005) Evaluating the environmental fate of pharmaceuticals using a level III model based on poly-parameter linear free energy relationships. *Science of the Total Environment – in press*

Zwiener, C; Frimmel, F (2003) Short-term tests with a pilot sewage plant and biofilm reactors for the biological degradation of the pharmaceutical compounds clofibric acid, ibuprofen and diclofenac. *The Science of the Total Environment309:201-211*