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OFFICIAL NEWSLETTER OF THE SOCIETY OF TOXICOLOGY OF CANADA

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TABLE OF CONTENTS

FROM THE EDITOR'S DESK (Bill Racz)	2
FROM THE PRESIDENT'S DESK (Genevieve Bondy)	2
REPORT FROM THE TREASURER (Gerald Cooke)	3
SOME CONSIDERATIONS ON TOXICOLOGY ADVOCACY (Roger Keefe)	4
AWARDS AND HONOURS	5
STC Award of Distinction	5
STC Student Travel Awards	6
STC Cantox Student Research Awards	6
NOTES FROM THE 40 th ANNUAL SYMPOSIUM (Paul Rowsell).....	7
REPORT ON THE ELEVENTH INTERNATIONAL CONGRESS OF TOXICOLOGY (Mallé Jurima-Romet and Douglas Arnold)	12
BOOK REVIEWS	14
UPCOMING EVENTS.....	14

VOLUME XXVIII, NUMBER 1

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From the Editor's Desk

Bill Racz

Demise of The Canadian Federation of Biological Societies: It was with some sadness that I learned of the closing of the doors of the CFBS at the end of 2008. Perhaps the organization had outlived its usefulness and had become an unaffordable lobby, but I remember far better days for the organization. CFBS was initially an organization comprised of the societies that for the large part represented the basic medical science disciplines, with a few other societies. The original mandate of the CFBS was to provide a forum for Canadian science and training venue for graduate students. I remember my first scientific presentation at CFBS, June 1968 at Queen's. Who knew that a few years later I would return as a member of faculty. My presentation, "*The Effect of Dihydropyridines on Heme Biosynthesis*", was placed in a session entitled "Blood". There I was presenting a biochemical toxicology paper to a group interested in clotting factors and who had little knowledge of my field, although there was interest, at least as judged by the two polite questions. My point is that there was a generation of us that cut some of our scientific teeth at CFBS. It was the showcase for Canadian biomedical science in the 1960's through the 1980's.

Another benefit of these CFBS meetings was that we met other Canadian scientists, some who worked in similar fields, but also scientists from other disciplines. A number of these individuals have remained friends to this day. Another benefit was that as young scientists we met some of the more colourful characters in Canadian science and being invited to join a group of the 'old guard' for dinner. I learned some of my scientific philosophy at these sessions. I will never forget a comment, in response to a rather provocative statement (not mine), during a vigorous discussion after two or three bottles of wine. "Show me the data". I have used this adage several times with graduate students.

With the fiscal constraints in the 1980's and onward, CFBS assumed an enhanced role to lobby the Federal Government for greater support of the

three federally-funded granting councils, as well as the support of science and innovation within government and the private sector. CFBS can take at least some of the credit for the increases in funding of NSERC and MRC (now CIHR) that occurred in the 1990's.

The emergence of 'specialized' scientific meetings reduced the relevance of the CFBS to essentially that of a lobby group, an activity that in 2008 could no longer be sustained. A process needs to be found to replace the lobby efforts of the CFBS; a process that speaks for all members of the STC, industry, government and academia. I did not see much new money for the granting councils in the recent budget, and as there are hard economic times ahead, science and innovation may be given a low priority.

(Please see Roger Keefe's report on lobby possibilities.)

From the President's Desk

Genevieve Bondy

Happy New Year! I'm pleased to report that the past year was a successful one for STC, culminating in a timely symposium that addressed issues that are becoming increasingly relevant for everyone working in the field of toxicology. Thanks to Lynne LeSauter and her program committee, who put together a stimulating program. Also in the past year STC continued with essential upgrades to its administrative and financial operations (thank you Elise!). Thanks also to all of our Executive and Committee members, who volunteer their time to support STC's activities. On a sadder note, 2008 was the last year of operations for the Canadian Federation of Biological Societies (CFBS), officially ending a longstanding alliance of Canadian life sciences societies. CFBS provided STC with a voice for science advocacy activities. To address this loss, the STC Executive is currently looking at alternatives to CFBS for advocacy on behalf of our members. Our Vice-President has elaborated on this issue in his column for this newsletter, and we welcome

comments from our members on how we can best provide science advocacy for toxicologists in Canada.

Speaking of input, you may have already received an e-mail requesting input on the 2008 symposium. Please send us your comments – we would love to be able to take them into account when we're planning the 2009 symposium and beyond. The 2009 symposium will address issues related to mixtures toxicology – concepts, mechanisms, research and regulatory issues. Our website will be updated in the near future – stay tuned for more details, and mark your calendar for December 7 and 8, 2009.

Report from the Treasurer

Gerald Cooke

A few words about STC finances.

As Treasurer of the STC, I presented the financial reports for the years 2006/07 and 2007/08 at the AGM in Montreal last December. The good news is that we are in 'good shape' at present. This is due in large part to STC's share of the profits from the ICT-XI meeting in Montreal (\$36,000) in 2007. Without this result of the efforts put in by those brave and stout hearted STC members who, all those years ago, took the initiative to propose, arrange and host the meeting, we would be in 'less good shape'! The December 2008 symposium was one of the best attended in recent memory (~150) and financially speaking will make a profit (once all the expenses are paid) which I estimate to be about \$8,000. Basically, apart from our GIC investments (which we would not want to touch unless absolutely necessary), we would be in a marginally profitable position annually.

The cessation of CFBS' existence that occurred at the end of 2008 means that their portion of the membership dues collected by STC will not be appearing on the membership renewal invoices for 2009. There was a discussion at the AGM of raising STC membership fees to support our financial situation for the long term. The decision was taken to raise the regular membership dues to \$100, associate membership dues to \$80, and

student and retired members' dues would increase to \$30. In most cases, your invoices for 2009 should show that you will be paying less than last year but STC will benefit greatly from this. (We have only a few members who were not paying their CFBS memberships through STC.)

The STC board is doing its best to keep expenditures down and we have to thank Imperial Oil Ltd. and Shell Canada Ltd. who generously covered the transport costs of their employees, Roger Keefe and Carol Drury, from Alberta to Montreal for our yearly 'face to face' board meeting last April. Conference calls and e-mails are also saving us some costs. We have also taken some measures that reduce the amounts we pay for Mastercard and Visa transactions fees when STC memberships and registrations are paid with these cards. Last year, that amount saved was about \$1,000! Unfortunately, for AMEX transactions we cannot reduce the fees we pay. We will continue to seek ways that will reduce costs where we can!

On a personal note, I unreservedly thank our Executive Secretary, Elise Boivin-Ford, for her excellent help in keeping the finances in order. Also, Marc Bulot of Polyhec Inc. who has helped us considerably with the implementation of our new accounting system and the transfer of information into it. Marc was also the source of some of the suggestions that the STC has implemented to reduce its expenditures. Finally, and on behalf of the STC, I thank Dr. Cindy Goodyer who stepped in at very short notice to audit our books for both 2006/07 and 2007/08.

Some Considerations on Toxicology Advocacy

Roger Keefe, Vice-President, STC

The Board of STC has been investigating how to replace the advocacy support formerly provided by the Canadian Federation of Biological Sciences (CFBS). CFBS ceased operations at the end of 2008. Four options are presented below to stimulate feedback from all interested STC members about your experiences – are there other organizations/options, what are good selection criteria, what are our advocacy needs, and why is this important?

Making a good choice if STC decides to join a consortium, is more important in the current, down economy than in better times. While an overview of the January 27 federal budget proposal does not identify cuts to research, one has to look at the details to find what's missing. For example, Genome Canada was not funded, possibly due to an oversight (Globe & Mail, January 29, p. A1). "Investments in knowledge infrastructure" are in the proposed budget, including funds for federal and other laboratories, Canada Foundation for Innovation, etc. STC needs effective advocacy to protect our research interests in competition with valid and other requests for 'economic stimulus' packages.

1. A new "Life Sciences Advocacy Group" is being proposed. CFBS facilitated a meeting to test interest among societies that were former members and prospective members, in a new organization for life sciences advocacy. The STC Board (G. Bondy) has been monitoring developments without making any commitments. If there is enough interest among other societies to build a viable new organization, STC might be interested as long as the fee structure is manageable, e.g. a flat fee based on number of members and within a practical range for STC.

2. "Research Canada" (RC) <www.rc-rc.ca> invited STC to join them, and the STC Board is seeking more information. They evolved from the Council for Health Research in Canada, and their value proposition appears to represent health sciences, weighted towards more clinical research. RC membership includes a large number of

charities, research institutes, public foundations, hospitals, regional health authorities and academic institutions, as well as professional organizations. RC sent a letter and brief to MPs on December 23, 2008 to influence the proposed federal budget. RC's impressive website includes a strategic plan, past and future advocacy positions, and media communications.

3. "Partnership Group for Science and Engineering" (PAGSE) <www.pagse.org> was formed by the Royal Society of Canada to represent science and engineering. They work closely with Industry Canada and NSERC and represent 24 professional associations. Their website lists their past advocacy positions, and it describes their "Bacon & Eggheads" breakfasts for MPs to raise the public profile of science. PAGSE interests do include some biology, focused more on the environment, but their public and political effectiveness may benefit all science and research.

4. "Canadian Consortium for Research" (CCR) <www.ccr-ccr.ca> is another voice supporting a broad range of research interests including 17 math and physical sciences, astronomy, nursing, psychology, brain & cognition, humanities and social sciences organizations. CCR also provided advice to MPs in a letter on December 17, 2008, regarding the federal budget proposal. Their concerns about that proposal were actually released to the media the same day the federal budget proposal was tabled in Parliament.

Among the choices for a consortium to represent STC, the larger organizations have effective mechanisms to promote science and research (grants, tax incentives, training, infrastructure and overhead), but any needs specific to STC may be neglected. On the other hand, STC needs might be better captured by other, often smaller, organizations that may not have the public or political profile of the larger ones. The STC Board invites other suggestions and advice to help us narrow the choices for members' consideration. Please look at these and other websites. Send our guidance to <roger.t.keefe@esso.ca>.

Awards and Honours

STC Award of Distinction

Barbara Hales

The Awards Committee was proud to honour Dr. Barbara Hales (McGill University) with the STC Award of Distinction in 2008. Dr. Daniel Cyr presented the award.



Dr. Barbara Hales received her B.Sc. (Hons.) in Biology at McGill University and M.Sc. at the Philadelphia College of Pharmacy and Science. She returned to McGill to complete a Ph.D. and a postdoc in the Department of Pharmacology and Therapeutics. Dr. Hales then joined the Department of Pharmacology and Therapeutics in 1979, as Assistant Professor, and subsequently rose through the ranks of Associate and Full Professor by 1992. Throughout her career, Dr. Hales has received numerous awards, including a Queen Elizabeth II Scientist Award (1981). She was an MRC Scholar from 1981 to 1986 and in 2002 was recognized for her teaching excellence at McGill, being named to the Faculty Honour List for Educational Excellence. She has made significant contributions to toxicology and teratology. With over 140 primary publications, including an impressive 38 book chapters, she has clearly been a leader in toxicology and teratology, not only in Canada but also at the international level. As such, she is an active contributor to the development of toxicology as Vice-President of the Teratology Society, Director of the IUTOX Executive Committee. She has also been on several toxicology related peer-review panels in

both Canada and the United States. She has contributed in particular to the Society of Toxicology of Canada as Chair of the Publications Committee (1989-1993); Member of the Awards Committee (1994, 1995); Member of the Committee to bring ICT to Montreal; and served as Vice-President (2001-2003), President (2003-2005); and Past-President (2005-2007). While Dr. Hales showed leadership in all of these functions, none was more evident than during her term as President of STC, during which she was instrumental in improving the financial well being of the Society and played an active role in the planning and preparation of the International Congress of Toxicology and as a liaison between the organizing committee and the IUTOX Executive. Her contributions to these were crucial to the overall success of this meeting.

Dr. Hales has also been an important mentor for Canadian Toxicologists. More than 45 trainees at all levels of education have trained in Dr. Hales' laboratory and most have gone on to productive careers in pharmacology and toxicology. She was instrumental in the development of both graduate and undergraduate level toxicology courses at McGill, which have introduced a large number of students who otherwise would not have been exposed to the field of toxicology. She has also played an important role as a mentor to young, and not so young, faculty members in toxicology throughout Canada. Dr. Hales has been an exceptional leader and spokesperson for Toxicology and for the Society for Toxicology of Canada. As a result of these and her many other contributions to education and medical research in Canada, she is unquestionably deserving of the Society of Toxicology of Canada's Award of Distinction.

STC Student Travel Awards

As always, STC is pleased to support our student members and to honour their achievements. Congratulations to all of our student award winners! The following students received STC Travel Awards for the 2008 symposium: Katherine Shoeman (University of Western Ontario), Anne Mullen Grey (University of Toronto), Kevin Sha (Queen's University), Andrew Slot (Queen's University), Jeanne Black (Queen's University), Steven Molinski (Queen's University), Helen Badham (Queen's University), Emily Tung (Queen's University), Marina Chan (Queen's University).



STC Cantox Student Research Awards

Cantox generously supports the STC Student Research Prizes. The recipients were: Kevin Sha, an M.Sc. candidate from Queen's University, and Helen Badham, a Ph.D. candidate from Queen's University. Their abstracts are reprinted below.

K. Sha & L.M. Winn

Valproic acid induces hyper-acetylated histones and DNA double strand breaks leading to increased homologous recombination repair.
Queen's University, Kingston, Ontario.

Use of the first-line antiepileptic agent, valproic acid (VPA) during pregnancy is associated with an increased incidence of major congenital malformations; however the molecular mechanism mediating VPA-initiated teratogenicity has not been elucidated. Our previous study showed an increase in the frequency of homologous

recombination (HR) repair in Chinese hamster ovary 3-6 (CHO 36) cells after exposure to VPA (Defoort et al., 2006). HR is not an error free process and can result in detrimental genetic changes. Since proper development requires tight regulation of gene expression, changes leading to disruption of this process may underlie a mechanism of VPA induced teratogenicity. In this study we evaluated if and how VPA affects DNA double-strand break (DSB) repair. To investigate whether VPA affects the activity of DNA DSB repair, CHO 33 cells containing the neo direct repeat recombination reporter substrate were transfected with either the *Saccharomyces cerevisiae* mitochondrial endonuclease I - SclI to induce a site specific DSB within the recombination substrate or the empty plasmid, pGem. Cells were then exposed to 5 mM VPA for 24 hrs and two weeks later, the frequency of HR was determined by counting the number of functional neo expressing G41 8-resistant colonies per cells plated. A significant increase in the frequency of HR was observed in the presence (I-SclI) or in the absence (pGem) of an artificially created DSB after exposure to VPA; however there was no increase in the fold difference in HR between VPA and vehicle (media) exposed I-SclI transfected cells compared to cells transfected with pGem suggesting that VPA does not affect DNA repair activity. To determine whether VPA induces DNA DSBs to elicit repair, CHO 33 cells were exposed to 5 mM VPA for 10, 16 or 24 hrs and γ -H2AX foci, a marker of DNA DSBs, was measured by immunofluorescence microscopy. A significant increase in the number of γ -H2AX foci per cell was observed for all time points with the greatest increase at 16 hrs after exposure to VPA. Recently VPAs ability to inhibit histone deacetylase (HDAC) has also been proposed as a possible mechanism of teratogenesis. Therefore, we also evaluated whether HDAC inhibition by VPA contributed to the increase in HR. The same recombination assay was carried out with 10, 50 and 100 nM of trichostatin A, a known HDAC inhibitor. Similar frequencies of HR to VPA were observed for all TSA treatment. Western blot analysis was also carried out to assess acetylated H3 and H4 levels at the time of DNA DSBs and a significant increase in acetylated histones was seen at all time points except for H3 at 24 hrs.

These results suggest inhibition of HDAC by VPA causes hyper-acetylation of histones leading to relaxed chromatin structure where DNA damage may occur in the form of DNA DSBs with subsequent increase in HR repair. Supported by CIHR.

H.J. Badham & L.M. Winn

In utero exposure to benzene causes reactive oxygen species-mediated alterations in hematopoietic progenitor cell growth.

Queen's University, Kingston, Ontario

Leukemia is the most prevalent childhood cancer and its incidence has increased by 20% within the last two decades. It is hypothesized that the primary cause of this increase in leukemia is *in utero* exposure to environmental pollutants such as benzene. Epidemiological studies have correlated *in utero* benzene exposure with an increased incidence of childhood leukemia. In addition, studies have shown that *in utero* exposure to benzene in mice increases the number of erythroid and myeloid progenitor cells in fetal tissue. The molecular mechanisms behind benzene-initiated leukemia after *in utero* exposure are unknown. One proposed mechanism is that the bioactivation of benzene causes an increased production of reactive oxygen species (ROS), which leads to deregulation of the cell cycle. Our first objective was to determine if *in utero* exposure to benzene causes an increase in ROS in fetal liver tissue, which is the primary site of hematopoiesis. Our second objective was to determine if deregulation of blood cell development after *in utero* benzene exposure is abrogated by pretreatment with the antioxidative enzyme catalase. Pregnant C57Bl/6N mice were injected i.p. on gestational days (GD) 8, 10, 12, and 14 with either vehicle (corn oil) or 200 mg/kg benzene. In addition, these mice were also injected i.p. with either PBS or 25 KU/kg PEG-catalase 16 hours prior to each oil or benzene injection. To measure ROS, a single cell suspension of GD 16 fetal liver tissue was incubated with the ROS sensitive dye dichlorofluorescein diacetate and fluorescence intensity was measured using flow cytometry. In addition, GD 16 fetal liver cells were plated in semi-solid media and incubated for 3-12 days after which colony-forming units (CFU) and blast-

forming units (BFU) of the erythroid lineage (CFU-E and BFU-E), and of the myeloid lineage (CFU-granulocyte, CFU-monocyte, and CFU-granulocyte/monocyte) were counted using a light microscope. Our results show that *in utero* exposure to benzene causes a significant increase in ROS in the fetal liver and this effect is abrogated by pre-treatment with catalase. In addition, catalase protects against benzene-induced alterations in CFU-E, BFU-E, CFU-M, CFU-G, and CFU-GM colony numbers. These results suggest that ROS play a key role in the development of *in utero*-initiated benzene toxicity. The growing incidence of childhood leukemia and the prevalence of benzene contamination in our environment emphasizes the need for further research in this area. Supported by CIHR.

Notes from the 40th Annual Symposium

***“Toxicity testing tomorrow:
What does the future hold?”***

Montreal, Quebec, Nov. 30 - Dec. 2, 2008

Paul Rowsell

For those of you who couldn't make it to the symposium, Paul Rowsell of Health Canada has kindly offered up his summary of the meeting. As you will read below, we enjoyed a thought-provoking two days devoted to the future of toxicity testing.

Our sponsors for the Annual Symposium were: **CANTOX Health Sciences International, Charles River Laboratories Canada, Global Tox International Consultants Inc., and Réseau de recherche en santé environnementale/ Environmental Health Research Network.** Thanks again to our sponsors, and all of the hard-working members of STC who come together to make our symposia so enjoyable.

Recently, the U.S. Environmental Protection Agency commissioned a report from the National Research Council (U.S.A.) on *“Toxicity Testing in the 21st Century: A Vision and a Strategy”* (2007). This was the topic of the 40th Annual Symposium of the Society of Toxicology of

Canada. The Program Committee, chaired by Lynne LeSauter of Charles River Laboratories, with Kannan Krishnan (Université de Montréal) and Mike Wade (Health Canada), constructed a stimulating overview of the many aspects dealt with in the report.

The problem, as we are all aware of, is the vast universe of novel chemicals introduced into the environment and encountered by humans. Testing of this large number of compounds using traditional toxicity testing is impractical. It was proposed that high throughput methods could be substituted for animal studies. Perturbations of toxicity pathways would be the measure of a compound's toxicity, not pathology. Eventually computational methods could take over: toxicology not *in vivo* or even *in vitro* but *in silico*. This meeting explored the current state of technologies that will alter the future of safety testing. The symposium proceedings presented attendees with much to consider regarding the challenges that we face to make this future a reality.

Daniel Krewski, University of Ottawa, opened the presentations and dialogue by giving a summary of the findings of the US NRC report, whose team of experts he chaired. He set out four strategies for future toxicity testing:

1. Keep the current paradigm: use many animals with high doses and accept the low through-put of these assays.
2. Leverage the newer methods in the context of traditional toxicity testing and add more *in vitro* tests. This strategy would make gains 'at the margins'.
3. Use limited animal testing but rely on medium to high through-put methods. The animal testing would be 'focused'. *In silico* methods would drive the testing.
4. Look at perturbation of established toxicity pathways. Use *in vitro* work with human cells. No research animals at all would be used.

The expert committee felt that option 1 is no longer viable; option 2 uses the new methods in too timid a fashion; and option 4 is desirable but

the science is not there yet. Option 3 is the recommended path.

This strategy will eventually allow risk assessment to be done without direct toxicity data. Instead chemical characterization of a compound will be used to computationally through structure-activity relationship analysis, classify the risk and kind of toxicity that is likely. Direct experimentation on toxicity pathways (using the example of the Nrf2 pathway) will give data on the likely consequence of exposure to the compound. This strategy will employ comprehensive *in vitro* testing. The pathway analysis used in this strategy will likely be definitive in 1-2 decades. Behind the current regulatory framework is a legal foundation: the new vision will require regulatory agencies to be guided by a new legal framework.

Ken Hastings of Sanofi Aventis spoke on the issues of biotechnology products and regulatory toxicology. He first went through the problems associated with testing many compounds. QSAR methods have been shown to be robust in predicting nephrotoxicity, cardiotoxicity and neurotoxicity. He then summarized an incident when an antiCD28 antibody set off a cytokine storm in six Phase I clinical trial subjects. Studies with non-human primates gave no evidence of harm, and little evidence of effect. Subsequent *in vitro* studies with human lymphocytes demonstrated the value of human *in vitro* tests versus traditional toxicity testing in regard to bioengineered proteins: they gave clear evidence of a cytokine storm. The non-intuitive nature of the test was the context needed for the effect to be seen: the antibody was required to be immobilized to set off the reaction. Traditional *in vitro* assays do not take into account the three dimensional nature of the tissues lymphocytes recognize. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is attempting to revise its standards to reflect the needs of the novel bioengineered products. Regulators must consider the context of the human dosing: for a limited duration for some cancer treatments, or lifelong, as some autoimmune treatments will require. Ideally one would use an animal model which exhibits similar cross-reactivity to human in its immune response

to the product. Epidemiology can act as an early warning system, as increased cancer rates in patients taking antiTNF- α seems to indicate. Use of a surrogate protein which is the homologue of human protein in the model animal is a possibility.

Kevin Crofton (EPA) discussed neurotoxicity testing in the past and possible future approaches. Currently there is a disjunction between regulatory and laboratory toxicologists. By the time a reproduction-development study has been completed, the regulatory decisions it was aimed to inform have been taken. The public health context has also changed with an increase in ADHD and autism diagnoses: are there environmental triggers? One response has been the current discussion of an extended one generation study, with limited neurotoxicology and developmental tests. This will increase the value of the test while reducing costs. Given the conservation of thyroid hormone signaling, other species such as zebrafish could be used for screening. The cellomics assay, which uses NS1 cells in 96 well plates can be used to study neurite outgrowth. A recent study used 320 chemicals at one concentration and could distinguish cytotoxic actions from increased or decreased neurite outgrowth effects. Both *C. Elegans* and zebrafish can be used in developmental assays, using immunohistochemical methods to mark aberrant development and even using an automated system in a 96 well context to distinguish chemical effects on behaviour. These systems have the advantage of rapid high through-put testing in a whole animal system. These systems also have pitfalls: the concentration of the compound added to the medium is not necessarily reflected in the concentration within the cell. Some compounds will adsorb to the plate. Lipophilic compounds may reach a much higher concentration in the cell than the medium. A promising system used by EVCAM is the so called “brain balls” which are spherical aggregates of neurons and glia in culture, allowing toxicity studies of glia-neuron interaction. Finally a short discussion of the funding environment was held, with the suggestion that funding agencies need to be more courageous in driving new methods in toxicology research.

David Dix (EPA) spoke about the EPA’s ToxCast program. The Toxic Substances Control Act (TSCA) has over 90,000 compounds in their inventory, although most are not in commercial use. Similar to the Canadian Domestic Substances List, ToxCast winnowed the list to about 11,000 chemicals and about 1,000 pharmaceuticals that were of concern. By considering their chemical nature, in Phase I, 320 chemicals were chosen to examine in high through-put assays. These were chosen as these chemicals were considered as being well characterized in toxicity. A ToxRef database is being constructed using 26 endpoints from mouse and rat studies. This database is being used to develop chemical ‘signatures’ which can be applied to classify the results of high throughput assays. Some of the assays in use are:

BioMap (BioSeek) – an ELISA-based system using multiple cell lines to generate 87 readouts and classify pathway perturbation using a library of signaling pathways;

Cellumen – a fluorescence based assay to identify toxicity;

HepG2 and primary hepatocyte assays;

Attagene – multiplexed reporter gene assay system;

NovaScreen – uses *in vitro* methods with 632 assays in combination with computational machine learning to develop toxicity profiles of chemicals.

These commercial systems were developed in screening pharmaceutical compounds. The known properties of the Phase I compounds have been used to validate the assays in toxicity profiling. ToxCast is using these systems to select predictive toxicity signatures of 700 additional compounds for Phase II of ToxCast. An issue with these *in vitro* methods is biotransformation of compounds – matching what happens in the whole organism remains a problem. ExpoCast is a program designed to predict human exposure to chemical compounds.

Holly Smith of Eli Lilly discussed pharmaceutical product testing. She particularly focussed on finding toxicity indicators early in drug development. By looking at the *in vivo* biomarkers of cardiotoxicity and myotoxicity,

cardioTroponin I, skeletal muscle Troponin I, My13, and Fabp3, one can sort out toxicity to cardiac muscle from skeletal muscle and even the toxicity to slow twitch versus fast twitch fibres. Similarly there are biomarkers that can characterize GI tract toxicities. Unfortunately, there is no ideal biomarker. One must use a panel of biomarkers.

Peter O'Brien of the **University of Toronto** spoke about using *in vitro* methods to screen for hepatotoxic drugs. This interesting talk exposed a mechanism by which idiosyncratic drug reactions occur. He has developed a primary hepatocyte, both induced and uninduced, assay system in which the addition of hydrogen peroxide and inflammatory cells such as Kupffer cells leads to a toxic reaction. He used this system to explain the toxicity of the Type II diabetes drug troglitazone in a significant fraction of patients. In a similar system, by adding ioniazid to his culture system 1 hour before exposing the cells to hydrogen peroxide, he could generate the hepatotoxicity this compound is known for. In summary oxidative stress and inflammatory reaction may lie behind many idiosyncratic drug reactions and it is possible to use an *in vitro* system to test for them.

A student speaker, **Katherine Schoeman**, from the **University of Western Ontario** spoke on methyl mercury and fish consumption. She compared the paradoxical findings in long-term studies in the Seychelles and the Faroe Islands. Both societies derive a large portion of their nutrition from ocean caught fish. One would expect that children test scores would be inversely proportional to levels of methyl mercury. That is what one finds in the Faroe Islands but in the Seychelles the test scores are directly proportional to methyl mercury levels. This underscores the importance of fish oils in brain development. She is testing attitudes to fish consumption in participating pregnant women in the MotherRisk program at the Hospital for Sick Children in Toronto.

A second student speaker, **Helen Badham**, from **Queen's University** spoke on benzene and childhood leukemia. She has studied mouse embryos acquired from benzene treated dams which were treated with PEG-catalase or simply

PEG. By examining colony forming units from the hematopoietic progenitor cells in the fetal liver, she was able to show an increase in erythroid CFU, in CFU-GM and CFU-G but a decrease in CFU-Me. Catalase protected against these aberrations in hematopoietic lineages. She concluded that reactive oxygen species are involved in benzene-induced leukemia.

Laura Andrews from **Genzyme** spoke on alternative methods to reduce animal use in the context of bioengineered products. About a decade after introducing Serazyme, which required the use of 80 rats and 6 non-human primates, Genzyme introduced a similar drug, Myozyme. This drug required the use of 400 rats, 100 mice, 72 rabbits and 112 non-human primates. Most of this increase was driven by regulatory safety requirements. She questioned the relevance of such extensive use of animals and noted the difficulty of having enough non-human primates per group to make the study statistically meaningful. The tremendous demand for non-human primates has led to alternative sourcing, with the result that the populations used by different labs are not homogenous. The relevance of the animal models themselves was questioned. Generally bio-engineered products are designed to be active in the human. For the animal model, a homologous protein would need to be devised. It could be costly and would not be identical to the preparation for the drug. Alternatively one could use humanized animal models. In this case the only humanized component is the target of the drug. Trying to find an animal model which exhibits similar tissue cross reactivity to humans is difficult. She gave an instance where there was pathological overgrowth in mouse esophagus to a growth factor product. Mice have a keratinized epithelium not found in primates. By using a small number of non-human primates, they demonstrated that pathological reaction was unlikely in humans. Despite this, the transgenic knockout knockin mouse has become the favourite animal model. Rather than using large numbers of non-human primates, *in vivo* imaging can be used with a single animal at various time points (MRI, PET scans, ultrasound, fluorescent imaging). She gave the example of fluorescent tagged tumour cells in a model of metastasis. Some testing strategies, e.g. developmental

toxicology, are not applicable to certain products: monoclonal antibodies will not pass the placenta so all you can test is maternal toxicity. Newer products pose new problems: drugs for MS will need to be given lifelong, and to vulnerable groups such as women of child bearing age. The Preclinical Guideline on Oncology Therapeutic Development (ICH S9), in development, will address these issues.

Ernie Bush (Cambridge HealthTech Assoc.) gave a talk on the use of the zebrafish model in toxicology. He pointed out that in the 1980's drug firms would lose 35-40% of their products due to Drug Metabolism and Pharmacokinetic (DMPK) surprises. Now the figure is <5%. He wondered if a similar improvement could be made in safety testing in this decade. Zebrafish (*Danio rerio*) are small tropical freshwater fish which have been used in developmental research. In toxicology the embryo, which is translucent, is the usual stage studied. They share 80-90% of human genes. Their metabolic activation potential for environmental chemicals is not established. Their primary use is in screening for developmental effects. There are a number of companies that use different, often automated systems to study the effects of chemicals and report back to the contractor. They have found use in exploring cardiotoxicity, reproductive toxicology, and some behavioural work with adult fish.

Kannan Krishnan (Université de Montréal) spoke on dose response modeling. He introduced the NOAPL – the no observable adverse perturbation level. Using quantitative structure-activity relationship (QSAR) techniques, predictions can be made of the likely effect of a compound. It is important to be able to know all of the compartments the compound will be partitioned into. Also the ability to predict the likely internal dose in the human from the *in vitro* system is being attained. Chemical moiety theory predicts a compound's properties from its chemical constituents. By looking at the partitioning into fat and into blood the models can be tested against real data. Virtual cell models have been created (the e-cell, which can model at least 495 processes) and are bringing us closer to the goal of biology *in silico*. Bayesian probability techniques can be used to refine the model,

incorporating factors derived from prior knowledge, keeping the modeling and the experimental data in sync. However, it will be difficult to isolate the key perturbation in the noise of multiple perturbations.

Lesla Aylward of Summit Toxicology spoke on the use of bio-monitoring equivalents as screening tools in human bio-monitoring. The classic example is lead where blood levels can be linked directly to the risk. This is not the case with most compounds. By using rodent models and PBPK modeling one can derive the Reference Dose (RfD) or the tolerable daily intake (TDI). The biomonitoring equivalence is then the biomarker level related to exposure. She reviewed several case studies (toluene, cadmium, 2,4-D, several halomethanes, and acrylamide). In the case of acrylamide, it forms adducts to hemoglobin. The level of the adduct becomes the biomarker. By calculating the area under the curve, and the known half-life of red blood cells, one can calculate the actual exposure. Using National Health and Nutrition Examination Study (NHANES) data this was found to be above the RfD. In a similar calculation the compounds were ranked according to priority of the public health risk, acrylamide was found to be a high priority, dioxin a medium priority, and the rest of low priority (References: Regul. Toxicol. Pharmacol. 2008 Aug; 51(3 Suppl): S4-15; J. Agric. Food Chem. 2008 Aug 13; 56(15): 6061-8). The choice of biomarker can be important as some are less sensitive and some of the analytical techniques are prohibitively expensive. More material is available at: <http://www.biomonitoringequivalents.net>.

William Fraser (Hôpital St-Justine) reviewed the MIREC (Maternal Infant Research in Environmental Chemicals). The study came out of an attempt to study the effects of antioxidant vitamins on preventing pre-eclampsia. They are recruiting expectant mothers to participate in a study of environmental contaminants in gestational and lactational sampling. They intend to sample at each trimester, at birth and at between 2 and 8 weeks post delivery. They will be sampling maternal hair, maternal blood, maternal urine, cord blood, and breast milk. The most difficult recruitment point is the first trimester. There are 10 sites across Canada

participating, generally associated with teaching hospitals, mostly urban. Food frequency questionnaires will be used. Work and home environments, mode of transport, will be profiled. Consumption of caffeine, fish, dairy, supplement use, calcium and iron sources will be estimated. Samples will be used to measure: mercury, manganese, lead, arsenic, cadmium, selenium, four species of phthalates, PCBs, organochlorines, PBB, PBDE, organophosphates, alkyl phosphates, PFOS, Bisphenol A, Dioxins/furans, perchlorate, cotinine, and nutritional status. SNP profiles will be measured. The patient will be able to get a contaminant profile but with the proviso that the risk entailed by those levels is unknown. If known risk levels of Pb, Cd or Hg are found the patient will be warned as soon as possible in order to reduce the risk. Physical measures of gender will be taken at birth (AGD, scrotal skin, aureole pigment). Finger length ratio, weight, head and chest circumference will also be measured at birth. TSH, T3, T4, TTR, steroid hormone levels, PUFA, trans fat levels will be measured from the blood sampling. The heart rate variability (Vagal tone) will be measured. The mother will be questioned on smoking habits, alcohol use, the length of breast feeding, and her BMI measured. All samples will be archived at the BioBank, in the hope that the study can be continued into childhood and adolescence.

Doug Haines (Health Canada) reviewed the Canadian Health Measures Survey and its biomonitoring component. Biomonitoring was a component of the Chemical Management Plan (2006). First Nations and Inuit Health also came on board. As a signatory of the Stockholm Convention on POPs, Canada is obligated to monitor its own population for POP exposure. It is also a component of the NAFTA side agreements, and our agreements to monitor the Arctic. Statistics Canada is leading the CHMS, with the support of Health Canada and PHAC. Cycle 1 runs from 2007-9, Cycle 2 from 2009-11, and Cycle 3 from 2011-13. Cycle 3 is not yet funded. This will be the first Canadian equivalent to NHANES. It is hoped to get benchmark data, establish time trends, and relate self reported data to environmental chemical load and to the other physical measures. It will try to represent the subpopulations in Canada. The aim is to enlist

5000 participants. The age groups targeted are: 6-11, 12-19, 20-39, 40-59, 60-79. The physical measures will be done through a mobile clinic in 15 randomly selected, representative sites throughout Canada. A general questionnaire will be used to gain information on general health, medications used, various habits with health implications, information on the age of the house, the sociodemographic characteristics, the education and income of the household and pesticide use. The participants will be questioned on cardiovascular disease, diabetes, infectious disease history. A fitness test will be given. Blood urine and DNA samples will be taken. Oral health will be assessed. Physical activity will be automatically measured for 1 week. Body measurements will be taken. Blood samples will be used to measure environmental chemical exposure, to assess nutrient status and blood lipid levels. Microbiology tests will be carried out. The chemicals to be measured are: lead, mercury, phthalates, PBDEs, phenoxyherbicides, PCBs, pyrethrins, organophosphates, organochlorine pesticides, PFOS, BPA, and cotinine. For most of these this study will establish baseline levels. The exposure data will also be valuable to programs such as the tobacco control program. Lead, mercury and cadmium levels will be reported back to the participants if they are above acceptable levels. Early results indicate that Lead, mercury and cadmium levels are generally low. Lead levels have declined since the 1978-9 Canadian Health Survey.

Report on the Eleventh International Congress of Toxicology (ICT-XI)

Mallé Jurima-Romet and Douglas Arnold

The Eleventh International Congress of Toxicology (ICT-XI) was held in Montreal from July 15-19, 2007. The Congress was hosted and organized by The Society of Toxicology of Canada (STC) and the National Research Council Canada (NRC), under the auspices of IUTOX. By all accounts, we believe that ICT-XI was highly successful. For example, there were over

1,500 participants from more than 70 countries in attendance, making this one of the largest and internationally diverse ICTs ever. On the Sunday preceding the official opening of the Congress, more than 300 delegates participated in the six Continuing Education sessions. Over the next 3.5 days, more than 150 invited speakers from 25 different countries participated in the Scientific Program, reflecting the extent to which internationally – recognized advances in the science of toxicology are being made. Nearly 1,000 posters were presented over three days while 56 companies and organizations participated in an informative and lively commercial exhibition.

The official opening of the Congress on Sunday evening was very well attended. The Deichmann Award lecture, delivered by Dr. Allan Okey of the University of Toronto, entitled: “*An Ah Receptor Odyssey to the Shores of Toxicology*”, was informative and engaging. Four keynote lectures, one on each morning of the Congress, were likewise well-attended. Several of the keynote lectures and symposia attracted attention from local radio and newspapers as well as from international science journalists who interviewed the speakers. Both the Deichmann Lecture and Dr. Moshe Szyf’s provocative keynote lecture on epigenetics were recently published in *Toxicological Sciences*. One of the many highlights of the scientific program was an entertaining debate on the topic of “Environment and Human Health Effects of Endocrine Disrupting Chemicals: Is There Really a Problem?”, with Dr. Frederick vom Saal and Dr. Linda Birnbaum arguing ‘For’ and the Chair of the Scientific Program committee, Dr. Daniel Cyr, arguing ‘Against’. An interesting aside was the chairperson asking for a show of hands prior to the debate to ascertain how the audience viewed this topic. While a majority of the attendees were ‘For’ initially, a show of hands at the conclusion of the debate favored the ‘Against’ argument.

The Palais des Congrès convention centre, located in downtown Montreal, was an excellent venue with the ICT-XI logistics being handled smoothly and seemingly effortlessly by the Palais and NRC personnel. One delegate commented afterwards: “*It flowed like a symphony*”. Graduate student

volunteers helped in a variety of roles, serving as ‘ambassadors’, directing delegates to meeting rooms and providing general information. Highlights of the social program included the Welcoming Reception, a sumptuous dinner and scenic boat cruise on the St. Lawrence River hosted by STC, and the Congress Banquet on Wednesday evening. Musical entertainment at the Banquet was provided by the Quebec-based, internationally – acclaimed Painchaud family who delivered an incredible performance featuring a diverse repertoire of classical, jazz, pop, and French-Canadian folk music, using a variety of instruments. Throughout the week, even the weather cooperated – the days were sunny and warm, and the evenings clear and cool, allowing delegates to enjoy their stroll along the streets while they took in the sights, sounds and tastes of the vibrant mid-summer Montreal atmosphere.

Financially, the Congress was highly successful, generating a large surplus that will become available for IUTOX to use for future programs and other scientific endeavors. The organizers acknowledge the generous contributions of the many companies and organizations that provided financial support. We are also grateful to the many people who volunteered their time and energy towards the planning and implementation of ICT-XI, including the members of the various subcommittees of the Organizing Committee, advisory committees, and students. In addition, the Organizing Committee gratefully acknowledges the assistance provided by IUTOX and SOT.

From the outset, the Organizing Committee’s objectives were to organize and host an ICT that would be scientifically rewarding, financially successful, and personally memorable for delegates and their families. We are proud to claim that ICT-XI met all three objectives!

Book Reviews

Bill Racz

“Human Toxicology of Chemical Mixtures”, Harold I. Zeliger, published by William Andrew, 2008.

The book considers how exposure to toxic mixtures affects the different physiological systems and organs, including the respiratory, cardiovascular, immunological musculoskeletal, nervous and reproductive organs. The author evaluates various sources of exposure, including air, water and soil pollution: *in utero* exposure; food additives and contaminants, cosmetics, domestic cleaning products, pesticides, industrial chemicals, electromagnetic radiation, and chemicals contained in tobacco smoke and alcohol.

The author has taken a chemical approach to discussing the various topics and as such lacks mechanistic detail from a biological perspective. The real value of the book is the breath of topics that are covered and the number of concepts that are included. The sections are fully referenced and thus the reader can find the original data to support the concepts. This reviewer found the lists of potential toxicants in the mixtures to which we are exposed interesting, for example comparing the list of chemicals in commercial and a home made loaf of bread was enlightening. Another strength of the book is the vast array of examples.

“Sittig’s Handbook of Toxic and Hazardous Chemicals and Carcinogens”, 5th Edition, Richard P. Polanish, published by William Andrew, Applied Science publishers, 2008.

“Sittig’s Handbook of Toxic and Hazardous Chemicals and Carcinogens” has been used by a wide audience for over a quarter of a century. This comprehensive work (2,850 pages) provides an easy to read description of some 2,100 commonly used chemicals. The chemicals are listed in alphabetical order and classified as a carcinogen, hazardous substance, hazardous waste, or toxic pollutant. The description of each chemical is expanded for the 5th edition. Each

chemical is presented in a ‘template’ manner with 28 fields covered for each entry. These fields cover: regulations, air and water limits, toxic effects, common routes of exposure, required protective equipment, environmental impact, and proper disposal to list a few. The entries are updated to reflect current knowledge. This text should be in the library of any organization that deals with hazardous materials. Industrial hygienists, safety directors, engineers, toxicologists, environmental specialists, transportation organizations as well as individuals charged with worker protection will find this two-volume set invaluable.

Upcoming Events

Society of Toxicology (SOT) 48th Annual Meeting, March 15-19, 2009, Baltimore Convention Center, Baltimore, MD.

The Society of Toxicology of Canada Annual Meeting, December 6-8, 2009, Montreal, PQ.

Society of Toxicology (SOT) 49th Annual Meeting, March 7-11, 2010, Salt Palace Convention Center, Salt Lake City, Utah.

XIIth International Congress of Toxicology, July 11-15, 2010, Barcelona, Spain.