Research Travel Grant Report

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AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Marine and Freshwater Toxins Analysis 3rd Joint Symposium and AOAC Task Force Meeting, University of Puget Sound, Tacoma, Washington, USA

LC-MS/MS Workshop on Lipophilic Marine Toxins, Washington State Public Health Laboratory, Seattle, USA

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Executive Summary

Harmful algal toxins are a recognised hazard to seafood safety. Several toxin families are subject to regulatory oversight, so the topic of reliable and reproducible detection and quantification of this structurally and functionally diverse group of compounds is of paramount importance for export and domestic seafood industries and seafood consumers. This is a rapidly developing field, both in terms of a growing understanding of the public health and trade implications attributable to known and novel algal biotoxins, as well as advances in analytical capability through instrumentation and skills. AOAC International, the Association of Analytical Communities, is the focal agency for analytical method development, method validation and related knowledge dissemination.

The objectives of this visit to the US were to:

1. Participate in the conference, both formally and informally.
2. Present a paper: *The laboratory mouse in harmful algal bloom research and food safety evaluation: past, present and future*.
3. Learn about new and emerging developments in the field and communicate same to Australian seafood industry and professional contacts.
4. Participate in training for advanced analytical techniques for lipophilic shellfish toxin measurement.

Outcomes Achieved to Date

- Novel contribution to the debate on appropriate analytical methods for saxitoxin and diarrhoetic shellfish toxin (DST) group analysis.
- Training in state-of-the-art mass spectrometric methods for DST analysis.
- Information exchange between global HAB toxin experts and local interests and expertise supported by ASCRC.
- Opportunities identified for continuing involvement in the discussion and debate on alternative methods for biotoxin analysis, with the aim of publishing a review and discussion paper on the mouse bioassay.
- Enhanced Australian capability to conduct priority research and development that minimises foodborne toxins risk and trade & access barriers.

Outputs Developed as a Result of Travel

- Presentation at the conference of a paper: *The laboratory mouse in HAB research and food safety evaluation: past, present and future*. This presentation will be written up as a review and discussion paper planned for inclusion in a special edition of the *Journal of AOAC International* that will feature work presented at this conference and the forthcoming AOAC Annual Meeting in Las Vegas, Nevada (30 September to 3 October 2012).

- Presentation at the “Blue green algae end of season workshop” organised by the Victorian Department of Sustainability and Environment, 10 July 2012. Ian Stewart was an invited speaker at this event, with travel support funded by Vic DSE and Vic Health. Ian Stewart presented a talk entitled: *Risk communication for cyanobacteria bloom development*. The talk was informed, in part, by discussions with Em. Prof. Wayne Carmichael at the Tacoma/Seattle meetings; Ian Stewart also distributed the new flyer *Algae Blooms in Oregon* developed by the Oregon Health Authority with advice and assistance from Prof Carmichael.
- Preparation of expert technical advice to Codex Australia delegates through SafeFish. The 32nd Session of the Codex Committee on Fish and Fishery Products will meet in Bali, Indonesia in October 2012. Drs McLeod and Stewart are contributing advice regarding the Proposed Draft Performance Criteria for Screening Methods for Marine Biotoxins in the Standard for Raw Bivalve Molluscs. Discussion and presentations of advances in analytical capabilities, national approaches to product testing for paralytic and diarrhoetic shellfish and regulatory responses were a significant focus of the Tacoma and Seattle meetings. Knowledge of these advances by Dr Stewart has enhanced the quality of the technical advice presented by SafeFish.

Travel Objectives

Some sectors of the Australian seafood industry are periodically but significantly impacted by algal biotoxins, either through intoxication-related stock losses or as a consequence of regulatory or guideline compliance failures due to excessive uptake of toxin/s into product tissues. The purpose of this conference travel was to:

1. Update knowledge on algal and cyanobacterial biotoxins
2. Communicate with experts in the field from around the world
3. Contribute to the proceedings by presenting a paper.

Background and Need

Considerable stock losses have occurred in southern Australian finfish aquaculture fisheries in the past through exposure to ichthyotoxic algal biotoxins, and some Australian shellfish fisheries are currently experiencing economic disruption because of unresolved challenges surrounding the toxicology and analytical quantification of “minor” saxitoxin analogues.

Marine microalgae can produce a range of potent toxins that are harmful to human health, with numerous fatalities and mass poisonings documented for over 200 years. National agencies with responsibility for food safety and public health in many countries apply regulatory oversight of fish and shellfish, both of local and imported origin, for the presence of specific algal toxins in marketed seafood.

State-of-the-art analytical competencies for harmful algal biotoxins are needed in order to fully support the Australian seafood industry’s ability to comply with export

requirements. AOAC International's Pacific Northwest meeting in Tacoma represented an opportunity for ASCRC to participate at an important gathering of global experts in the field of algal toxin analysis and research. This is a rapidly-developing discipline, so the opportunity to represent ASCRC at this conference benefits both the core business and reputation of the organisation through its support for Dr Stewart's attendance.

Results

A travel itinerary is provided in Appendix 1 and a summary of conference presentations as Appendix 2. Ian Stewart delivered a presentation entitled *The laboratory mouse in HAB toxin research and food safety evaluation: past, present and future*. Drs Stewart and McLeod are currently discussing an invitation from AOAC to contribute a paper to the *Journal of AOAC International* which is planned as a special edition arising from the AOAC Pacific Northwest Conference and the forthcoming AOAC Annual Meeting to be held in Las Vegas, Nevada: http://www.aoac.org/meetings1/126th_annual_mtg/main_2.htm

Dr Stewart took the opportunity to engage in discussions with several experts on a range of topics.

- Dr Ron Manger, director of the Biologics Production facility of the Fred Hutchinson Cancer Research Center in Seattle was keen to hear of Ian's previous work on the mouse neuroblastoma assay (MNA). Dr Manger and colleagues first developed and published the MNA in the 1990s and continues to use it in his lab's work. The ability to establish and maintain the MNA in Australia would be a valuable enhancement of our local capability for detecting ciguatoxins in finfish, which would align with our separate proposal for assessing and validating the current Sydney fish market ciguatera management guidelines.
- Elizabeth Tor, Senior Analytical Chemist, California Animal Health & Food Safety Laboratory, University of California Davis. This lab was investigating a poisoning event by a hepatotoxic cyanotoxin, microcystin-LR. One of the diagnostic challenges for the microcystin group of toxins is that of protein binding and bioavailability of the covalently-bound fraction. Free and bound microcystin analysis will be discussed by Dr Stewart in his presentation at the 9th International Conference on Molluscan Shellfish Safety, to be held in Sydney in March 2013.
- Dr Sherwood Hall, US Food and Drug Administration. Dr Hall was a lead participant in the debate on analytical methods for saxitoxin-group toxin analysis and a proponent of the receptor-binding assay. Dr Hall has a great deal of experience with the mouse bioassay and agrees that the MBA should be phased out for non-research applications. Dr Hall offered constructive and detailed feedback on Ian Stewart's presentation, which will be invaluable for subsequent development of the case to be presented in a peer-reviewed publication.

Dr Stewart participated in two training sessions. The first was a Rapid Test Workshop, run by Em. Prof Wayne Carmichael (Wright State University) and David

Deardorff from Abraxis LLC. The workshop featured hands-on experience with immunoassays (ELISA and qualitative stick-type field tests) for various cyanotoxins, domoic acid, histamine and okadaic acid-group toxins.

Dr Stewart also received training and instructional materials for liquid chromatography and mass spectrometric techniques, conducted jointly by Drs Pearse McCarron from Canada's National Research Council, Paul McNabb from New Zealand's Cawthron Institute, and Jerry Zweigenbaum from Agilent Technologies. This workshop was held at the Washington State Public Health Laboratories in Seattle and featured detailed presentations on the theory and practice of LC-MS/MS techniques and their application to the field of diarrhoetic shellfish toxin group analysis. The workshop also featured a theoretical food poisoning outbreak investigation for group participation, presentation and discussion.

Outbreaks of diarrhoetic shellfish toxin poisoning have occurred in Australian commercial and recreational fisheries; such events can be anticipated to occur sporadically again in the future. A fully supported marine biotoxins diagnostics capacity in Australia is essential to safeguard our export industry and biosecurity requirements; support for training and skills updates are an important aspect of our overall capability and an enhanced ability to provide expert technical support to government and industry in emergency response situations.

Summary of Conference Presentations (See Appendix 2)

Analytical chemists, biologists, public health workers and government agency representatives from North America, Europe, Japan, Australia and New Zealand presented on a range of topics pertaining to marine and freshwater algal toxins and their detection and quantification in seafood. Much of the focus of the meeting was devoted to saxitoxin and diarrhoetic shellfish toxins analysis, and the challenges for chemical and antibody-based techniques to address the functions of bioassay methods. Japanese experts expressed the opinion that the mouse bioassay will continue to be favoured there for routine shellfish safety testing in the foreseeable future. Presenters from the USA and New Zealand extolled the benefits of the saxitoxin receptor binding assay as a functional bioassay. Presentations featured emerging biotoxins such as palytoxin, with a regulatory limit proposed by the European Union. A new guideline for Pacific ciguatoxins in fish flesh has been published by the US Food and Drug Administration, though there is currently no confirmatory analytical capability that can determine compliance with this limit.

Outcomes

- Enhanced Australian capability to conduct priority research and development that minimises foodborne toxins risk and trade & access barriers
- Enhanced contribution towards complete acceptance of Australian biotoxin testing capabilities by regulatory agencies in countries to which Australia exports seafood

- Enhanced international awareness of ASCRC as a supporter of research into risk management issues for the seafood industry
- Expanded professional network
- Determining future directions for seafood safety testing in the EU, USA, Japan and Canada
- Enhanced international profile of ASCRC and the broader Australian seafood sector by presenting CRC-supported research at a prestigious international conference
- Identification of potential collaborators for ciguatera toxin and cyanotoxin research projects
- Training in the theory and application of state-of-the-art analytical techniques for measuring diarrhoetic toxins in seafood matrices

Recommendations

International conferences such as the AOAC Pacific Northwest Meeting are a constructive and worthwhile opportunity for research staff to engage with international experts, maintain and update their knowledge and skills, develop their professional networks and promote the activities of supporting agencies such as ASCRC.

Industry Recommendations:

- Drs Stewart and McLeod to develop and submit to Sydney Fish Market cost estimates for their provisional proposal for testing Australian finfish for ciguatera toxins, as an assessment and validation of the SFM ciguatera risk management program.
- Seek opportunities, e.g. SafeFish, Australian Seafood Magazine, ICMSS, to inform industry on the emerging topic of cyanotoxins in fish and shellfish, in light of the new Victorian Government guidelines.
- Disseminate through SafeFish information on new US regulatory limits for ciguatoxin concentrations in finfish.

Regulator Recommendations:

- Incorporate knowledge gains into technical advice (through SafeFish) provided to Codex Australia representatives on reference methods and screening tests for paralytic and diarrhoetic shellfish toxins.
- Advise FSANZ regarding new US FDA guidelines for ciguatoxins in finfish.

Appendix 1: Itinerary

Saturday 16 June 2012	Depart Adelaide
Saturday, 16 June 2012	Arrive Seattle
Sunday, 17 June 2012	Transfer to campus accommodation, University of Puget Sound, Tacoma
Monday, 18 June 2012 to Friday, 22 June 2012	AOAC Marine and Freshwater Toxins meeting
Friday, 22 June 2012	Transfer to Edmonds Harbor Inn
Saturday, 23 June 2012	Tour of Boeing factory, Everett WA, with other conference delegates
Monday, 25 June 2012 and Tuesday, 26 June 2012	LC-MS/MS training workshop at Washington State Public Health Laboratories
Wednesday, 27 June 2012	Commence return travel to Australia
Friday, 29 June 2012	Arrive Sydney, Adelaide

Appendix 2: Notes from the conference; Conference highlights

Opening address, Jim Hungerford. AOAC Pacific Northwest is continuing its laboratory training program:

- Seattle 2006: Lawrence method for PSTs
- Seattle 2010: automated Lawrence method & PCOx
- Seattle 2012: LC-MS/MS for lipophilic toxins

Takeshi Yasumoto: Plenary: Determination of toxins involved in ciguatera and related seafood toxins

- Mouse bioassay is used for CTX determination in Japan; 0.025MU/g
- Program to produce toxins for analytical standards: purchased >300kg of various fish species from local fishers, with considerable impost on their research budget. This highlights the challenges in securing sufficient source material (potentially ciguatoxic fish in this case) for extraction and purification of toxins
- *O. punctata* produces CTX-4 & 4B – non-oxidised congeners
- LC-FD used for low-level quantification because terminal OH group can be labelled
- Poisoning due to humphead parrotfish *Scarus ovifrons*: 17 outbreaks, 6 fatalities 1962-2012. Symptoms: rhabdomyolysis, myoglobinuria, dyspnoea, dysmobility, hepatic & renal dysfunction. Similar poisonings reported from boxfish and others. Cause not identified; palytoxin & ovatoxin analogs not detected by LC-MS/MS. Taurochenodeoxycholic acid (TCDC) was the cause of haemolysis and mouse lethality; TCDC is non-toxic orally, therefore unlikely to cause rhabdomyolysis
- Chemiluminescence assay of CTX-3C: overestimates due to non-specific binding were observed. Endogenous biotin in synaptosomes interferes with the assay

Takeshi Tsumuraya: Production of monoclonal antibodies for sandwich ELISA detection of Pacific ciguatoxins

- Hokama *et al* 1977: produced monoclonal antibodies from natural toxins, but these cross-reacted with okadaic acid and other structurally-related compounds
- Proposal is to use synthetic haptens
- Small ring fragments can be used as synthetic haptens for generating anti-ciguatoxin antibodies

Jennifer McCall: Development of a fluorescence-based receptor binding assay for site 5 of VSSCs in rat brain synaptosomes

- Problems with radioligand assays: cumbersome and expensive; slow analysis time; generation of radioactive waste; requirements for radiation-compliant facilities
- These problems led to the development of fluorescent probes
- Attached fluorophores to PbTx-2; varied in brightness and binding affinity

Mark Poli: A sandwich ELISA for quantification of palytoxin and analogs

- Palytoxin originally isolated from soft corals, *Palyotha* spp., also produced by the dinoflagellate *Ostreopsis*
- EFSA suggested regulatory limits: 30ppb in edible parts
- Excellent calibration curve using 11 concentrations
- ELISA is highly specific, with minimal cross-reactivity to other marine HAB toxins

Spencer Fire: Determination of PSP toxins in shellfish by receptor binding assay – inter-laboratory collaboration

- There is a two orders of magnitude difference in toxicity across known congeners
- Inter-lab validation study: RSD = 33%
- RSD better (to around 20%) across labs using the method routinely
- Good correlations between the receptor binding assay (RBA) and mouse bioassay
- Both RBA and Lawrence method tend to over-report activity compared to MBA

Sherwood Hall: Scientific and practical support of receptor binding assays for marine toxins

- Why use the RBA?: It is sensitive and accurate – responds to concentrations in the potency range of mouse intraperitoneal injection.
- Advantages of increased sensitivity: earlier detection, better management
- IAEA support: because of its commitment to peaceful uses of atomic energy the IAEA Technical Assistance Program has been supported in ten “less developed” countries
- Challenges to implementation: supply of radiolabelled STX; ownership and sale of STX; licensing via NIH
- RBA uses a commercially-supplied rat brain preparation. The product is stable when frozen.

- Sherwood raised the possibility of using abattoir products as a source of receptors to further reduce the requirement for laboratory rodents. Discussion ensued about the genetic heterogeneity that might be introduced as a result, further discussion of use of inbred *vs.* outbred rats and contribution to assay variability

Shigeru Sato: Skit, a quantitative ELISA for PSTs

- The mouse bioassay is used in Japan for routine shellfish monitoring
- Initial STX antigen: hapten designed by Johnson *et al* (1964).
- Current ELISA still shows variable affinities across toxin components
- This particular ELISA cannot be guaranteed for regulatory purposes in its current form

Emanuel Hignutt: Implementation of alternatives to the mouse bioassay for PSTs in Alaska

- MBA detection limit in Alaska State Environmental Health Laboratory: 340ppb
- Dungeness crabs: mice not dying, but very high STX levels by PCOx; unknown congener, co-elutes with GTX-4
- Ran x7 Dungeness crab samples – needs more investigation for matrix interferences and LC-MS/MS identification

Paul McNabb: Discovery of tetrodotoxin in the common grey side-gilled sea-slug *Pleurobranchaea maculata*

- 5th August 2009, Auckland area: warning signs were posted for dog owners to keep their pets off the beach
- Beach almost empty, panic atmosphere, extensive press coverage
- Baiting programs for feral animals (rats, stoats, ferrets) were put on hold because people thought that dog deaths were caused by baiting
- TTX found in pipis x7, oyster x1
- 431 environmental samples comprising a large range of invertebrates were examined, *n*=10 were TTX-positive
- TTX found in all organs and tissues of *P. maculata*

Wade Rourke: Animal-free shellfish toxin testing – the Canadian perspective

- Canadian Shellfish Sanitation Program (CSSP): jointly operated by the Canadian Food Inspection Agency, Department of Fisheries & Oceans and Environment Canada. Health Canada is the regulator
- 10,000 samples per year tested for PSTs

- PCOx method implemented in November 2009: analysed 7,300 samples over 31 months. 70% had no detectable toxins
- Mouse bioassay LOQ: 400ppb
- C-toxins: average C-toxin contribution was 1.8% of total toxicity
- Long-term evaluation of data required to determine the need to monitor for C-toxin toxicity
- Scallop digestive glands from Argentina were known to have high toxicity by MBA. PCOx revealed an unknown STX congener
- Pinnatoxins: most shellfish on the East coast contain PnTx-G & associated esters. Also seen on West coast but at lower levels

Clara Hard: DSP in the Salish Sea, Washington State

- Washington State's Sentinel Mussel Monitoring Program commenced in 1999
- PSP and DSP testing uses the mouse bioassay at present
- Recently established a HPLC-MS/MS method for DSP testing
- 1793: captain Vancouver records PSP fatality in British Columbia
- 2008: Shellfish fisheries closed due to OA in Texas Gulf oysters
- 2011: DSP outbreak in BC, Canada
- Public communication: BC CDC website updates; multiple media requests
- Most prevalent toxins in 2011: DTX-1 and DTX-1 esters
- Mussels seem to take up DSTs quicker than oysters and clams, but this requires confirmatory research
- This was a large illness outbreak but the response was efficient and effective

Pearse McCarron: LC-MS with Q-Trap reveals complex toxin profiles in Canadian mussel samples

- Rapid polarity switching on 5500 Q-Trap
- Liquid-solid extraction: exhaustive extraction; sample weighed in tube; extraction with multiple portions of solvent
- Matrix Solid Phase Dispersion (MSPD): patented process (1989) – see Barker 1988 *J Chromatogr*
- Uses "Fast MSPD method" for combined extraction of lipophilics and domoic acid
- Extract with MeOH for lipophilics; 50:50 aqueous MeOH for DA

Jonathan Deeds: Regulatory implications of DSTs in US shellfish

- Gorge Bay, BC 2011: 30,000lb of product recalled

- Some 60 illnesses were attributable to consumption of commercial product
- Jellett strip: no mention of cross-reactivity with *Dinophysis* toxins in the product literature
- Abraxis OA ELISA: no mention of hydrolysis for detection of fatty acid esters
- OKAtest (Spain)
- Tropical Technology Centre DSP Rapid Kit (Japan)
- FDA guidelines require testing for total DSP toxins (free + esterified)

Toshiyuki Suzuki: Analysis of DSTs and other lipophilics in Japanese bivalves – performance and correlation of LC-MS *vs.* mouse bioassay

Number of harvesting closure days for bivalves in Japan, 2010

	Incidents	Closure days
DSTs	32	1451
PSTs	21	1480
Total	53	2951

- High rate of false positives (≈40%) by mouse bioassay are a problem for the shellfish industry
- Low rate of false negatives (≈4%) have been protective of human health
- LC-FD method for OA using column switching to simplify and automate cleanup

James Hungerford: Marine and Freshwater Toxins Task Force Meeting

- The US FDA has determined action levels of 0.01ppb P-CTX-1 equivalents for Pacific ciguatoxins
- No groups anywhere in the world have as yet reported LoQ capabilities to achieve analysis by LC-MS methods at 0.01ppb P-CTX
- The FDA action level is presumably set in anticipation of rapidly developing advances in instrument sensitivity by UPLC-MS/MS for ciguatoxin analysis

David Boyle, Program for Appropriate Technology (PATH): Rapid molecular testing outside the laboratory

- Cheap, easy-to-use real-time PCR for field applications
- Facilitating the development and application of novel diagnostics for developing countries
- Isothermal amplification technique
- Biomarkers for micronutrient deficiency

- Maternal & infant infection
- www.path.org "PATH is an international, non-profit organization that transforms global health through innovation."

Blaine Rhodes: Washington State response to the Fukushima radiation releases, March-April 2011

- 20th March: ^{131}I detected by in-situ rooftop sampler at Washington Public Health Laboratories
- 30th March: USEPA reports ^{131}I in Washington milk – levels at or below routine detection limits
- Sr & Cs monitored in cattle. Uptake from grass, deposited by rain

Wayne Carmichael: CyanoHABs and cyanotoxins – detection methods for minimising toxicity to the environment, animals and humans

- Microcystins: MWt 909-1037, ∴ work very well for raising antibodies
- *C. raciborskii* and cylindrospermopsin are seen in the USA
- Anatoxin-a: MWt 105 ∴ more difficult to raise an antibody
- Anatoxin-a(S) – unstable in the environment
- ELISA & PPIA kits: Zeu-Immunotec (Spain) – PPIA kit; Abraxis – MCs, CYN, STX & Antx-a (soon)
- PPIA: activity-based test, ∴more closely related to toxicity than ELISA

Sébastien Sauvé & Dipankar Ghosh: Accelerating the analysis of cyanotoxins in water

- Ultrafast laser diode thermal desorption
- **Check forthcoming paper: Lemieux oxidation & 96-well plate method ⟶ direct presentation of sample for laser desorption ⟶ LC-MS. No sample prep or cleanup required

Jens Dahlmann: LC-MS/MS with QTrap and TOF

- Predictive Multiple Reaction Monitoring software for detection of congeners for which standards are not available

Randy Self: Rapid qualitative screening of phthalates in food products by DART/MS

- DART: Direct Analysis in Real Time
- High temperature, meta-stable He is used to desorb and analyse sample directly into the stream

- Used to qualitatively separate positive samples from negatives, thus considerably increasing throughput
- Identified positives can then be reanalysed quantitatively
- Self RL 2012 – paper on phthalates

Ed Paski: Workshop on sampling solid materials

- VIM International
- www.bipm.org Bureau International des Poids et Mesures
- Bias: estimation of a systematic measurement error
- Repeatability *cf* reproducibility – definitions are different: repeatability is same operator, same lab, and same timeframe. Reproducibility is ability of different operator or lab or time to replicate results. Check BIPM-VIM for definitions

LC-MS/MS workshop on lipophilic toxins, Washington State Public Health Laboratories, 25-26 June 2012

Jerry Zweigenbaum, Agilent: Atmospheric pressure ionisation and mass filters

- Components: ionisation → mass analyser → detector
- Ionisation: generation of gas-phase ions (atmospheric pressure or high vacuum)
- Transfer of compounds or ions from atmospheric pressure to high vacuum
- Mass analyser: separation of ions by their mass to charge ratio under high vacuum
- Detector: convert ion flux in electrical current
- Ionisation and sample introduction: compounds need to be introduced in high vacuum which is needed to perform separation by m/z
- APCI – Atmospheric Pressure Chemical Ionisation – now largely obsolete, superseded by electrospray ionisation
- **NB: APCI won't form Na^+ adducts, *cf* electrospray
- Sensitivity: maximise signal wrt noise
- Mass analysers: principle is mass to charge ratio
- Resolving power is $M/\Delta M$; resolution is $\Delta M/M$
- Resolution is the ability of a mass spectrometer to distinguish between ions of different m/z ratios. Greater resolution corresponds directly to the increased ability to differentiate ions
- Mass analysers: quadrupole mass analyser – four parallel rods
- Time of Flight mass analyser: a uniform electromagnetic force is applied to all ions at the same time, causing them to accelerate down a flight tube

- Fourier-transform MS: ions are trapped electrostatically within a cubic cell in a constant magnetic field. Application of a RF pulse between the excite plates induces a covalent orbital (“cyclotron”) motion
- Matrix effects: caused by competition for the droplet surface; acid/base chemistry in the liquid phase; adduct formation; competition for buffer ions

Paul McNabb, Cawthron Institute: Extraction efficiency

- Single extraction is dispersive, the sample and solvent are assumed to have equal affinity for the analyte
- A multiple extraction is exhaustive, the sample is assumed to have released all its analyte

Features of LC-MS:

- Peaks can co-elute
- Peak area – and height – is variable between determinations from the same solution
- Peak area is variable between days
- Peak area is affected by co-eluting compounds
- Clean-up: problem if you do reverse-phase cleanup and then go to reverse-phase chromatography, can get the same interfering compounds coming through

NB** PMcN plots residuals as well as his calibration curve – if residuals are evenly distributed above and below zero line, then OK

Pearse McCarron, NRC Canada: Matrix effects

- A major problem in LC-MS analysis of complex samples is the suppression or enhancement of electrospray ionisation resulting in over- or under-estimation of analyte concentrations
- NRC Canada produces a wide range of Certified Reference Materials and matrix-specific reference materials