

## Evolution of Chemical Contaminant and Toxicology Studies, Part 1 - An Overview

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### ABSTRACT

*The study of environmental chemical contaminants and their toxicological effects has changed dramatically over the last 50 years. Initially studies concentrated on trying to identify what contaminants were actually present and to develop quantitative methods to determine the concentrations (total) present. Health impacts were often investigated independently in medical research centres. With improving analytical techniques, studies of the speciation of contaminants began and the specific forms that were creating the major problems were gradually identified. Continuing improvements in analytical chemistry, together with a move towards more integrated and multidisciplinary research now sees chemists, biologists, toxicologists and health researchers working closely in teams to identify the specific agents of major concern and their pathways, transformations and mode of action. These changes in approach are reviewed. Issues that still require significant research such as cumulative impacts are also discussed.*

**Keywords:** Toxicology, chemical contaminants, colourimetry, electrochemical determinations, separation science, hyphenated methods.

## 1 INTRODUCTION

A review of published (and some unpublished) information from the Pacific Islands (e.g., SPREP, 2002a,b) shows that the vast bulk of contaminant research has been of the quantification type. There is very little completed work on the toxicology or on integrated health investigations. This would seem to be an important area where regional chemists could develop new research directions having both important regional benefits and strong academic outputs. This paper describes the evolution of the environmental toxicology as a discipline, an evolution that was strongly supported by advances in analytical technology.

## 2 THE EVOLUTION OF ENVIRONMENTAL TOXICOLOGY

Environmental toxicology is a young field that has developed rapidly over the past 40 years. Environmental toxicology involves the studying of sources, pathways, transformations, and effects of chemicals that are harmful in the environment. The study of these harmful effects extends from individuals and populations of organisms to the ecosystem level. The study of the toxic effects of environmental contaminants really began in the 1960s, evolving from the traditional field of human or classical toxicology. The first writings of toxicology relate back to prehistoric times when various chemicals, or mixtures of chemicals, were used as both tonics and poisons, with particular references to arsenic and mercury based compounds (Przygoda *et al.* 2001). Historically, most of the positive applications of arsenic and mercury were overshadowed by their 'usefulness' as a poison to resolve

various personal or political problems (Wright & Welbourne, 2002).

The first students of toxicology were physicians and alchemists. The Swiss physician Paracelsus (1499-1541) was particularly well-known for the formulation of the dose-response relationship. Paracelsus observed that some chemicals administered to patients at low concentrations had a therapeutic effect, whereas high concentrations produced toxic effects (Wright & Welbourne, 2002). One of the next major advances in toxicology came from the Spanish physician Mattieu Orfila (1787-1853). Orfila published a comprehensive paper on the toxicity of natural substances, which identified a relationship between the toxic symptoms of the patients (pathology) and the chemical content of the tissues. Orfila further studied the action of antidotes and proposed that the human body has mechanisms for eliminating poisons (Wright & Welbourne, 2002). This led to the work of the French physiologist Claude Bernard (1813-1878), who introduced a more strategic approach to toxicology by performing controlled experiments on animals. The findings and theories produced by Orfila and Bernard over a one hundred year span highlight the foundation of toxicology as a science.

With the mid nineteenth century came the chemical revolution, with a rapid expansion in the extraction of natural chemicals and production of new synthetic compounds (Wright & Welbourne, 2002). Toxicology developed concurrently, still focusing on the two base themes: (i) testing of new pharmaceutical products, and (ii) the identification of potentially harmful effects from the new natural and synthetic compounds. This era marked the beginning of occupational toxicology, with direct links

between the workplace and employee health being proposed and tested.

It was during the chemical revolution that an awareness of animal toxicology evolved in Europe and North America, as it was confirmed that a variety of chemical contaminants were causing wildlife deaths. Environmental contamination was recognized as a serious issue by the British government, and a royal commission was established to assess the potential contaminants in industrial wastewater (Wright & Welbourne, 2002). However, it was not until 1962 with the release of Rachel Carson's book '*Silent Spring*', that a true public awareness of environmental toxicity was established. '*Silent Spring*' was written for the lay-person and clearly describes the impacts of harmful chemicals on wildlife, namely the disappearance of birds from waterways (Carson, 1962).

In the late 1960s René Truhaut used the term 'ecotoxicology' to describe the study of environmental toxicity. Truhaut (1975) defined ecotoxicology as a branch of toxicology that focuses on the toxic effects caused by both natural and artificial substances on living organisms. Truhaut went on to formally distinguish between classical toxicology and ecotoxicology, describing the study of ecotoxicity as a multistep process, which includes: (i) the entry, distribution and fate of pollutants within the environment; (ii) the entry and fate of pollutants in living organisms of the ecosystem; and (iii) the harmful effects of the chemical pollutants on the constituents of ecosystems (which include man). Each of these steps is complicated by potential biological or abiotic transformations of the parent pollutant.

It was during the mid 1970s that scientists began to realize that the environmental monitoring of toxic substances from anthropogenic sources was producing a growing inventory of data on the quantities of substances in the terrestrial and aquatic environments (Kovach, 1975). Kovach stated that before this information could be used to evaluate the biological effects, knowledge was required of the chemical form of the pollutants, the mechanisms by which the pollutants enter and move through organisms, the abiotic and bio-transformations of the parent compound, the nature of the toxic reactions within biological tissues, and the changes in physiological and physical behaviour of the contaminated individual. This was the time when many scientists acknowledged that quantification, in itself, was not sufficient to define and describe toxicological problems (Wright & Welbourne, 2002).

Interest in developing physico-chemical models of contaminant behaviour in the environment also expanded in the 1970s. Various physical and chemical parameters (such as pH, temperature, dissolved oxygen) were known to govern contaminant speciation in the environment, and attempts to predict contaminant species, and hence the potential toxicity of the environment, were made through modeling. Thirty years on and these models are still being refined, and are the focus of many environmental toxicity studies.

The evolution of environmental toxicology as a discipline is clearly demonstrated through this brief review. A definite change in scientific approach in toxicology to incorporate the environment, both biological and abiotic, resulted in a new multi-disciplinary field.

During this period, especially when the need for contaminant speciation became apparent, rapid developments in technology and analytical chemistry produced new opportunities in instrumentation. The analytical instrumentation developed during the technology revolution had an enormous impact on the field of ecotoxicology, and will be reviewed briefly below.

### 3 DEVELOPMENTS IN ANALYTICAL CHEMISTRY

Prior to the mid-20<sup>th</sup> century, analysis of toxicologically important chemical species was essentially by classical 'wet' methods. This is exemplified by determination of the identity of a gaseous arsenic compound in the 1930s (Cox, 1975). The development of instrumentation involving either spectroscopic or electrochemical interactions with analytes has yielded methods of much greater sensitivity, greater flexibility, instrumental identification of analyte species as well as quantification and higher throughput of determinations. The other important analytical development was in the area of separation methods. These methods are necessary for sample cleanup and/or separation of the analyte from complex matrices. More importantly, separation methods allow the determination of different chemical forms of toxicants. The combination of the separation and detection systems gives toxicology investigators powerful tools for determining both the presence (and quantity) of different forms of the toxicant in different materials and the mechanism of toxicant action.

#### 3.1 COLOURIMETRY

During the 1930s through to the 1960s many methods were developed for the determination of metals based on the formation of highly coloured or fluorescent complexes (Perrin, 1964). The formation of the highly fluorescent selenium diaminonaphthalene complex used in selenium determination and the colourimetric determination of lead as a dithizone complex are two examples of these methods (Allen *et al.* 1974). The presence and the concentrations of metal species were determined by visual colour comparison (using Nessler tubes) or spectrophotometry/fluorimetry. Atomic spectroscopy eventually overtook the use of these methods.

However, the application of such reactions to produce colour responses has found use in a variety of simple chemical tests, sometimes called spot tests, widely applied for screening purposes. In the last 10 years, spot test research has upsurged because of low cost portable colorimetric systems. Spot tests are now available for a range of elements with detection limits often as low as  $10^{-5}$ - $10^{-6}$  mol/L (e.g., Rossi *et al.* 2000; Gotzl & Riepe, 2001). Diagnostic spot tests are used as screening methods from which decisions were made as to whether further investigations were needed. There are many current examples of these diagnostic tests: forensic, clinical, food quality, air, soil or water analysis (Jungreis, 2000).

Spectroscopic determinations of trace metals proceeded from flame photometry to flame atomic spectroscopy during the 1960s (Alkemade *et al.* 1979; Walsh, 1955) using both emission and absorption techniques. Although

atomisation of analyte metals in the flame (most commonly air-acetylene) was convenient and reproducible, it proved to be relatively inefficient in terms of the atom population generated, and therefore was not as sensitive as could be achieved by other means of atomisation. Flame atomisation was also found to be prone to various chemical and spectral interferences.

Electrothermal atomisation of metal analytes using a variety of 'mini furnaces' in the light path became commercially available in the early 1970s. In this method the analyte in a few microlitres of sample is atomised in accordance to a set temperature program, in the light path yielding a transient signal. Electrothermal atomisation yields sensitivity enhanced over flame methods by two to three orders of magnitude depending on the element. The most common furnaces used are cylindrical graphite tubes (L'vov, 1991; Koirtyohann & Kaiser, 1982).

Further atomisation developments for specific elements occurred in the 1970s, with the introduction of hydride generation (HG) for As, Se, Sb, Sn, Pb, and Bi, and cold vapour (CV) atomisation for Hg.

The use of the inductively coupled plasma (ICP) as an atomisation tool developed in the late 1970s (Fassel, 1978; Floyd *et al.* 1980) with atomic emission detection (ICP-AES). The advantages of ICP over flame and electrothermal atomisation are fewer inter-element interferences due to the higher temperatures, and good emission spectra for many analytes under one set of operating conditions, yielding the possibility of simultaneous (or rapid sequential) determination of many elements. In addition, elements forming compounds resistant to thermal decomposition at temperatures found in flame and electrothermal atomisation are atomized in the plasma. ICP-AES permits analyte determinations over a substantially wider range of concentration than is possible with flame and electrothermal atomic spectroscopy.

The coupling of ICP to mass spectrometric (MS) determination as an important tool for trace element analysis began in the early 1980s. The ICP serves as both atomiser and ion source for the spectrometer. ICP-MS instrumentation offers low detection limits and high selectivity. Quantification can be performed either by use of calibration curves for relative response of analyte to an internal standard or by isotope dilution methods. Because this method allows the determination of different isotopes of an element, it can be used as a tool for distinguishing sources on the basis of relative isotope abundance.

### 3.2 ELECTROCHEMICAL DETERMINATIONS

Stripping voltammetry (SV) has developed since the 1960s to become one of the most sensitive methods for trace metals determination. The sensitivity arises from the initial analyte deposition stage allowing preconcentration of the analyte metals. A major step forward in the 1960s involving development of differential voltammetry allowed an improvement of 2 – 3 orders of magnitude in sensitivity. Stripping voltammetric methods of analysis in operationally defined metal speciation have had direct application in modeling bioavailability of toxic metals (Whitfield & Turner, 1979).

### 3.3 SEPARATION SCIENCE

Early liquid chromatography was performed essentially as a wet benchtop chemical method using glass columns to support the stationary phase and passing the eluent or mobile phase through under gravity feed. To ensure a none-too-tedious flow rate, the particle size had to be large (in the order of 0.20 mm). Theoretical considerations established early on that more efficient chromatography would be achieved with the use of small particle size for the stationary phase. The technology necessary to produce these small particles was not available until the late 1960s. In addition, the smaller particle size restrained the flow of eluent severely, necessitating the use of high pressure pumping systems for the mobile phase. The requirements for pumps in these systems are high; these pumps must generate high pressure (up to 40 MPa), give pulse-free output, generate flow rates between 0.1 and 10 mL/min, resist corrosive chemicals. These systems became known as high performance liquid chromatography (HPLC).

Because of the versatility available in terms of stationary phase and separation modes, HPLC is the most widely used of all separation techniques. The importance of this technique is reflected in its application across many different fields of research (Skoog *et al.* 1998).

### 3.4 HYPHENATED METHODS

Many types of chromatography can be coupled with a detection system directly, allowing powerful separation methods to be immediately followed by detection. In early systems the chromatographic eluent was collected in a fraction collector after passing a nonselective and non-destructive detector. The fractions were then subjected to further spectroscopic or electrochemical methods for identification or quantification. Today many instrumentation arrangements allow the selective detector to continuously monitor the eluent. Usually these instruments are computer controlled and need computing facilities to store spectral data for later display and / or analysis.

With respect to trace metals, these hyphenated systems allow separation of different forms of the analyte trace metal prior to detection. Where separation is coupled to mass spectrometry, identification of compounds is enhanced by mass spectral information. Thus high separation power is attached to high selectivity and exceptional sensitivity, for example the use of HPLC coupled to electrospray ionisation MS-MS to determine organoarsenicals (Pergantis *et al.* 1997).

During the past four decades there have been enormous advances in the capabilities of analytical instruments that have driven rapid developments in toxicological research, particularly with respect to trace metals. These advances have seen major improvements in traditional methods, providing greater sensitivity and accuracy with decreased signal-to-noise influences, and producing more reliable and robust methods. Extensive improvements are particularly evident for instruments involving either spectroscopic or electrochemical interactions with analytes, yielding methods of much greater sensitivity, greater flexibility, instrumental identification of analyte species as well as quantification and higher throughput of determinations.

The evolution of environmental toxicology has occurred in parallel with technological advancement. This exciting interdisciplinary field is still in its infancy, and establishing itself within the realm of traditional and applied sciences. In general, the current students of environmental science are young enough to accept that environmental toxicology is a recognized discipline of scientific importance. These students have not had the life experience to witness or understand the rapid development of this discipline (Wright & Welbourne, 2002).

As noted in the introduction, environmental toxicology information is almost non-existent for Pacific Island situations. Apart from one or two papers based on work in the smaller north Pacific islands (e.g., Burger *et al.* 2001), remarkably little relevant information can be gleaned from the published literature. There is an urgent need for scientists and health workers in the region to initiate appropriate studies on important issues in the region, e.g., the impacts of organochlorine compounds in regional ecosystems, mining-induced metals mobilisation, marine food chain bioaccumulation of toxic substances, and contamination caused by inappropriate waste handling. These studies are essential for the maintenance of the health of Pacific Islanders and also for protection of the regional industries based on clean and green images, especially where commodities are exported.

#### 4 CONCLUDING REMARKS

Environmental toxicology as a 'new science' has come a long way in the past four decades for a variety of reasons, not the least of which was the great need to address some real and pressing issues. Important advances in analytical techniques and in biochemistry have aided the evolution of this multidisciplinary research. We can now characterize molecules of toxicant attached to various biological moieties, rather than simply gather an inventory of elemental concentrations in the environment which has little toxicological relevance. In addition, our growing understanding of the geology, chemistry and biology of earth systems has lead us to recognize the baseline 'clean' situation and the 'polluted' or disturbed one. However, this is still just the tip of the ice-berg. There are over 200,000 man-made organic compounds in existence (Wright & Welbourne, 2002), and the toxicity of the majority of these is unknown. There are also many factors which impact on the toxicity of contaminants that are not fully understood. These factors include the synergistic and antagonistic effects of other compounds, the effect of changes in the physical environment (such as pH and temperature), and environmental and biological transformations of these compounds during their passage throughout various ecosystems.

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