

ACTRA 2008 Scientific Session

EARLY ADAPTIVE RESPONSE IN MICE LIVER CAUSED BY ARSENIC EXPOSURE

Arthur D, Ng JC, Moore MR, Abu-Bakar A

University of Queensland, National Research Centre for Environmental Toxicology, Queensland, CRC Contamination Assessment & Remediation, South Australia

Arsenic is known to cause cancer in a variety of essential organs in humans, as well as chronic diseases at least some of which can be induced by oxidative stress. The protective mechanisms mounted by our bodies against arsenic toxicity are not fully understood. However, arsenic is known to induce two different enzyme systems – the haem oxygenase and cytochrome P450. These enzymes are believed to protect against oxidative damage by regulating the antioxidant bilirubin. This project investigates the basic biology of arsenic-induced cytochrome P450-mediated bilirubin oxidation pathway. Mice treated with a single dose of arsenite at time points 1, 3, 6, 18, 24 and 48 hours, results in an induction of both haem oxygenase-1 (HO-1) and Cyp2a5 (cytochrome P450) enzymes in the liver. Protein and mRNA levels of these enzymes were identified with western blots and real time QRT-PCR, respectively with the highest levels at 3 (HO-1) and 6 hours (Cyp2a5). This was in agreement to Cyp2a5 activity which also peaked at 6 hours. Total arsenic and MDA (product of lipid peroxidative damage) were also determined in the liver using ICP-MS and GC-MS, respectively. Total arsenic in the liver increased 1 hour after treatment, followed by a gradual decrease to 48 hours. Interestingly, MDA levels in the liver successively decreased at each of the time points and were lower than the control. Therefore, the induction of Cyp2a5 in mice by arsenic may have a protective effect against lipid damage. This work suggests that Cyp2a5 and HO-1 induction may be part of an early adaptive response in mice to arsenic exposure.

PHYSIOLOGICALLY BASED EXTRACTION TEST (PBET) FOR SITE SPECIFIC RISK ASSESSMENT OF ARSENIC AND LEAD AT A MINE SITE

Diacomanolis V¹, Noller BN², Sadler R³, Ng JC¹

¹National Research Centre for Environmental Toxicology-EnTox, University of Queensland, ²Queensland Health Forensic and Scientific Service, ³Centre for Mined Land Rehabilitation, University of Queensland

A site-specific human health risk assessment focusing on arsenic and lead soil concentrations was conducted at a mine site in Australia after decommissioning and rehabilitation. *In-vitro* PBET determination of bio-accessibility of individual soils and *In-vivo* bioavailability measurement of composite wastes were employed in this risk assessment study. Arsenic bioavailability is depending on its oxidation states. The absolute bioavailability (ABA) of arsenic in this study ranged 1.6 – 8.9%, arsenate (As^v), the oxidized form of arsenic which is more likely to be found in mine waste materials, was found to be <5% whereas arsenite (Asⁱⁱⁱ) could be as high as 8.9%. Conservatively, ABA of 10% for arsenic is used for the exposure assessment. The bioavailability of lead was in the range of 0.6 – 1.4%. Similarly, ABA of 2% was used for the risk assessment purpose. In our experience, PBET data (BAc) were generally one order of magnitude higher than those of ABA, except for arsenic. For a more pragmatic approach, *in-vitro* bioaccessibility (BAc) data obtained using the PBET method was used for this site specific risk assessment and new health investigation levels for arsenic and lead were proposed.

HUMAN HEALTH RISK ASSESSMENT OF CHLORINATION DISINFECTION BYPRODUCTS IN DRINKING WATER USING A PROBABILISTIC APPROACH

Connell D

Griffith University, Griffith School of the Environment, Queensland, d.connell@griffith.edu.au

The presence of chlorinated byproducts in drinking water is a public health issue. A risk assessment was carried out on chloroform (THM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), bromoform (TBM), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) using published exposure data from 25 different countries. Adverse health effects from these byproducts were reported in the scientific literature using epidemiological data on human populations as well as animal investigations. The exposure data was processed using probabilistic techniques giving distributions of the data over the range of exposure situations. However the adverse effects data did not allow this type of evaluation and so was expressed as ranges within which adverse effects were observed to occur. The risk was characterised by a comparison of the exposure and adverse effects data and indicated that small increases in the incidences of some cancers in males developmental effects on infants are possible.

ACTRA 2008 Scientific Session

THE TOXICITY ASSESSMENT POTENTIAL OF EUGLENA GRACILIS

McKay AR^{1,2}, Moore MR^{1,2}, Whitehouse M³, Wang JP^{1,2}, Ng JC^{1,2}

¹The University of Queensland, National Research Centre for Environmental Toxicology, ² CRC-CARE (Contamination Assessment and Remediation of the Environment), ³ School of Biomolecular & Physical Sciences, Griffith Univ, Nathan, Queensland

Euglena gracilis Z and its mutant strain SMZ have plant- and animal-like characteristics respectively. Arsenic (As), cadmium (Cd) and cyanide (CN) are common mining waste products. Aurocyanide (AuCN) is produced in extracting gold from ores with sodium cyanide (NaCN). The very stable AuCN is biologically active and has been proposed as an anti-inflammatory and an anti-arthritic drug. The potential hazard of environmental AuCN to aquatic organisms remains unclear. This study aimed to compare the toxicities of the above mining related contaminants in *Euglena spp* as a model for environmental hazards to aquatic organisms. Strains Z and SMZ were incubated under a range of concentrations of sodium arsenite (0 - 4mM), cadmium chloride (0 - 400µM), potassium aurocyanide (0 - 313µM) and sodium cyanide (0 - 625µM) to determine the IC_{50s} measured using spectroscopy at 610nm (growth) and 490nm (enzymatic function). Cadmium was found to be more toxic than arsenite to Z and SMZ and had a similar toxicity as AuCN. Sodium cyanide was more toxic than As, Cd, and AuCN to SMZ but non-toxic to Z. Therefore the toxicity of AuCN is due to the compound itself and not the cyanide moiety as expected because AuCN has a very high dissociation constant. This study also confirms that cyanide ion generally has a low toxicity to plants suggesting the photosynthetic mechanism of Z may play a role in its resistance to cyanide toxicity.

RISK ASSESSMENT OF PBDES BASED ON AUSTRALIAN HUMAN BIOMONITORING RESULTS

Nugent KW and Satya S

Existing Chemicals, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Marrickville, New South Wales

In Australia, manufacture and importation of industrial chemicals is regulated by the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). NICNAS conducts scientific assessments of the risks to human health and the environment and makes recommendations on how these risks can be managed to enable safe use. Industrial chemicals already in use are reviewed by NICNAS on a priority basis when health and/or environmental concerns are raised. NICNAS is assessing two commercial polybrominated diphenyl ether (PBDE) mixtures, pentabromodiphenyl ether (pentaBDE) and decabromodiphenyl ether (decaBDE). The PBDE mixtures consist of ranges of individual congeners with different degrees of bromination. These congeners differ in toxicity profile, and particularly in bioaccumulation potential. NICNAS undertook an interim risk assessment of the tetra- to hexabrominated congeners, found in the commercial products pentaBDE and octabromodiphenyl ether (octaBDE). While PBDE congeners are not acutely toxic, the lower brominated congeners have effects on the liver and particularly the thyroid hormone system. The Department of Environment, Water Resources, Heritage and the Arts (DEWHA) commissioned a consultancy which measured the serum levels of the most common PBDE congeners in pooled serum in Australia, and NICNAS undertook a risk assessment based on these biomonitoring results. The toxicity profile of the range of PBDE congeners, the methodology of the risk assessment and the resulting risk conclusion and uncertainties are presented.

ACTRA 2008 Scientific Session

RECENT AUSTRALIAN STATISTICS ON DURATION OF RESIDENCE

Frangos J¹ and Arunachalam D²
¹Toxikos Pty Ltd, ²Monash University

The duration of residence is an important exposure factor for environmental risk assessments intended to assess a resident's exposure to a chemical substance. Historically the primary information on the population movements in Australia has been drawn from the census held every five years. The census data reveals that Australian's move residence, on average 11-13 times during their lifetime. At face value this statistic contrasts the enHealth default of 70 years for duration of residence. However there is a high degree of variation associated with the average and there is insufficient data available within the census dataset to properly characterise the duration of residence. The key issue is that the census provides data on population mobility (ie proportion of the population that changed residence at least once within the survey period) and not the duration of residence. Insufficient data availability to assess the statistical distribution is one of the likely underlying reason for the current enHealth default. Recently a new dynamic dataset offers the opportunity to provide an up-to-date data set to fill the data gaps on duration of residence. The HILDA (household, income and labour dynamics in Australia survey) large-scale, representative household-based panel (i.e., longitudinal) surveys designed to collect a large amount of information about households and the members of those households. It is intended to be both representative of the Australian population (or at least a significant proportion of it) and provide information on the dynamic nature of events and how they interact in influencing the changing behaviour and fortunes of Australian households, families and individuals. This dynamic data set has not been previously available and is considered a deficiency of existing datasets. A preliminary analysis of the HILDA dataset found that the 50th, 90, 95, and 99th percentile duration of residence for Australians are 5, 25, 35, and 52 years respectively. These preliminary results are consistent with US surveys of similar design and intent and indicate the default duration of residence around 25 to 35 years rather than 70 years.

UNCERTAINTY IN HUMAN HEALTH RISK ASSESSMENTS

Wright J¹ and Issa J²
¹Environmental Risk Sciences, New South Wales, ²Cintox Australia Pty Ltd, New South Wales

The assessment of risks to human health is associated with the consideration of uncertainty particularly within the stages of exposure assessment as well as hazard/dose response assessment. In many cases the issue of uncertainty is brushed over in these assessments, or is addressed by considering overly conservative assumptions to provide greater confidence that the outcome will be protective of human health. However all aspects of uncertainty should be considered in greater detail to ensure that the potential for health effects are neither over nor underestimated. The potential for uncertainty within the exposure assessment aspect of a risk assessment can vary significantly. Lower levels of uncertainty may be associated with the assessment of consumer product and workplace exposures to a chemical used within the business as there is often data on exposure levels or information available on the activities undertaken such that exposure can be reasonably estimated. For environmental contaminants, however, where concentrations in different media may vary and behaviour/activity patterns vary, the assessment of exposure can be associated with a higher degree of uncertainty. These assessments typically then adopt overly conservative assumptions to address these uncertainties. The assessment of chemical hazards/dose-response aspect of these assessments is also associated with uncertainty. These uncertainties may be associated with the database of information available for use in a dose-response assessment and those applied in relating studies in animals to humans including sensitive groups. Uncertainties and limitations however can also be associated with the use of dose-response assessments that have not been updated with recent information or studies, and those where the potential for mixtures affects the use of the information. Hence it is important that uncertainties inherent in the dose-response assessments themselves are adequately considered as well as issues associated with whether the information is current and considers issues such as mixture effects. The presentation will look at these uncertainties with case examples where risk has been overestimated and potentially underestimated.

ACTRA 2008 Scientific Session

DIRECTIONS FOR REGULATORY SCIENCE IN THE 21ST CENTURY

Frangos J
Toxikos Pty Ltd

Over the past 80 years a large volume of toxicology data has been generated both in the public and non public domain. In 1920 only one scientific journal was dedicated to the discipline of toxicology while a recent count estimated over 120 scientific journal titles. The volume of unpublished toxicology reports is also voluminous when one considers 31,000 new substance notifications in Europe alone in the period 1979 to 1996. There is a growing emphasis in understanding this data in order to facilitate the community expectation for safer chemicals while at the same time refining the use of animal testing. This review focuses on the growing use within chemical registrations schemes around the world of "intelligent" testing strategies. Such testing strategies use a hazard assessment approach including the use of in vitro and in silico toxicology techniques. Many of the major chemical registration authorities around the world have been using and integrating in vitro and in silico methods within their new chemical and existing chemical review programs. An overview of some of the current and upcoming techniques used by US EPA, US FDA, Health Canada and the European Union will be described including the role of quantitative structure activity relationships to predict mammalian toxicity endpoints within these schemes.

HAEMOLYTIC AGENTS ASSOCIATED WITH SAGO HAEMOLYTIC DISEASE IN PNG

Pue AG¹, Fletcher MT², Greenhill AR³, Warner JM³, Blaney BJ², and Ng J¹

¹*The University of Queensland, ENTOX*, ²*Queensland Department of Primary Industries and Fisheries*, ³*James Cook University, Townsville*

Sago Haemolytic Disease (SHD) was first recognised in Papua New Guinea in 1974 as the acute haemolysis of red blood cells (RBC), occurring in people within 20 h after consumption of stale sago starch. Its symptoms include a rapid onset of fever, vomiting, jaundice and production of brown reddish urine and in severe cases is often fatal. Previous studies have shown the disease is associated with fungal contamination of stored old sago but attempts to elucidate the etiological agent of SHD have been unsuccessful.

The present investigation sought to determine the chemical identity of haemolytic compound(s), their mode of action on RBC and their role in causing SHD. Suspect haemolytic fungi have been identified using a RBC haemolysis bioassay. Methanol extracts of these fungal cultures contained haemolytic compounds which have been separated and purified by solvent partitioning and chromatography.

A series of long chain free fatty acids ranging from C₁₆ to C₂₀ have been identified in the haemolytic fractions. The haemolysis of human RBC by high levels of free fatty acids has previously been reported and is attributed to changes in cell permeability associated with detergent-like activity of fatty acids. Free fatty acids in blood are carried by serum albumen, and the albumen protects against these haemolytic effects. It has been hypothesised that SHD may occur under conditions where the free fatty acid/ albumin ratio at least temporarily exceeds the threshold as to cause red cell breakdown. Further studies are being conducted to test this hypothesis.