Canadian Society for Pharmaceutical Sciences

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4th Annual Symposium

Beyond Bioequivalence! Canadian & International Issues in Biopharmaceutics & Pharmacokinetics.

June 14 - 16, 2001

CROWNE PLAZA OTTAWA

OTTAWA, ONTARIO, CANADA

CONFERENCES: BALLROOMS B, C, THURSDAY, FRIDAY, SATURDAY

EXHIBITS: BALLROOM FOYER, THURSDAY, FRIDAY, SATURDAY

POSTERS: BALLROOM A, FRIDAY

Sponsors

Health Canada (Therapeutic Products Directorate), AstraZeneca, DuPont Pharma, GlaxoSmithKline, Merck Frosst Canada, Novartis Pharmaceuticals, Pfizer Canada, Pharmacia

Exhibitors

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Original Articles Published In Last Issue (J Pharm Pharmaceut Sci (www.ualberta.ca/~csps) 4(1) 2001)

Thursday, June 14, Laurentian Room

0700h CSPS Executive Meeting

Thursday, June 14, 15, 16, Ballroom Foyer

0800h Exhibitors

Thursday, June 14, International Ballrooms B & C

0830h *Objectives*, Iain J. McGilveray, McGilveray Pharmacon, Ottawa, Ontario, Canada

0835h *Welcome*, Robert Peterson, Director-General, Therapeutic Products Directorate, Ottawa, Ontario, Canada

Session 1: Oral and non-oral formulation development in drug development. *Chairs: Elizabeth Vadas, Merck Frosst Canada & Co.; Vinod Shah, U.S. Food & Drug Administration*

0845h An overview: place of formulation in drug development, integrating with toxicology, early human studies and clinical trials. Elizabeth Vadas, Merck Frosst Canada & Co., Montreal, Quebec, Canada

0915h Percutaneous studies-application of pharmacokinetics in development and formulation changes. Lester Harrison, 3M Pharmaceuticals, St. Paul, Minnesota, USA

0945h Dermatopharmacokinetics in evaluation of topical formulations. Vinod P. Shah, U.S. Food & Drug Administration, Rockville, Maryland, USA

1015h Coffee/Tea Break.

1035h Pharmacodynamic protocols to assess relative potency of effect of inhaled corticosteroids. Frederick E. Hargreave, Firestone Institute for Respiratory Health, St. Joseph's Healthcare and McMaster University, Hamilton, Ontario, Canada

1105h Topical formulations: TPD guidelines. Paul Roufail, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada

1135h Panel

1200h Lunch Break

Session 2: Pharmacokinetic issues for new drug submissions. *Chairs: Jake Thiessen; Faculty of Pharmacy, University of Toronto, Ontario; Neil Maresky, Bayer Canada*

1300h Development surprises: the thrilling world of cytochromes and transporters. Leslie Z. Benet, Department of Biopharmaceutical Sciences, University of California, San Francisco, California, USA

1340h ICH common technical document, PK content and overview. Iain J. McGilveray, McGilveray Pharmacon, Ontario, Canada

1355h Main PK studies required for filing an NDS/NDA. Éric Masson, Anapharm Inc., Sainte-Foy, Québec, Canada

1425h Pharmacokinetic issues in global product development – an industry perspective. Neil S. Maresky, Vice-President, Medical and Scientific Affairs, Bayer Inc., Toronto, Ontario, Canada

1500h Coffee/Tea Break.

1520h The pursuit of better medicines through genetic research. Terri Arledge, US Department Head, GlaxoSmithKline, Research Triangle Park, North Carolina, USA

1550h Drug interactions: *in vitro* case studies. Brian C. Foster, Office of Science, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada

1605h Clinical drug interactions: study design and data analysis. Keith Gallicano, Axelson BioPharma Research Inc. and CroMedica Prime, Vancouver, British Columbia, Canada

1620h TPP Clinical trial regulation update. Christine Nestruck, Clinical Trials and Clinical Access Programme, Therapeutic Products Directorate, Ottawa, Ontario, Canada

1650h-1715h Panel/Questions

Eric Ormsby, Risk Management Methods, Office of Science, Health Canada, Ottawa, Ontario, Canada

1900h Dinner & awards: The Pinnacle. Cash bar.

Music of Ben Segal.

Award recipients: Fakhreddin Jamali and Kamal Midha.

Friday, June 15, International Ballroom A

0800h Posters

Friday, June 15, International Ballrooms B & C

Session 3: Regulatory and federal/provincial bioequivalence and interchangeability issue. *Chairs: Norman J. Pound, Therapeutic Products Directorate, Canada; Silvia Alessi-Severini, Alberta Blue Cross, Canada*

0830h Bioequivalence: Therapeutic Products Directorate perspective. Norman J. Pound, Division of Biopharmaceutics Evaluation, Therapeutic Products Directorate, Health Products & Foods Branch, Ottawa, Ontario, Canada

0900h Interchangeability: provincial government perspective. Silvia Alessi-Severini, Scientific and Research Services, Clinical Drug Services and Evaluation, Alberta Blue Cross, Edmonton, Alberta, Canada

0930h Bioequivalence and interchangeability: industry perspective. Derek Ganes, Taro Pharmaceuticals Inc., Bramalea, Ontario, Canada

1000h Coffee/Tea Break. Poster Viewing.

Authors will be available by their posters.

1100h Scientific basis for biowaivers based on BCS. Ajaz S. Hussain, Office of Pharmaceutical Science, CDER, FDA, Rockville, Maryland, USA

1130h Post-marketing changes and approval: industry perspective. Anne Tomalin, CanReg Inc., Hamilton, Ontario, Canada

1200h Panel Discussion

1230h Lunch Break.

Authors will be available by their posters.

Session 4: Pharmacokinetic and biopharmaceutic issues involving biologics/biotechnology products.

Chairs: Keith Bailey, Ottawa, Ontario, Canada; Daan Crommelin, University of Utrecht, Netherlands

1330h Compendial issues for biotechnology products. Roger L. Williams, United States Pharmacopeia, Rockville, Maryland, USA

1410h Characterization of biotech products by mass spectrometry and other methods. David Kwok, BRI Biophharmaceutical Research Inc., Vancouver, British Columbia, Canada

1440h Delivery issues with biopharmaceuticals. Daan J. A. Crommelin, Biofarmacie en Farmaceutische Technologie, Universiteit Utrecht, The Netherlands and B.V. Leiden, The Netherlands

1510h Pharmaceutical equivalence of biologic drug products. Salomon Stavchansky, Alcon Centennial Professor of Pharmaceutics, College of Pharmacy, Division of Pharmaceutics, The University of Texas at Austin, Texas, USA

1540h Coffee/Tea Break. Poster Viewing.

Authors will be available by their posters.

1600h Comparative pharmacokinetic studies in the development of biotechnology drugs. Andrew Storey, Vice-President, Quality, Clinical and Regulatory Affairs, Cangene Corporation, Winnipeg, Manitoba, Canada

1630h The essential non-comparability of innovator and second-source biotechnology products: interferons as a test case. James N. Bausch, Director of Protein Analysis, Schering-Plough Corporation, Kenilworth, New Jersey, USA

1700h A Canadian regulatory perspective on biocomparability. Anthony A. G. Ridgway, Manager, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Ottawa, Ontario, Canada

1730h Panel/Wrap-up. Keith Bailey, Nepean, Ontario, Canada

1800h CSPS Members Annual General Meeting. (this will be a half-hour meeting immediately following the panel/wrap-up)

Saturday, June 16th, 2001

Session 5: Issues in bioequivalence. *Chairs: Fakhreddin Jamali, University of Alberta, Edmonton, Canada; Michael Spino, Apotex Inc., Toronto, Ontario, Canada*

0830h Bioequivalence: current concerns and issues. Jake J. Thiessen, Faculty of Pharmacy, University of Toronto, Ontario, Canada

0910h Complicated cases. Need for revised criteria? Fakhreddin Jamali, University of Alberta, Edmonton, Alberta, Canada

0940h Bioequivalence of narrow therapeutic range drugs. Scott Walker, Coordinator, Research and Quality Control, Pharmacy, Sunnybrook and Women's College Health Sciences Centre; and Associate Professor, Universityof Toronto, Toronto, Ontario, Canada

1010h Coffee/Tea Break.

1030h Bioequivalence measures and parameters: criteria for comparisons, IBE & ABE. John W. Hubbard, PharmaLytics Inc., A Drug Metabolism, Drug Disposition Institute, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

1100h Measures for the determination of bioequivalence: exposure, absorption rate and curve shape. Laszlo Endrenyi, Department of Pharmacology, University of Toronto, Ontario, Canada

1130h Panel

1200 *Closing: Concluding Remarks*
Elizabeth Vadas and Iain McGilveray

Robert Peterson
Director-General, Therapeutic Products Directorate, Ottawa, Ontario, Canada

Dr. Peterson received an M.D. degree and a Ph.D. in Pharmacology from Yale University in 1974, having completed the National Institutes of Public Health sponsored Medical Scientist Training Program at that university. He was a resident in pediatrics at the Yale-New Haven Hospital until 1976 at which time he became a postdoctoral fellow in Clinical Pharmacology and Neonatology at the University of Colorado. He subsequently joined the Department of Pediatrics at the University of Colorado as Assistant Professor, where he was Director of the Section of Pediatric Clinical Pharmacology. In 1983, Dr. Peterson joined the Departments of Pediatrics and Pharmacology at the University of Ottawa, Faculty of Medicine as Associate Professor and Medical Director of the Ontario Provincial Poison Information Centre at the Children's Hospital of Eastern Ontario. In 1987 he became Director of the Children's Hospital Research Institute. He became Professor of Pediatrics and Pharmacology in 1989, and in 1990, Chairman of the Department of Pediatrics and Pediatrician-in-Chief. He has authored numerous papers/chapters in pediatric clinical pharmacology/toxicology. Dr. Peterson completed a Master's of Public Health in the Department of Health Policy and Health Care Management at Harvard University School of Public Health in 1996. He joined, Health Canada in January 1999, and is Director General of the Therapeutic Products Directorate

Robert Peterson
Director-General, Therapeutic Products Directorate, Ottawa, Ontario, Canada

Le D[r] Peterson a obtenu son diplôme de médecine et un doctorat en pharmacologie à l'université Yale en 1974, après avoir suivi, dans cet établissement, le programme de formation de médecins chercheurs parrainé par le National Institutes of Public Health. Résident en pédiatrie à l'hôpital Yale-New Haven jusqu'en 1976, le D[r] Peterson a obtenu une bourse de recherche postdoctorale en pharmacologie clinique et en néonatologie à l'université du Colorado. Par la suite, il a été professeur adjoint au département de pédiatrie de l'université du Colorado, où il dirigeait la section de pharmacologie clinique pédiatrique. En 1983, le D[r] Peterson est entré aux départements de pédiatrie et de pharmacologie de la Faculté de médecine à l'Université d'Ottawa à titre de professeur et de directeur médical du Centre d'information antipoison pour la province de l'Ontario à l'Hôpital pour enfants de l'est de l'Ontario. En 1987, il a été nommé directeur de l'Institut de recherche de l'Hôpital pour enfants. Il a ensuite été professeur de pédiatrie et de pharmacologie en 1989, et en 1990, directeur du département de pédiatrie et pédiatre en chef. Le D[r] Peterson a écrit de nombreux articles et chapitres sur la pharmacologie et la toxicologie cliniques pédiatriques. Il a obtenu une maîtrise en santé publique au Department of Health Policy and Health Care Management du Harvard University School of Public Health en 1996. Le D[r] Peterson est entré au Santé Canada en janvier 1999, au poste de directeur général .Programme des produits thérapeutiques.

An overview: place of formulation in drug development, integrating with toxicology, early human studies and clinical trials.

Elizabeth Vadas, Merck Frosst Canada & Co., Montreal, Quebec, Canada

Formulation development starts long before a new molecule is first introduced in man. During the discovery process the absorption characteristics of a new molecule must be evaluated in a number of species, most importantly in those which will be used in toxicology studies. Sometimes a simple vehicle will suffice, if the molecule is to be administered orally. Even in the case of oral administration there may be a need for thorough evaluation of the effect of particle size, and of the chemical form of the molecule; e.g. free base, free acid or salt forms. Definition of the human formulation(s) must precede toxicology studies when the molecule is slated for non-oral administration. If the drug is intended for delivery as an aerosol, injectable or dermal preparation, the toxicology studies will be carried out via the appropriate route of administration. This requires formulation development prior to the toxicology studies. The first clinical studies, incremental single dose and incremental multiple dose safety and tolerability studies cover a wide dose range often requiring the use of several formulations. As clinical trials progress the dose range narrows and ultimately the clinically safe and efficacious dose is defined. Parallel to the clinical development the formulation composition and manufacturing processes are defined and optimized based on feedback from the clinical studies. Formulation development must be intimately integrated with toxicology and clinical studies if it is to result in a clinically safe and efficacious, commercially viable dosage form which meets all regulatory requirements.

Elizabeth Vadas
Merck Frosst Canada & Co., CSPS

Elizabeth B. Vadas obtained her Ph.D. in Physical Chemistry from McGill University. Following postdoctoral training she joined Merck Frosst in 1980 as a senior research scientist in the department of Pharmaceutical Research and Development. Over the years she has been involved in the development of many new chemical entities discovered at the Merck Frosst Centre for Therapeutic Research while taking on increasing management responsibilities. Currently Dr. Vadas is Executive Director of Pharmaceutical Research and Development, a department which has grown from 18 to over 70 scientists in the last 10 years under Dr. Vadas' leadership. Most notable of her department's scientific accomplishments are the development efforts supporting the leukotriene and Cox-2 programs leading to the regulatory approval of SINGULAIR® in the former and VIOXX™ in the latter; two new therapeutic breakthroughs discovered and developed in Canada. Dr. Vadas' main scientific interests are in the area of pharmaceutics, particularly in solid state chemistry and physics, drug excipient interactions and aerosols. She is an Adjunct Professor of Pharmaceutics at the Faculty of Pharmacy, University of Montreal. She also has lectured and published widely. Professional activities and honours: USP Aerosol Advisory Panel member 1989-1990, 1992-1995, USP Committee of Revision member 1990-1995, Louis W. Busse lecturer, School of Pharmacy, University of Wisconsin, 1991, Elected Fellow of American Association of Pharmaceutical Scientists 1996, "Nouveaux Performants" Management Award 1998, Elected to the Executive of CSPS as Member-at-Large (1998-2000), Canadian Health Manager Award 1999, CSPS DuPont Leadership Award 2000, Elected President CSPS 2001-2002.

Percutaneous studies – application of pharmacokinetics in development and formulation changes

L. Harrison, Section Head, Clinical Pharmacokinetics, 3M Pharmaceuticals, St. Paul, Minnesota, USA

The current Cmax and AUC metrics are not adequate to measure the bioequivalence of either formulation changes or of generic products for transdermal drug delivery. One must recognize the unique nature of transdermal drug delivery in that drug delivery is occurring throughout the entire application interval, currently as long as seven days. A further complication is that the rate of transdermal drug absorption is not constant, but is frequently changing as excipients and penetration enhancers are depleted. Bioequivalence can be assessed for transdermal products by adopting the systemic exposure approach. One needs to measure peak exposure (Cmax) and total exposure (AUC) over the application interval, and also early exposure (partial AUC from time zero to Tmax of the reference). These three metrics would be adequate if the pivotal bioequivalence trial was done at steady state. If a single application study serves as the bioequivalence test, then a fourth metric of late exposure (concentration at the end of the application interval) is recommended. It is possible that equivalence of early, peak and total exposure can be obtained following a single patch application trial without achieving equivalence for late exposure, with the result that the steady-state profiles could be significantly different.

Lester Harrison
3M Pharmaceuticals

Lester Harrison is Division Scientist and Section Head, Clinical Pharmacokinetics, in the Pharmacokinetics and Drug Metabolism Department of 3M Pharmaceuticals, St. Paul Minnesota. He has overall responsibility for the human pharmacokinetic and pharmacodynamic studies done by 3M Pharmaceuticals, which are primarily topical, transdermal and oral inhalation studies. A major focus of his workgroup is performing and analyzing the clinical studies necessary to meet the regulatory requirements for bioavailability and bioequivalence on a global basis. Dr. Harrison received his Ph.D. in biological chemistry from the University of Michigan, Ann Arbor, in 1973. He subsequently completed two postdoctoral fellowships, one in microbiology; and one in biopharmaceutics and pharmacokinetics at the State University of New York at Buffalo. He joined the Drug Metabolism Department of 3M Pharmaceuticals in 1976. While at 3M, he has published more than 50 peer-reviewed research papers in the fields of pharmacokinetics and drug metabolism.

Dermatopharmacokinetics in evaluation of topical formulations.

Vinod P. Shah, Senior Research Scientist, Office of Pharmaceutical Science, United States Food and Drug Administration, Rockville, Maryland, USA

Methods to establish bioequivalence (BE) in descending order of preference are pharmacokinetics (PK), pharmacodynamics, comparative clinical trials and in vitro studies. For topical dermatological drug products, currently comparative clinical studies are required to establish BE for most of the dosage forms (except corticosteroids). PK when applied to drug concentration measurements in stratum corneum (SC) is termed dermatopharmacokinetics (DPK). Tape stripping procedure is a tool used to measure drug concentration in SC and to determine drug uptake and disappearance (elimination) from the SC after topical application. In general, the method is applicable and feasible for all topical dermatological drug products, it measures the drug concentration in the vicinity of the site of action in the skin. Pros and cons of DPK, and current regulatory research efforts at FDA will be discussed.

Vinod Shah
United States Food and Drug Administration

Dr. Shah is a Senior Research Scientist in the Office of Pharmaceutical Science, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA). He received his Pharmacy degree from Madras University, India and Ph. D. in Pharmaceutical Chemistry from the University of California at San Francisco (UCSF). Dr. Shah has served in different capacities in CDER/FDA since 1975. He is a recipient of several CDER/FDA awards including FDA's Award of Merit in 1981 and 1988, and FDA's Scientific Achievement Award in 2000. Dr. Shah has published more than 175 scientific papers and is a co-editor of two books. Dr. Shah has developed Regulatory Guidance's for Pharmaceutical Industry in areas of dissolution, bioanalytical method validation, topical dermatological drug products, and BA/BE for oral drug products. Dr. Shah is a member and Fellow of American Association of Pharmaceutical Scientists and is a recipient of AAPS Distinguished Service Award in 2000.

Pharmacodynamic protocols to assess relative potency of effect of inhaled corticosteroids

F.E. Hargreave, K. Parameswaran, Firestone Institute for Respiratory Health, St. Joseph's Healthcare and McMaster University, Hamilton, Ontario, Canada

Assessing the relative potency of effect of inhaled corticosteroids and their devices is problematic. No publication has identified how this can be conveniently achieved. Three closed workshops, with international participation in two, have been sponsored by Health Canada in Seattle (1995) and Toronto (1996 and 2000); a publication followed the second (1). The workshops discussed 3 possible models, study design, outcomes and analysis. The models have been of allergen inhalation in subjects with very mild asthma, of steroid reduction in subjects with moderate to severe asthma, and of uncontrolled asthma in subjects with mild to moderate asthma. A cross-over design with 3 doses of each comparator has been considered preferable to reduce sample size and identify the steep portion of the dose-response curve with each product. Outcomes have expanded from physiological measurements to induced sputum eosinophils and exhaled NO. Initial studies with allergen inhalation proved unsuccessful. Current studies utilizing each of the models will be presented.

1. Boulet L-P, Cockcroft DW, Toogood J, Lacasse Y, Baskerville J, Hargreave FE. Comparative assessment of safety and efficacy of inhaled corticosteroids: Report of a Committee of the Canadian Thoracic Society. Eur Respir J 1998; 11:1194-1210.

Frederick Hargreave

Firestone Institute for Respiratory Health, St. Joseph's Healthcare and McMaster University

Achievement: Pioneer in the measurement of asthma severity, and components. His research has paved the way for major advances in asthma diagnosis and treatment. Born: Nov. 20,1938. Family: Married, with three children. Pastimes: Reading, hiking, photography, rugby (as a spectator), gardening. The Researcher Dr. Frederick E. Hargreave was born in Hong Kong where his father worked for a large British multinational corporation. His family returned to their native England during the Second World War, where Dr. Hargreave pursued a medical degree, and eventually, came to study at London's world renowned Institute for Diseases of the Chest (now called the National Heart and Lung Institute). There he developed an interest in the problem of asthma and allergic reactions to airborne particles. Dr. Hargreave was persuaded to leave England in 1969 to join McMaster University's new medical school, in Hamilton Ontario. The decision turned out to be a fortuitous one for McMaster and Dr. Hargreave – today he has over 260 published scientific articles, and leads a team of over twenty researchers and support staff. Dr. Hargreave lives near Hamilton, Ontario, Canada, with his wife, Alix, three cats, a dog and a rabbit. His three children, Clare, Erica and Peter are away at University. Quotes: "In the past, the only method we had for measuring inflammation in asthma was bronchoscopy - putting a tube down into the lungs. Only a few people would agree to have this done for research. Fortunately, we now have less invasive methods. "We're at a position now of beginning to understand asthma much better and of being able to treat it much better. There are even some studies which suggest that if inflammation is the cause and you can treat it early, then potentially you can prevent asthma from becoming severe or even occurring on a regular basis."

Topical Formulations: TPD Guidelines

Paul Roufail, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada

The Therapeutic Products Directorate has been developing guidance documents on several areas related to topically administered formulations. The objectives of these guidance documents are to assist the pharmaceutical industry in the development and filing of submissions and ensure consistency in the procedures used for the evaluation of these formulations. This presentation will give an update on the status of these activities and discuss some of the issues related to each of them.

Paul Roufail

Therapeutic Products Directorate, Health Canada

Paul Roufail, Ph.D. is Acting Manager of Clinical Group 2,Bureau of Pharmaceutical Assessment in the Therapeutic Products Directorate of Health Canada. He received his Ph.D. in Pharmacy from Basel University, Switzerland, in 1965. He worked for three years at Merck Frosst Laboratories, Montreal, Canada, and was Assistant professor for two years in the College of Pharmacy, Dalhousie University in Halifax, N.S., before joining the Canadian Health Products and Foods Branch in 1971.

Development Surprises: The Thrilling World of Cytochromes and Transporters

L.Z. Benet, Department of Biopharmaceutical Sciences, University of California, San Francisco, California, USA

Intestinal Phase I metabolism and active extrusion of absorbed drug have recently been recognized as major determinants of oral drug bioavailability. Both cytochrome P-450 3A4 (CYP3A4), the major phase I drug metabolizing enzyme in humans, and the multidrug efflux pump, MDR or P-glycoprotein (P-gp), are present at high levels in the villous enterocytes of the small intestine, the primary site of absorption for orally administered drugs. CYP3A4 and P-gp are also both present in the liver. These proteins are induced or inhibited by many of the same compounds and demonstrate a broad overlap in substrate and inhibitor specificities, suggesting that they act as a concerted barrier to drug absorption, as well as working coordinately in hepatic elimination. Clinical studies from our laboratory have demonstrated that the bioavailability of three immunosuppressive agents, cyclosporine, tacrolimus and sirolimus, can be increased by concomitant administration of ketoconazole, a potent CYP3A inhibitor (K_i approximately 1μM) and moderate to potent inhibitor of P-gp (K_i ranges from 5-120 μM). Conversely, concomitant administration of rifampin, a potent inducer of CYP3A and P-gp, markedly decreased the bioavailability of cyclosporine, tacrolimus and sirolimus. More recent studies in cell systems transfected with human enzyme and transporters provide potential models to predict significant interactions and elucidate the interactive nature of these elimination proteins, and also highlight the potential for multiple transporters and enzymes working at apparently cross purposes.

Leslie Benet
Department of Biopharmaceutical Sciences, University of California

Dr. Benet, Professor of Biopharmaceutical Sciences, University of California San Francisco and founder and Chairman of the Board of AvMax, Inc., received his AB, BS and MS from the University of Michigan, and Ph.D. from the University of California. He has received five honorary doctorates: Uppsala University, Sweden (Pharm.D., 1987), Leiden University, The Netherlands (Ph.D., 1995), University of Illinois at Chicago (D.Sc., 1997), Philadelphia College of Pharmacy and Science (D.Sc., 1997) and Long Island University (D.Sc., 1999). His most recent work has addressed the cooperative effects of metabolic isozymes of cytochromes P-450 and antitransport proteins as related to immunosuppressive, anticancer, anti-AIDS and anti-parasitic drugs, as well as drugs of importance to women's health. He continues his studies to explain the immunotoxicity of NSAIDs through acyl glucuronide and acyl CoA intermediates. Dr. Benet is a Fellow of AAPS, AAAS and APRS. In 1985 he served as President of the Academy of Pharmaceutical Sciences. During 1986, Dr. Benet was a founder and first President of the AAPS. In 1987 he was elected to membership in the Institute of Medicine of the National Academy of Sciences. In 1989 he received the first AAPS Distinguished Pharmaceutical Scientist Award; in 1991, the Volwiler Research Achievement Award of the American Association of Colleges of Pharmacy; in 1995, the Rawls-Palmer Award of the American Society for Clinical Pharmacology and Therapeutics; in 2000, the APhA Higuchi Research Prize and the AAPS Wurster Award in Pharmaceutics. Dr. Benet has published over 400 scientific articles and book chapters, holds 9 patents and served as editor of 5 books.

ICH common technical document, PK content and overview.

Iain J. McGilveray, McGilveray Pharmacon; Consultant and Adjunct Professor, Faculty of Medicine, University of Ottawa, Ontario, Canada

This talk will be a very brief outline introducing the major topics for the afternoon. There is no specific ICH guidance's for human pharmacokinetics, but the recent common technical document "Efficacy" (CTD/E) lists the expected organisation of human study reports, with short descriptions. As well as bioanalytical method reports, PK studies expected include BA and BA, plasma protein binding, *in vitro* hepatic metabolism and interaction, healthy subject and patient PK reports, special population and extrinsic factor (food effect, etc.). PD and PK/PD relationship studies have also a listing in the CTD/E and there is an example of a tabular summary. The detailed guidelines for the studies are left to the regions to develop and the FDA has most. However, all regions and TPD have recent guidance's on Drug-Drug Interactions: Studies *in Vitro* and i*n Vivo*.

Iain McGilveray
McGilveray Pharmacon, University of Ottawa, CSPS

Dr McGilveray obtained a B.Sc. in Pharmacy and a Ph. D in Pharmaceutical Science in Glasgow, Scotland, followed by a NATO fellowship at the U. of Illinois. He was also a visiting scientist, training in pharmacokinetics, with Sidney Riegelman at the U. of California, San Francisco. He has been scientist, section head and latterly Division Chief, Biotherapeutics, Bureau of Biologics and Radiopharmaceuticals, working in Health Canada for over 30 years. His research interests have included, biopharmaceutics, bioanalytical methodology, dissolution, pharmacokinetics, metabolites, pharmacodynamics and chiral effects, but principally have been focused on bioavailability and bioequivalence and he has contributed to more than 150 research publications. He has organized and participated in many scientific conferences internationally and was chair of the first Bio-International Conference (Toronto 1989), and on the planning committee of both the 1990 and (January) 2000 AAPS-FDA workshops on Bioanalytical Methods Validation. Dr McGilveray is a previous Expert Member of the FIP Board of Pharmaceutical Sciences (to 1996) and Fellow of the AAPS and Canadian Institute of Chemistry. He is past-chair (1999) of the Regulatory Sciences section of AAPS, serving on several annual meeting and workshop program committees, including the Pharmaceutical Sciences Congress of the Americas (Orlando, March 2001). He has participated in several DIA workshops and received the Distinguished Career Award in 1998. He is interested in new scientific approaches to drug regulation and harmonization of standards, having been advisor to WHO and FDA, most recently on biopharmaceutics drug classification. He is adjunct professor (medicine) University of Ottawa and a consultant and will be chair of the CSPS June 2001 symposium in Ottawa.

Main PK studies required for filing an NDS/NDA

Éric Masson, Anapharm Inc., Sainte-Foy, Quebec, Canada

Pharmacokinetics and pharmacodynamics are surrogate markers of efficacy and safety. During drug development, there is a least one PK study, with the majority of submissions having more than 20. Most PK studies have impact on regulatory decision, and package insert or product monograph. PK studies can be used as exploratory, or confirmatory in drug development. In addition, PK data can help bridging pre-clinical to clinical data, as well as early clinical results (Phase I) with late, confirmatory results (phase III). Several innovative design, and methods allow rapid assessment of PK. PK studies can help reducing drug development time, and late failures by characterizing the impact of some factors (e.g., formulation changes, demographic characteristics) on outcome (safety/efficacy). This presentation will provide several examples of how PK studies have helped speed-up the development and approval of drugs.

Éric Masson

Anapharm Inc., CSPS

Éric Masson is Senior Director of Scientific and Regulatory Affairs at Anapharm, a Canadian Phase I CRO. Since joining the company over 3 years ago, he has been involved in the design and conduct of over 200 clinical pharmacology studies. His research interest included trial simulations, pharmacokinetics and pharmacodynamics, and drug development. He still affiliated with Laval University, Faculty of Pharmacy, teaching some of the courses to graduate students in drug development. Before that, he was assistant professor in pharmacy (Université Laval, 1995-97), post-graduate fellow (St. Jude Children's Research Hospital, 1993-95), clinical pharmacist and adjunct clinical professor (Université Laval 1994-5). He obtained his post-bachelor Pharm.D. degree from SUNY Buffalo (1992), and, degree and license in pharmacy from Université Laval, Québec (1990). Éric is also Vice-President of the Canadian Society of Pharmaceutical Scientists.

Pharmacokinetic Issues in Global Product Development – An Industry Perspective

N.S. Maresky, Vice President, Medical and Scientific Affairs, Bayer Inc. Toronto, Ontario, Canada

The clinical standards for evaluating new chemical entities (NCEs) has traditionally involved pharmacodynamic and pharmacokinetic studies, as well as studies evaluating drug-drug interaction, enzyme induction, and kinetics in special test populations. Advances made in the areas of bioinformatics, combinatorial chemistry and high throughput screening have resulted in a sharp increase in the number of (NCEs) entering into clinical drug development. It has become increasingly important for pharmaceutical companies to make early Go / No Go decisions and only allow NCEs with higher probability of success to enter the more expensive Phase IIb and III stages of development. Results of preclinical in-vitro metabolism studies and early clinical pharmacology studies are being used to weed out compounds with significant enzyme induction or drug – interaction potential. It is also becoming increasingly important to study drug candidates in patients early in the development process to determine the pharmacokinetics and pharmacodynamics in the target population and use the findings to facilitate Go / No Go decisions for Phase IIb and III studies. Pharmacokinetic assessments that have to be conducted in special populations pose a different set of challenges. For instance, under the Pediatric Rule of 1998, FDA may require clinical evaluation of new and approved drugs in pediatric patients. Bayer's experience in conducting these critical, and often difficult, pharmacokinetic studies will be discussed in this presentation. Examples to be discussed deal with (a) the characterization of metabolic profile of an investigational compound as part of a multiple dose pharmacokinetic study, (b) characterization of penetration into site of action and interaction potential of an investigational compound in the target patient population and (c) ethical and logistic issues in conducting pediatric pharmacokinetic studies, and (d) the extrapolation of bioequivalence to therapeutic equivalence.

Neil Maresky
Bayer Inc

Neil Maresky was born in Welkom, South Africa. He completed his Medical Training at the University of Witwatersrand in Johannesburg. After his internship at Baragwanath Hospital, Neil underwent a two year of Cardiology rotation, one of which was at the First Military Hospital in Pretoria, the other at the Cardiovascular Research Laboratory at Baragwananth Hospital in Soweto. Thereafter, Neil went into his own practice as a Family Physician, for two years. In 1993, Neil immigrated to Canada, and entered the pharmaceutical industry as an Associate Director at Ciba. He then progressed to the position of Director Drug Safety, and Epidemiology . Neil joined Bayer in 1998, and is now the Vice-President of Medical and Scientific Affairs. He is married with two children. neil.maresky.b.@bayer.com

The Pursuit of Better Medicines through Genetic Research

Terri Arledge, US Department Head, Drug Development Genetics, Glaxo Smith Kline, Moore Drive, Research Triangle Park, North Carolina, USA

Pharmacogenetics holds the promise of personalized therapy. We envision a day when physicians can use pharmacogenetic data rather than population data to make more informed decisions about therapy. Physicians will be more likely to identify patients who can best respond to medicines in the situations where differences in medicine response can be explained by genetic variability. Pharmacogenetics is a new tool for drug development. While this is a powerful new tool, it will be added to, rather than replace, the already existing methods of drug evaluation (PK, PD, etc). We expect to work within the regulatory foundation that already exists – specifically one built on strong science - i.e. reproducibility, good clinical and laboratory practices; all of the ethical understandings we already have for drug development, including maintaining confidentiality of patient data. How do we in industry envision the steps to take us from dream to reality? Hypothesis generation is the first step. This means that any genes that could possibly be involved in a drug's response are listed. Response is used here in the broadest sense - genes involved in absorption/distribution/metabolism/excretion pathways, target receptors, genes involved in the drug's mechanism of action. At this stage an initial relationship between a response to drug and a specific genotype(s) would be identified for further investigation. With the availability of SNP maps, individual genes would not have to be identified. Instead it will be possible to look at SNP profiles and compare this to medicine response in order to identify subpopulations of patients who have differing benefit:risk assessments for a given drug. We may not ever need to know the underlying science behind the differences in SNP profiles and how they give rise to different responses to certain medicines. It may be enough to know that there is an association between a given profile and a response to medicine. Further study, of course, could yield valuable information for the development of similar medicines in the drug development pipeline. Once a pharmacogenetic relationship to medicine response is identified in drug development, this needs to be confirmed. Having a certain result in a pharmacogenetic test (or medicine response profile test) could become part of the selection criteria for including patients in confirmatory trials. Pharmacogenetics may allow sponsors to prove efficacy on a much smaller population of patients. For example, if a subgroup of patients were identified who would respond to the medicine being evaluated, the confirmatory trials could be designed recruiting only patients of that pharmacogenetic subgroup. Confirmatory trials would not need to be as large in order to prove efficacy statistically. Seriousness of the disease being treated and whether the medicine response profile measures safety or efficacy responses are important factors that will need to be considered when conducting confirmatory trials. These factors will have important implications on the degree of association between pharmacogenetic finding and medicine response and will have implications on whether other pharmacogenetic subgroups should be included in these clinical trials. Implied, but not directly stated thus far, is that evaluation of a pharmacogenetic hypothesis requires parallel development of a pharmacogenetic in vitro diagnostic test and validation of this test. This aspect will not be discussed in any detail in this talk. If a test is necessary for safe and effective use of a given medicine in the marketplace, inherent in this parallel process is coordinated development and regulatory review.

Terri Arledge
GlaxoSmithKline

Dr. Arledge is the U.S. Department head for the Drug Development Genetics Department at Glaxo Smith Kline. The role of the department is to develop pharmacogenetic strategies for key development programs, interpret pharmacogenetic data and ensure appropriate processes exist for collection of data. Dr. Arledge joined Glaxo as a clinical development research scientist in 1988. She was responsible for clinical development studies and programs for nine years (primarily phase 2-4). The vast majority of her clinical development experience is in GI and respiratory drug products. She then spent 18 months in a position that focused on global strategy for respiratory products before joining the Clinical Genetics Division as a Therapeutic Area Advisory in June 1998. As a Therapeutic Area Advisor, Dr. Arledge was responsible for developing and implementing a pharmacogenetic strategy for the GI/Metabolic products in drug development. At the end of 1999, she assumed the role of US Department Head in Drug Development Genetics. Dr. Arledge received her undergraduate degree in Biological Sciences from North Carolina State University and her DVM (Doctor of Veterinary Medicine) from Tuskegee University. She spent some time in veterinary practice (both food and companion animal practice) before joining the pharmaceutical industry.

Drug interactions: *in vitro* case studies

Brian C. Foster, Office of Science, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada.

There are many classes of phytochemical xenobiotics with claimed health benefits. The relative concentrations of each class within a plant will vary and are governed by genetic, geographic, and environmental factors. Traditional use of most natural health products haveuse of most natural health products has proven safety, but their modern/current pattern of consumption and uses in the global context has changed. Natural health products are generally regarded as safe by many consumers but anecdotal and published reports suggest that they can affect absorption, metabolism, distribution and elimination of drugs and other xenobiotics which enter the body. Problems can occur when drugs and other xenobiotics, which compete for the same active sites are taken together. This can increase or decrease absorption and toxicity, change pharmacologic activity, or shunt the products and their metabolites through other pathways, adversely affecting the safety and efficacy of these compounds. Our primary objective was to establish the potential of various commercial herbal extracts and tinctures (CHETs) to affect the metabolism of human cytochrome P450 isoforms in vitro, and ascertain the risk potential of these products for generating possible adverse interactions with conventional drugs. Serial dilutions of over 120 CHETs and 13 relevant plant biomarker compounds and positive controls were analysed for their ability to inhibit P450 mediated-metabolism in GENTEST Supersomes of a reference substrate using a fluorometric microplate assay. Roughly 95% of the CHETs and 50% of the pure compounds examined have markedly inhibited CYP3A4, CYP3A5 and 3A7 metabolite formation. Many also strongly affected 2C9, 2C19, and 2D6 metabolism. Although most compounds were inhibitory in this system, some like fresh garlic extracts stimulated 2C9*2 metabolism. Within the CHETs examined, goldenseal and St. John's wort, Ginkgo, wild cherry, and cat's claw had the highest inhibitory activity with the four isoforms. Among the pure compounds, naringenin and dillapiol were the most potent inhibitors of CYP3A4 metabolism. High throughput methods can assess the probability of identifying the likelihood of interactions occurring between CHETs and conventional drugs metabolized by the same isozyme, but additional test substrates are required to ensure that all substrate types are characterized. The safety and efficacy of therapeutic products, particularly those with a narrow therapeutic index, when taken concomitantly with natural health products need to be examined further through clinical studies.

Brian Foster
Health Canada

Dr. Foster is a Senior Science Advisor in the Office of Science, Therapeutic Products Directorate, Health Canada. He received his Ph.D. in Medicinal Chemistry at the University of Alberta in 1981 with Professor R.T. Coutts through research on alternative models for drug interactions and metabolism. Since joining Health Canada, his research was in the areas of dissolution, toxicology and drug metabolism (phenotypic and genotypic). Dr. Foster was a co-investigator in an Inuit population study and led a study to develop specific nucleotide polymorphic tests and sequencing protocols for the detection of crucial mutations in the prion gene associated with Creutzfeldt-Jakob disease. His current research interest is in the area of drug disposition and how this effects the safety and efficacy of conventional therapeutic products. Dr. Foster is also an Adjunct Professor, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa. He has a joint Health Canada - University of Ottawa laboratory in the Faculty of Medicine. He has assembled a multi-disciplinary team with expertise in clinical care of elderly and terminally ill populations, ethnobiology, pharmacogenetics, pharmacokinetics, and physiology to study the effect of herbal and nutraceutical products on drug disposition in HIV/AIDS and elderly populations. He is a founding member of the Canadian Natural-Sourced Medicines Research Network, the Drug and Environmental safety Consortium, the Canadian Network for Herbal Medicine Research, and the University of Ottawa Centre for Research in Biopharmaceuticals. brian_foster@hc-sc.gc.ca

Clinical Drug Interactions: Study Design and Data Analysis

Keith Gallicano, Axelson Biopharma Research Inc., Vancouver, British Columbia, Canada

The design and analysis of clinical drug interactions are important factors to assess the presence or absence of an interaction and are pivotal to provide reliable information in product monographs and for proper labeling. Several factors must be considered in designing drug interactions, such as the study design, subject population (healthy volunteers or patients), route of administration, mechanism of interaction (inhibition, induction or activation), pharmacokinetic and pharmacodynamic characteristics of the interacting drugs, and frequency (single and multiple dose), duration, dose size, timing, and order of co-administration of the regimens. Most published studies and studies submitted to regulatory agencies are either a 2-period crossover or longitudinal (fixed-sequence) design, which is appropriate to determine if an interaction is absent or present. However, extended longitudinal studies of more than 2 periods may be required for drugs such as ritonavir that produce complex time-dependent interactions or for inhibitory metabolites that have longer half-lives than the parent drug. A switchback longitudinal design where the washout phase is investigated after stopping an inhibitor or inducer is useful to determine the residual effects of an interaction. Longitudinal parallel designs that incorporate a test group and a reference group (split-plot) are increasing being used in patient studies to account for time changes from baseline in long duration studies. The currently accepted bioequivalence approach (i.e., the inclusion of the 90% confidence limits for the ratio/difference of the means or medians within some prespecified equivalence range) is recommended for data analysis. The range of clinically acceptable variation defines the equivalence range (clinical no-effect boundary), which is ± 20% by default but may be wider or narrower depending on the patient population, and the therapeutic index and pharmacokinetic variability of the affected drug. No dose adjustment is required if the confidence limits fall within the no-effect boundary, and the boundary doses not have to be symmetrical around the mean difference on the raw and logarithmic scales. This presentation will highlight the differences in the bioequivalence approaches to assessing drug interactions and provide examples of how the interpretation of the magnitude of a drug interaction differs with different designs.

Keith Gallicano

Axelson Biopharma Research and CroMedica Prime

Dr. Gallicano is Vice President of Axelson Biopharma Research, a small bioanalytical and pharmacokinetic firm located in Vancouver, B.C., and Scientific Director of CroMedica Prime, a phase 1 clinical unit located at Vancouver General Hospital. He received his Ph.D. in chemistry from the University of British Columbia (UBC) in 1980. Shortly thereafter he completed an industrial research fellowship investigating bioanalytical methods for the isolation, identification and quantitation of drugs in race horses. In 1986 he joined the Royal Canadian Mounted Police (RCMP) and trained as a Forensic Chemist, specializing in the analysis and comparison of materials, such as petroleum, paint, glass, building products, headlamps and explosives, from scenes of crime. From 1988 to 1997 Dr. Gallicano was a Research Scientist in the former Bureau of Drug Research, Health Protection Branch (HPB), Ottawa, where he pursued his interests in development and validation of bioanalytical assays and in clinical pharmacokinetic studies, particularly those involving drug interactions, of drugs used in HIV therapies. In 1997 he left HPB as a senior Research Scientist and Head of the Biopharmaceutics and Pharmacokinetics Section to join the Clinical Investigation Unit, Division of Infectious Diseases, Ottawa General Hospital as a clinical research scientist and the University of Ottawa as an Assistant Professor of Medicine. He returned to Vancouver in 2000 to take on his current positions. Dr. Gallicano has co-authored more than 55 publications, including research papers, reviews, and book chapters. He is a member of the Editorial Board of the *Journal of Chromatography* and the *British Journal of Clinical Pharmacology*, and an external reviewer for several Canadian granting agencies. Dr. Gallicano has given numerous invited lectures on pharmacokinetic and pharmacostatistical aspects of drug interactions, as well as chairing or co-chairing international meetings on this topic.

TPD clinical trial regulation update.

Christine Nestruck, Clinical Trials and Special Access Programme, Therapeutic Products Directorate, Ottawa, Canada

Christine Nestruck
Clinical Trials and Special Access Programme, Therapeutic Products Directorate

Dr. Nestruck obtained her M.Sc. in Physiology from the University of Western Ontario and her Ph.D. in Veterinary Clinical Studies from the University of Saskatchewan. Her post-graduate experience includes a post-doctoral fellowship in Biochemistry at McGill University, the position of chercheur boursier at the Institut de Recherches Cliniques de Montréal, research assistant at the Max Planck Institutes and University Hospital Goettingen in Germany, and several years as senior scientist with the Atherosclerosis and Hyperlipidemia Research Group at the IRCM. Dr. Nestruck spent several years in the pharmaceutical industry as Manager Clinical Research with Boehringer Mannheim Canada and then Director Clinical Research with BioChem Pharma and Biochem Vaccines. Dr. Nestruck joined the Clinical Trials and Special Access Programme as a reviewer of Investigational New Drug Submissions in 1997 on a temporary basis and rejoined in August 1998. She was Acting Manager from July 1999 - March 2000.

Eric Ormsby
Risk Management Methods, Office of Science, Health Canada, Ottawa, Ontario, Canada

Eric has worked in Health Canada for over 20 years, almost entirely in the Therapeutic Products Programme. Educated at the University of Guelph, he received an undergraduate degree in quantitative genetics, followed by a Masters in statistics. He is currently the acting manager of the Office of Science. His areas of expertise are statistical aspects of bioequivalence studies, method validation and laboratory experimental design and risk management with related interests in Quality Management and Information Management.

Keith Bailey
Nepean/Ottawa, Ontario, Canada

Dr Bailey is a private citizen with thirty years' experience of public service in scientific research, policy development, and executive management at Health Canada. His formal education was at St Catherine's College Oxford, in the Honour School of Natural Science- Chemistry: B.A., 1963; D. Phil., 1965. Following two years of post-doc teaching and research at Oxford and two years at Trent University, he joined Health Canada as a Research Scientist. In the then Food and Drugs Directorate, he was first engaged by the research laboratories in the synthesis, physicochemical characterization and pharmacological (QSAR) and forensic assessment of classical and novel psychotropic substances (cannabinoids, LSD analogues, amphetamine and phencyclidine analogues, etc.). He became Chief of the Drug Identification Division and subsequently the Drug Toxicology Division before appointment as Bureau Director, Drug Research Laboratories (renamed The Bureau of Drug Research) in 1984. He published over 50 original articles in scientific journals and presented over 100 reports at scientific conferences during a twenty year active research career. He was Director, Bureau of Drug Research (100 laboratory personnel) from 1984 -1994 and Director of the Bureau of Biologics and Radiopharmaceuticals (120 laboratory and drug submission review personnel, responsible for all biological, biotechnological and radiopharmaceutical drug approvals in Canada) from 1994 -1999. As a senior manager with the Therapeutic Products Programme (TPP), Dr Bailey was intimately involved in developing TPP's policy course and Programme strategy, and effecting policies at the Bureau and Programme level. He chaired several key committees at Programme (e.g., TPP Policy Committee) and Health Protection Branch (e.g., HPB Biotechnology Committee) level. His recent focus as Director, Bureau of Biologics and Radiopharmaceuticals, was particularly on life sciences and the impact of the rapidly-advancing areas of molecular genetics, blood safety, and xenotransplantation on regulatory science and risk/benefit assessment. He also applies his time to community services, choral singing, musical theatre, and organic gardening.

Bioequivalence: Therapeutic Products Directorate Perspective

Norman J. Pound, Division of Biopharmaceutics Evaluation, Therapeutic Products Directorate, Health Products and Foods Branch, Ottawa, Ontario, Canada

The Division of Biopharmaceutics Evaluation, is responsible for the regulation and submission review for drugs which rely on comparative bioavailability (bioequivalence) studies as evidence of their safety and efficacy. This presentation describes the organization of the Therapeutic Products Directorate and the regulation of subsequent-entry (generic) drugs in Canada. Current comparative bioavailability and bioequivalence guidance's are presented; together with an overview of emerging issues.

Norman Pound

Division of Biopharmaceutics Evaluation, Therapeutic Products Directorate, Health Products and Foods Branch, Health Canada

Dr. Pound is Policy Advisor, Division of Biopharmaceutics Evaluation, Therapeutic Products Directorate, Health Products and Foods Branch, Health Canada. He was Manager of the Division from June 1994 to February, 2001. This Division is responsible for the regulation and submission review for drugs which rely on comparative bioavailability (bioequivalence) studies as evidence of their safety and efficacy. Dr. Pound is a graduate (B.Sc. and M.Sc.) of the College of Pharmacy at the University of Saskatchewan (1966/68). He received his Doctorate in Pharmaceutical Chemistry from the University of Alberta in 1970. He joined the Health Protection Branch, Health Canada, in 1970. Since that time he has held a number of positions, initially as a research scientist, then as an officer in the Field Operations and Drugs Directorates. This work primarily involved the analysis and quality control of pharmaceuticals, with particular emphasis on issues related to the Federal/Provincial Drug Quality Assessment Program (QUAD) and liaison with the Provincial Ministries of Health. Prior to becoming the Manager of the Division of Biopharmaceutics Evaluation, Dr. Pound served as Chief, Pharmaceutical Assessment and Cosmetics Division, Bureau of Nonprescription Drugs, Therapeutic Products Programme during which time he was responsible for the chemistry and manufacturing of nonprescription drugs, and the regulation of cosmetics and disinfectants. Dr. Pound is also a member of several professional associations and is currently serving on several international committees involved primarily with regulatory affairs and bioequivalence. norman_pound@hc-sc.gc.ca

Interchangeability: Provincial Government Perspective

Silvia Alessi-Severini, Scientific and Research Services, Clinical Drug Services and Evaluation, Alberta Blue Cross, Edmonton, Alberta, Canada

Under the Canada Health Act, provincial and territorial governments in Canada are constitutionally responsible for the administration and delivery of health care services. As a result, funding for drugs in Canada falls largely under provincial or territorial jurisdiction, as does the designation of interchangeability of subsequent-entry products. The majority of the publicly funded drug programs in Canada impose mandatory substitution (switching) between the innovator and subsequent-entry drug products as well as among subsequent-entry drug products. Substitution may be waived for an individual patient on an exception or special authorization basis if a clinical case can be made by the prescriber to do so. In these instances, it is unlikely that an adverse drug reaction (ADR) report is made. Therefore, our ability (or lack thereof) to measure patient outcomes resulting from imposed substitution or switching between drug products can be questioned. In this presentation, the relationship between the federal and provincial review of subsequent-entry products will be discussed as will the different review processes between the provinces and current harmonization efforts that are underway. Issues of provincial interest will be touched on which will include interchangeability of pharmaceutical alternatives, requirements for non-oral dosage forms and 'old drugs', and the impending CFC to non-CFC transition and phase-out of metered dose inhaler formulations. Interchangeability challenges and prescriber acceptance of interchangeability designations of narrow therapeutic range drugs and drugs with complicated or variable pharmacokinetics will also be featured.

Silvia Alessi-Severini
Scientific and Research Services, Clinical Drug Services and Evaluation, Alberta Blue Cross

Dr. Silvia Alessi-Severini graduated from the University of Parma (Parma, Italy) with a degree in Pharmacy and a degree in Pharmaceutical Chemistry and Technology. As an analytical chemist in the Laboratory Medicine Department at the University Hospital (Parma, Italy), she was responsible for therapeutic drug monitoring and toxicological analyses. In her overseas experience, Dr. Severini also consulted for the pharmaceutical industry in the area of regulatory affairs. In 1993 she received her Ph.D. in Pharmaceutical Sciences from the University of Alberta (Edmonton, Alberta, Canada) where she also completed her post-doctoral training (Department of Pharmacology, Faculty of Medicine) as a fellow of the Alberta Cancer Board. Her research work included the development of enantioselective analytical methods applicable to pharmacokinetic studies and the description of intracellular metabolism and kinetics of antineoplastic agents. Dr. Severini has been a scientific consultant to Alberta Blue Cross since 1996. In this role, she has been involved with the review of hundreds of drug product submissions and has been providing scientific support to the Alberta Health and Wellness Expert Committee on Drug Evaluation and Therapeutics. Dr. Severini is also Adjunct Professor at the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta where she coordinates the undergraduate toxicology course and lectures in the areas of applied pharmacokinetics and evidence-based medicine. She is married and has two daughters.

Bioequivalence and interchangeability: industry perspective

D. Ganes, A. Yacobi, Taro Pharmaceuticals Inc., Bramalea, Ontario, Canada

Interchangeability is the domain of state and provincial governments and formularies, hospitals, and third-party payers. Generic substitution of narrow therapeutic index (range) drugs, such as warfarin, has been vigorously debated. Generic warfarin sodium tablets which are bioequivalent and therapeutically equivalent with Coumadin®, were approved by the FDA in 1997 (Barr, Geneva) and 1999 (Taro), and by the Therapeutic Products Directorate (TPD) in 2000 (Taro, Apotex). Generic warfarin represents 19% of the $453 million $US (yr 2000) market for warfarin. In Canada, the warfarin market is $43 million $CD (yr 2000). DuPont waged an intense campaign against FDA approval, and interchangeability of generic warfarin and Coumadin®. In 1997, the FDA concluded that DuPont had presented false and misleading information, resulting in misbranding of Coumadin®. Taro conducted three bioequivalence studies in 92 healthy subjects to support its ANDA. Taro conducted two single dose fasting crossover bioequivalence studies, at the 2 and 10 mg strengths. Taro's warfarin tablets meets the FDA's bioequivalence requirements, and are bioequivalent to Coumadin®. Taro conducted a single dose fasting four period replicate study at the 5 mg strength in order to demonstrate bioequivalence, switchability and interchangeability of Taro's warfarin with Coumadin® (Yacobi *et al*, 2000). This study indicated that the products were bioequivalent, performed similarly in individual subjects (no subject by formulation interaction), and demonstrated within-subject variability similar to Coumadin®. Therefore, Taro's warfarin tablets are switchable, and interchangeable, with Coumadin®. Since approval, an estimated 40,000 patients have switched to Taro's warfarin tablets, and there have been no reports of adverse events from such switches. Internationally, TPD has implemented the most stringent bioequivalence requirements for narrow therapeutic range drugs. Taro conducted four bioequivalence studies in 87 healthy subjects to support its ANDS. Four single dose crossover bioequivalence studies were conducted at the lowest (1 mg) and highest strengths (10 mg), and in the fed and fasting state. Taro's warfarin tablets meet the TPD's stringent bioequivalence requirements, and are bioequivalent to Coumadin®.

Derek Ganes
Taro Pharmaceuticals Inc.

Dr. Ganes received his B.S.P. (1980), M.Sc. in drug metabolism (1983) and Ph.D. in drug metabolism and pharmacokinetics (1987) from the University of Saskatchewan. He completed a postdoctoral research fellowship in pharmacokinetics (1989) at the University of Michigan. He joined American Cyanamid Company (1989), as a Senior Scientist in Clinical Pharmacokinetics, Pharmacodynamics Research. He contributed to the development of new drugs in the infectious disease, cardiovascular, oncology and CNS therapeutic areas. He joined Biovail Corporation International (1994) as Director, Biopharmaceutics, Contract Research, and conducted bioequivalence studies for Biovail and other pharmaceutical companies. He joined Taro Pharmaceuticals Inc. as Director, Regulatory Affairs, Canada (1996), and was promoted to Vice President, Regulatory Affairs, North America (1997). He oversees drug product registration in the Canadian, U.S., European and international markets, formulary submissions, and is involved with new drug development. Dr. Ganes has authored 15 articles in peer reviewed journals. His broad experience includes bioanalysis, toxicokinetics, clinical pharmacokinetics and pharmacodynamics, population modeling, bioequivalence and drug product registration.

Scientific Basis for Biowaivers Based on BCS

Ajaz S. Hussain, Office of Pharmaceutical Science, CDER, FDA, Rockville, Maryland, USA

The FDA/CDER's BCS guidance document provides a means for justifying biowaivers for *rapidly dissolving* products (85% in 30 minutes in 900 ml of 0.1 N HCl, 4.5 pH and 6.8 pH media, in USP I or II) of *highly soluble* (highest dose strength soluble in 250 ml in pH 1-7.5 range) and *highly permeable* (extent of absorption equal to, or greater than 90%) drugs that are not considered to exhibit a *Narrow Therapeutic Range*. The *high permeability* plus *high solubility* attributes are utilized in this guidance to minimize risk of bio-in-equivalence due to solubility or permeability limited absorption processes. Low permeability drugs exhibit incomplete absorption from small intestine and, therefore, small intestinal residence time of these drugs (dissolved) is considered critical. Relatively minor differences in the time needed for complete *in vivo* dissolution of low permeability drugs can potentially reduce the time available for their absorption during small intestinal transit. The *rapid dissolution* (in three different pH conditions) and built-in profile similarity criteria are to ensures that dissolution *in vivo* is not likely to be rate limiting and minimal differences in product disintegration time (to minimize the likelihood of differences in gastric emptying) are observed between the pre- and post change products. The multi-media dissolution is recommended to account for observed physiologic (and pathologic) variability in gastric fluid pH and gastric emptying process. Since the time of drug administration (during bioequivalence studies and use by patients) is not synchronized (and should not be for practical reasons) with the gastric motility pattern, gastric emptying in some subjects could occur almost immediately after administration. In such cases dissolution would occur in small intestine following emptying. If one of the two products being compared exhibits a different dissolution rate (compared to the other product) in intestinal pH then the effect of variable gastric emptying on *in vivo* dissolution may not be a truly random phenomenon. This may increase the likelihood of bio-in-equivalence when only a single *in vitro* dissolution condition (e.g., 0.1 N HCl) is used to compare two products. The multi-media dissolution criteria are intended to minimize this possibility. The permeability attribute of drugs plays a significant role in the request for biowaivers. In addition to reasons stated above, permeability contributes to the development of "sink condition" for *in vivo* drug dissolution. Drug dissolution *in vitro* in a relatively large volume, 900 ml, is likely to be a better emulation of *in vivo* dissolution process of a highly permeable drug. High permeability attribute also reduces the probability of excipient affecting bioavailability due to an effect on gastrointestinal membranes and/or motility.

Ajaz Hussain

Office of Testing and Research, CDER, United States Food and Drug Administration

Dr. Ajaz Hussain is the Director Office of Testing and Research, CDER, FDA. He currently on assignment as Acting Deputy Director of the Office of Pharmaceutical Science in CDER. He is the Chairperson for the FDA's SUPAC-IR (Revision) and the Biopharmaceutics Classification Working Groups, and as the Technical Director of the Product Quality Research Institute (PQRI). He holds adjunct faculty appointments at the Purdue University and University of Maryland and is a Fellow of AAPS. Prior to joining the FDA he served as Associate Professor of Pharmaceutics at the University of Cincinnati. He trained at the Bombay College of Pharmacy**,** University of Bombay (B.Pharm.) and received his Ph.D. in biopharmaceutics from the University of Cincinnati. His personal research interests include, design and evaluation of controlled release oral and transdermal drug delivery systems, computer-aided formulation design, and application of pattern recognition tools such as artificial neural networks to solve complex pharmaceutical problems.

Post-Marketing Changes and Approval: Industry Perspective

Anne Tomalin, CanReg Inc., Hamilton, Ontario, Canada

Changes to formulations, packages and sourcing are common occurrences in the pharmaceutical industry. This presentation will discuss the impact that these changes have on the commercialization of the product in Canada and will particularly focus on those changes that require bioequivalence. Dissolution profiles will be discussed for solid oral dosage forms. The US Scale-Up and Post Approval Changes (SUPAC) Guidelines will also be discussed. Dosage forms other than solid oral dosage forms, e.g., suppositories, liquids and parenterals will also be reviewed. Regulatory nuances related to New Drug/Old Drug requirements, and related provincial issues will be touched upon.

Anne Tomalin
CanReg Inc.

Anne Tomalin, B.A., B.Sc., President of CanReg Inc., has practised exclusively in the area of regulatory affairs in Canada since 1971. Over that time, she has worked with three international pharmaceutical companies to obtain registrations for prescription pharmaceuticals, OTCs and medical devices. Anne founded CanReg Inc., a regulatory affairs consulting company, in September 1996. Four and a half years later, CanReg has 45 full time employees, and is one of the most successful regulatory consulting firms in Canada. Prior to founding CanReg, Anne was employed for 20 years with Searle Canada, A Unit of Monsanto Canada Inc., as a Business Unit Director. Responsibilities in the last several years at Searle included regulatory affairs, provincial government, reimbursement strategies, managed care, customer interface, legal and information services. Prior to joining Searle, Anne was employed by Hoffmann-LaRoche Limited for three years, and prior to Roche, Anne was employed by Wyeth Ltd.

Compendial Issues for Biotechnology Products.

Roger L. Williams, United States Pharmacopeia, Rockville, Maryland, USA

Complex active ingredients include macromolecules and mixtures that are either naturally sourced or that are produced by means of recombinant technology. Assuring the safety, efficacy, and quality of complex drug substances poses special challenges for pharmaceutical manufacturers, regulatory agencies, and pharmacopeias. Characterization and setting specifications are difficult and are closely related to safety and efficacy. A spectrum of moieties exist that range from relatively small moieties capable of reasonably complete characterization to extremely complicated substances where characterization is difficult or impossible. The more challenging the characterization for the ingredient, the more the 'process becomes the product.' Fortunately, advances in analytical methods and a better understanding of the concepts underlying equivalence determinations have allowed, for pioneer manufacturers, the opportunity to assure equivalence in the presence of change and, for multisource manufacturers, the possibility of creating pharmaceutical products that can be shown to be duplicates of a corresponding pioneer product. In considering the impact of change on a pharmaceutical product containing complex active ingredients the focus on equivalence is usually more related to pharmaceutical equivalence rather than bioequivalence, given that many of the dosage forms are solutions. To study pharmaceutical equivalence in the presence of change, key issues relate to identification and strength, i.e., is the complex active ingredient the 'same' and does it have the same strength (potency) pre- and post-change. Issues of identification and strength are both regulatory and compendial issues, and both devolve on a good understanding of the science/technical challenge of assuring sameness. Depending on the ingredient and the degree of change, a range of tests, procedures and acceptance bounds may be needed to assure that the complex active ingredient is the same in the presence of a one or more changes, to include changes that arise with generic substitution. Necessarily, the assurance of equivalence relies on a comparison of data, using specified goalposts and pre-determined criterion for the comparison. At this time, comparisons of means without explicit consideration of variance are the usual practice. The presentation will consider variance terms that arise in a 'switchability' setting where reliance on clinical trial methods (PK, PD, clinical studies) may be used to determine pharmaceutical equivalence. A conceptual understanding will be presented for the basis of a 'molecule by subject' interaction term. Finally, the presentation will consider compendial issues and why a substance/preparation monograph is both useful from a public health perspective but is also insufficient to assure equivalence in the presence of change.

Roger Williams
United States Pharmacopeia

Roger L. Williams, M.D., is Executive Vice President and Chief Executive Officer of the United States Pharmacopeia. Dr. Williams received his B.A. degree at Oberlin College and his medical degree and training in internal medicine at the University of Chicago. He served in the United States Army, both in Korea and at Walter Reed Army Institute of Research, where he conducted anti-malarial drug research. In 1977, he completed a clinical pharmacology training program at the University of California, San Francisco, and became a faculty member at the University, a position he retains. In 1989, he spent a year studying an anti-HIV drug at Genelabs as Vice President for Medical Affairs. He joined the Food and Drug Administration in 1990 as the Director of the Office of Generic Drugs in the Center for Drug Evaluation and Research. In 1993, he moved to an Associate Director position in the Center and in 1995 became Deputy Center Director for Pharmaceutical Science. In this capacity, he had oversight for the Center's Office of New Drug Chemistry, Office of Generic Drugs, Office of Clinical Pharmacology and Biopharmaceutics, and Office of Research and Testing. Many of his efforts at FDA were directed towards the provision of guidance's, based on information generated through applied regulatory research, to assist pharmaceutical sponsors and FDA reviewers in understanding science and technical information needed to support regulatory submissions and decisions. He chaired or co-chaired several CDER coordinating committees and was responsible for the Advisory Committee for Pharmaceutical Science. While at FDA, Dr. Williams led many national and international efforts that promoted stakeholder input into Agency policy. He was responsible for activities that led to the formation of the Product Quality Research Institute, which is a collaborative activity, located in the American Association for Pharmaceutical Sciences, between FDA, the pharmaceutical industry, and academia that will generate research information to support drug development and regulatory policies. Dr. Williams was active in many international activities and was the Agency's lead representative to the International Conference on Harmonization. He also worked actively with the World Health Organization and with the Pan American Health Organization. Dr. Williams is a fellow of AAPS, an expert member of the FIP Board of Pharmaceutical Sciences, and has been a member of the WHO Expert Committee on Pharmaceutical Preparations. He has authored or co-authored over 150 reports in the areas of clinical pharmacology and product quality. Dr. Williams is board-certified in internal medicine and clinical pharmacology and is a member of Phi Beta Kappa and Alpha Omega Alpha.

Characterization of Biotech Products by Mass Spectrometry and Other Methods

David Kwok, BRI Biopharmaceutical Research Inc., Vancouver, British Columbia, Canada

Biotechnology derived drugs products including recombinant therapeutic proteins, monoclonal antibodies or natural protein isolates often require careful considerations in determining the use of appropriate test methods for product and quality control specification testing. In the context of cGMP guidelines, concerns on viral contamination, needs for characterization of the cell substrates and characterization of the expression construct are also subjects of quality control considerations. In the design of product specifications with respect to activity, safety and efficacy of a biotechnology derived drug product, chemical specifications are often analytical method specific. Chemical purity of a biotechnology derived product and purity of reference standards are often challenging to define due to heterogeneities resulting from glycosylation, deamidation or other post-translational modifications of a protein. To meet the needs of product specification testing, a variety of physiochemical, biochemical and immunochemical analytical methodologies are often required and will be reviewed. The utility and application of recent developments in mass spectrometry in providing complimentary and confirmatory data on amino acid sequence, peptide map, glycosylation profile, molecular weight determination and purity assessment will be presented.

David Kwok
BRI Biopharmaceutical Research Inc.

David Kwok is the founder and President of BRI Biopharmaceutical Research Inc., a contract research company established in 1999 with a mission to provide an integrated analytical service to accelerate drug discovery and pharmaceutical product development. BRI routinely provides analytical services in in-vitro ADME screening assays, CMC, formulation development and development/validation of LC/MS/MS methods for biological samples. Dr. Kwok received his Ph.D. in 1991 from the Faculty of Pharmaceutical Sciences at the University of British Columbia. He served in a variety of management and scientific posts as a Research Scientist at the Therapeutic Products Program, Health Canada, involving herbal and botanical quality control method development, antimicrobial drug resistance and veterinary drug residues analytical methodologies.

Delivery Issues with Biopharmaceuticals

Daan J.A Crommelin, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands and OctoPlus B.V. Leiden, The Netherlands

Biopharmaceuticals share a number of (bio)pharmaceutical and pharmacokinetic features. The vast majority of these compounds are administered parenterally, in addition, they are inherently physically and chemically unstable. In this lecture new approaches to administer these high molecular weight actives will be discussed, including: prolonged release formulations ('stick to the needle, but reduce the injection frequency') and approaches using alternative routes of administration ('needle free'). Poly lactic/glycolic acid (PLGA) based microsphere systems for prolonged protein release (hGH) were the first to enter the market. But, second generation microsphere systems, e.g. microspheres based on cross-linked dextrans may offer considerable advantages. Alternative routes of administration are being evaluated at present. Intradermal, needle-free, gas driven delivery devices offer interesting options. Besides, the potential of the pulmonary route for the systemic delivery of therapeutic proteins is evaluated in a series of clinical trials.

Daan Crommelin
Utrecht University

Professor Daan Crommelin is full professor at the Department of Pharmaceutics at Utrecht University. He is also Scientific Director of the Utrecht Institute for Pharmaceutical Sciences (UIPS) and adjunct professor at the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Crommelin is CSO of OctoPlus B.V. a company specialized in drug formulation. His research is focused on formulation of biopharmaceuticals, advanced drug delivery and drug targeting strategies. He published over 260 articles in peer reviewed journals.

Pharmaceutical Equivalence of Biologic Drug Products

Salomon Stavchansky, Alcon Centennial Professor of Pharmaceutics, College of Pharmacy, Division of Pharmaceutics, The University of Texas at Austin, Austin, Texas, USA

The last decade has witnessed unprecedented advances in biological technology leading to new and challenging paradigms in the regulatory approval process of biologic drug products. At the heart of managed care are cost, access and quality of new technologies and products. This paradigm has triggered a series of regulatory initiatives that have catalyzed changes in the regulation of biologics and classical drugs resulting in a faster rate of drug approvals. Historically, different pathways and regulatory centers have been used to approve biologics and classic drug products. Biologics were equated to the manufacturing process. In other words, the sameness of the biological process ensured the biologic product, changing the manufacturing process triggered new efficacy trials. Recently, the concept of comparability protocols has evolved to assure that manufacturing changes do not affect safety and efficacy. Essentially, comparability protocols could reduce the burden of time that companies have to wait before implementing a change. The key question is what constitutes characterization (e.g., impurities and definition of active ingredient) of a biologic product and whether characterization is sufficient to ensure therapeutic equivalence of biologics. Compounded with the advances in technology is the genesis of generic biologics. The question for regulatory agencies is how to establish pharmaceutical and therapeutic equivalence of biologics. Assurance of pharmaceutical equivalence and bioequivalence may not assure efficacy of biologics. Thus, other measures of efficacy such as biomarkers and/or surrogate markers may have to be included in the final equation concerning the regulatory approval of generic biologics. This presentation will provide a brief discussion of potential pharmaceutical equivalence problems of biologic drug products

Salomon Stavchansky

The University of Texas at Austin, College of Pharmacy, Division of Pharmaceutics

Salomon A. Stavchansky, Ph.D., received his B.Sc. degree in Pharmacy from The National Autonomous University of Mexico (*Suma cum laude*) in 1969, and his Ph.D. in Pharmaceutical Sciences from the University of Kentucky at Lexington in 1974. His dissertation work and subsequent postdoctoral work at Sloan-Kettering Institute for Cancer Research involved the synthesis of carbon-II phenytoin with the purpose of following the *in vivo* distribution of phenytoin and the identification of gliomas and neuroblastomas. Dr. Stavchansky worked as an analytical chemist at the National Medicine Center in Mexico where he developed an immunofluorescence antibody diagnostic test for toxoplasmosis. In 1968 he joined the research and development group of Syntex Laboratories. In 1970 Dr. Stavchansky was admitted to the graduate program of the University of Kentucky. Dr. Stavchansky joined the University of Texas in 1974 where he was awarded the Teaching Excellence Award and has received the Alfred and Dorothy Mannino Fellowship in Pharmacy and the James T. Doluisio Centennial Fellowship, and is now holder of the Alcon Centennial Professorship. Dr. Stavchansky is a fellow of the American Association of Pharmaceutical Scientists, Honorary member of the Mexican Pharmaceutical Association, Honorary Member of the United States Pharmacopeial Convention, and has served as advisor and external tutor of The Council of Science and Technology of Mexico. Dr. Stavchansky presently serves as chair of the USP Expert Committee on Bioavailability and Nutrients Absorption He recently served on the Advisory Committee to the Office of Generic Drugs of the Food & Drug Administration and on the Pharmaceutical Science Advisory Committee. Dr. Stavchansky was a member of the review committee on drug delivery systems for The National Institute of Child Human Development and The National Institute on Drug Abuse and is presently an *ad-hoc* reviewer for the National Institute of Health and reviewer for NIH Small Business Grants. Recently, Dr. Stavchansky served in the expert panel of the World Health Organization and the Pan American Health Organization in the area of bioavailability and bioequivalence drugs and biologics. He served as a special advisor to the National Agency of Sanitary Vigilance in Brazil during the drafting of the regulation of generic drug products. Dr. Stavchansky was also a member of the Bioavailability Steering Committee of the American Pharmaceutical Association. He presently serves as Chairman of the Translation Committee of the USP into Spanish. Dr. Stavchansky has directed and participated in research concerning the development of analytical methodology for the measurement of drugs in biological fluids, the absorption, metabolism, distribution, and elimination of drugs from biological fluids in animals and man, and the evaluation and design of drug delivery systems. This work has resulted in over 100 publications on bioequivalence and drug absorption, biopharmaceutics, pharmacokinetics, physical pharmacy, and pharmacy education for national and international journals, textbooks and conferences. Dr. Stavchansky has supervised or co-supervised the post-graduate (M.Sc. and Ph.D.) training of several students. Dr. Stavchansky has been an active member of AAPS since its inception and has served as Program Chairman of AAPS and also of the PPDM section of AAPS. In addition, he served as Chairman of The Awards Committee of AAPS and also of PPDM. He has been a member of The Fellows Committee of AAPS and of The Finance Committee of AAPS. Dr. Stavchansky presently serves as Co-Program Chair of the Pharmaceutical Congress of the Americas sponsored by AAPS. Dr. Stavchansky's professional memberships have included New York Academy of Sciences, APhA, AAPS, AAAS, Sigma Xi, Rho Chi, and Beta Alpha Phi International Honor Society.

Comparative Pharmacokinetic Studies in the Development of Biotechnology Drugs

Andrew Storey, Vice-President, Quality, Clinical and Regulatory Affairs, Cangene Corporation, Winnipeg, Manitoba, Canada

Comparative Pharmacokinetic studies can provide useful information to support that a new product is comparable to a similar existing product, thereby supporting that the new product will be safe and effective. Cangene has utilized Pk studies to support licensure of both plasma-derived and recombinant DNA products. The merits, challenges and potential pitfalls of utilizing these types of studies will be discussed.

Andrew Storey

Quality, Clinical and Regulatory Affairs, Cangene Corporation

Andrew Storey is responsible for Cangene's Clinical Development Projects, obtaining licensure of products globally and the assurance of Product Quality. He is responsible for all QA, Clinical and Regulatory activities for the company's products, including hyperimmune and biotechnology drugs. Andrew has been with Cangene for 15 years and is currently focused on drug development, involving 16 clinical trials for 7 of Cangene's commercial or developmental products. Cangene has utilized approaches such as Bioequivalence and Comparative Pharmacokinetic studies as tool to support product licensure.

The Essential Non-Comparability of Innovator and Second-Source Biotechnology Products: Interferons as a Test Case

James N. Bausch, Director of Protein Analysis, Schering-Plough Corporation, Kenilworth, New Jersey, USA

Biotechnology pharmaceuticals, such as Interferons, are complex pleiotripic molecules whose origins begin with the human version of the cytokine. During their development they have been re-engineered at the DNA level to satisfy the needs of high-level production in foreign hosts. The biochemical and biophysical characteristics of these molecules is therefore inextricably intertwined with the processes of production and purification. The multi-function nature of the Interferons necessitates that their activities be tested across many biological systems when any alterations (i.e., source change, process alteration) are proposed. Although hi-tech, the analytical testing for these products can not provide absolute assurance that the tertiary structure for a second source Interferon is identical to the innovator product. Additionally, the tests can not assure the complete clearance or even identical profiles for low-level host cell impurities. Based upon immunogenicity and efficacy concerns, all second-source Interferons should be tested in full clinical trials to insure the safety and proper pharmacokinetic action of these life-savings drugs.

James Bausch
Schering-Plough Corporation

Dr. Bausch is the Director of Protein Analysis in the department of Analytical Development at Schering-Plough Corporation in Kenilworth, New Jersey. He received his Ph.D. in 1977 from Rutgers University in New Brunswick, New Jersey in the field of Protein Chemistry. His graduate work centered on lectin-protein chemistry and lectin interaction with cell surfaces. He participated in two postdoctoral fellowships in the areas of Molecular Virology and Recombinant DNA Technology at Columbia University, NYC. He joined Schering Corporation as a Senior Scientist in 1982 and has held increasing levels of responsibility. He was promoted to his current position in 2000. Dr. Bausch has 28 employees under his supervision including 8 PhDs. Dr. Bausch's group at Schering Corporation is involved in the development of pharmaceutically active protein products and viral vectors which are derived from recombinant DNA technology. Dr. Bausch and members of his group have published several papers relating to the chemical analysis of these recombinant proteins.

A Canadian Regulatory Perspective on Biocomparability

Anthony A.G. Ridgway, Manager, Biotherapeutics Division, Bureau of Biologics & Radiopharmaceuticals, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada

Demonstration of biocomparability is important when significant changes are introduced into a manufacturing process for a biological drug and, if successful, may relieve the manufacturer of the need to repeat preclinical or clinical studies. It is heavily influenced by the ability to manufacture proteins to high levels of purity and the capability of modern analytical techniques to thoroughly characterize them. How much characterization abrogates how much human testing and what are some of the prominent clinical issues? The Canadian regulatory perspective will be presented along with some consideration of relevant ICH guidance on biotechnology products.

Anthony Ridgway
Biologics and Genetic Therapies Directorate, HPFB

Dr. Anthony Ridgway was born and educated in the U.K. and obtained his B.Sc. and Ph.D. in biology from McGill University, Montréal. From 1982-85 he conducted post-doctoral research at the Cancer Research Laboratory (CRL), University of Western Ontario, London, Canada, and continued as Assistant Professor, CRL and Dept. of Microbiology and Immunology, from 1985-91. From 1989-91 he held a National Health AIDS Research Scholar award. Academic research activities included work on the structure and expression of oncogenes, retroviral regulatory elements, HIV regulatory genes, and inducible expression vectors. In 1991, Dr. Ridgway joined the Health Protection Branch of Health Canada as Head of the Biotechnology Section in the Bureau of Biologics and Radiopharmaceuticals (BBR). He is currently Manager of the Biotherapeutics Division, BBR, whose responsibilities include product testing, bioassay research and regulation of biopharmaceuticals including: cell and gene therapies, hormones, cytokines and monoclonal antibodies. He has been active with ICH* since 1993, serving on Expert Working Groups addressing the quality of biotechnology products. In June 2000, he was elected to the US Pharmacopoeia Committee of Experts on Gene Therapy, Cell Therapy and Tissue Engineering. (*International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use)

Michael Spino
Apotex Inc.

Dr. Michael Spino is the Senior Vice President, Scientific Affairs, for Apotex Inc., where he is responsible for overseeing the research and development of both generic and innovative drugs at Apotex. Dr. Spino serves as the Chairman of the Scientific Advisory Committees for both the Canadian Drug Manufacturer's Association and the International Generic Pharmaceutical Alliance. He is also a Professor in the Faculty of Pharmacy, University of Toronto where his research is focussed on drug disposition and biotransformation. He received his B.Sc.Phm. degree from the University of Toronto in 1967 and his Pharm.D. degree from the University of California, San Francisco Medical Center, followed by a Post Doctoral Research Fellowship in Clinical Pharmacology in Toronto. Dr. Spino has served on several Expert Advisory Committees to the Canadian Health Protection Branch, including, the EAC on Bioequivalence, which developed the Guidelines that serve as the basis of the current Regulatory requirements for generic drugs in Canada. Today Dr. Spino's research is based on the pharmacokinetics, biotransformation, and efficacy of both currently marketed drugs and investigational new drugs.

Bioequivalence: current concerns and issues

Jake J. Thiessen, Faculty of Pharmacy, University of Toronto, Ontario, Canada

Earlier this year, the Therapeutic Products Programme (TPP) re-established the Expert Advisory Committee (EAC) to act as a forum of advice and a sounding board for management and scientists of the TPP. The EAC carries a broad mandate that encompasses guidance's for new drug submissions and abbreviated new drug submissions. Bioavailability and bioequivalence (B&B) is an intersecting region for three primary sectors: the research community of scientists and statisticians, the pharmaceutical industry, and regulatory agencies. While B&B is a comparatively mature scientific and statistical field, new twists and even controversies emerge, as seen by the issue of individual bioequivalence. Concerns about competitive products in patients have lead to particular interest in special populations that include gender and age. The TPP has two official B&B guidelines (Part A: Oral Dosage Formulations Used for Systemic Effects; Part B: Oral Modified Release Formulations). Yet, while various recommendations for approving "unusual" products have been proposed by a previous iteration of the EAC, these remain unofficial, despite the fact they appear to be the basis for many TPP decisions. The challenge is to revisit the fundamentals of Report C and assist the TPP in completing its official portfolio of B&B guidelines. There may also be room to re-examine the guidelines found in Reports A and B. The extrapolation of B&B to product selection and interchangeability still remains an issue in various Canadian jurisdictions. A more effective method needs to be implemented so that that reimbursement agencies and clinicians are more confident with the bioequivalence designation and its role in product substitution. B&B information in product monographs is another aspect that warrants attention. Finally, it is important that Canadian regulators, the industry and professionals harmonize in their acceptance of B&B, especially in the light of the challenges to be faced with the emerging biotechnology products.

Jake Thiessen

Faculty of Pharmacy, University of Toronto

Dr. Thiessen is a professor and the associate dean at the Faculty of Pharmacy, University of Toronto, Canada. He is a pharmacist who earned his professional degree from the University of Manitoba. Thereafter he obtained graduate degrees from the University of Manitoba (1969) and the University of California, San Francisco (1974). In his doctoral studies he specialized in pharmacokinetics. His independent and collaborative investigations have focused on theoretical pharmacokinetic concepts and practical clinical pharmacotherapy concerns. His research interests in recent years have been directed to new approaches in cancer treatment. Furthermore, he has been working on new molecules to treat patients with iron overload. Dr. Thiessen is recognized for his understanding and experience in the theoretical and practical issues of bioequivalence. His extra-university responsibilities have included an appointment to the Ontario Ministry of Health's Drug Quality and Therapeutics Committee and the1990 Pharmaceutical Inquiry of Ontario. In 2001, he was appointed as Chair of the new Expert Advisory Committee on Bioavailability and Bioequivalence, which serves the Therapeutic Products Programme (Health Canada).

Complicated cases. Need for revised criteria?

Fakhreddin Jamali, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

The principles of bioequivalence have been well accepted as a method to establish therapeutic equivalence and assure the safety of products interchangeability. While, in general, there is no controversy as to the success of this approach, the use of such techniques may require further examination when applied to particular types of drugs or products. For example, for drugs with long $t_{1/2,}$ the requirement to follow plasma concentration for a certain number of $t_{1/2}$ may prove impractical and, in fact, unnecessary. It has been shown that data generated from a certain segment of the area under plasma concentration-time curve may provide the necessary information with accuracy and certainty. The determination of bioequivalence of some types of modified release formulations also requires reconsideration. Modified release formulations are often intended for chronic therapy and hence, the steady-state concentration is the parameter considered most important in the determination of bioequivalence. According to the present guidelines, bioequivalence is achieved if only Cmax and AUC values estimated for both products are in agreement, i.e., the rate of absorption and the shape of the plasma concentration-time curve do not have to be similar. In addition, Cmax is assumed to be a reflection of Tmax, and indeed, a better estimate of the rate of absorption. Both these assumptions, although valid in certain conditions, cannot be generalized for the entire category of modified release formulations. For certain drugs, e.g., some cardiovascular agents, even under the steady-state conditions, the rate of release, hence absorption, is important and from the therapeutic viewpoint, the specific timing of the peak concentration may influence the therapeutic outcome. In addition, Cmax does not always reflect the rate of absorption, e.g., timed-release formulations. In addition to the total AUC and Cmax, a comparison of AUC segments during the absorption phase may be found useful in these cases.

Fakhreddin Jamali
University of Alberta, CSPS

Dr. Jamali is a professor and the associate dean at the Faculty of Pharmacy and Pharm. Sci. of University of Alberta. He received his Doctor of Pharmacy in 1969 from University of Tehran, Iran followed by an MSc (1973) in pharmaceutics and a PhD (1976) in pharmacokinetics from University of British Columbia, Vancouver, Canada. He joined the faculty at the University of Tehran (1976-81) and then in 1981 moved to the University of Alberta. His research interests include effect of pathophysiological changes on the action and disposition of drugs, stereochemical aspects of drugs action and disposition, basic and clinical pharmacology of anti-rheumatic, analgesic and cardiovascular drugs, and toxicology of nonsteroidal anti-inflammatory drugs. He has published more than 160 refereed articles and has been an invited speaker at many conferences, and has trained over 20 PhDs. He is a Theme Leader in the Canadian Arthritis Network (Networks of Centres of Excellence), is a Fellow of American Association Pharmaceutical Science and American College of Clinical Pharmacology, and for his research achievements, he has received the McKeen Cattel Memorial Award of the American College of Clinical Pharmacology, the McCalla Professorship of the University of Alberta and the McNeil Award of Assoc Fac Pharm Canada. Dr. Jamali has served as a consultant to many pharmaceutical houses and is a member of the Health Canada's TPD Expert Advisory Committee on Bioavailability and Bioequivalence. He is the founding president of Canadian Soc. Pharm. Sci. and editor of J. Pharm. & Pharm. Sci. (www.ualberta.ca/~csps), and has served in the editorial board of J. Clin. Pharmacol., Chirality and Am. J. Therapeutics and AAPS PharmSci. He teaches pharmacokinetics and is involved in pharmacy curriculum development. http://www.ualberta.ca/~pharmc/jamali.htm

Bioequivalence of Narrow Therapeutic Range Drugs

Scott E. Walker, Coordinator, Research and Quality Control, Pharmacy, Sunnybrook & Women's College Health Sciences Centre; and Associate Professor, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

The Canadian regulatory authority currently defines a narrow therapeutic range drug as one that commonly exhibits adverse effects which limit the therapeutic use in doses close to those required for therapeutic effect. For these drugs, the ratio of the lowest concentration at which toxicity commonly occurs to the median concentration providing therapeutic effect is not greater than 2. Drugs in this category include theophylline, warfarin, carbamazepine and valproic acid. The current Canadian criteria for a narrow therapeutic range product requires that the 95% confidence interval (CI) of the test-to reference ratio ($\mu T/\mu R$) of AUC_{0-t}, and C_{max} fall completely within the 80-125% boundary before the generic is considered bioequivalent. These criteria generate at least two issues for NTI products. Under the current criteria, most generic products gaining approval in Canada today must generally have a relative bioavailability ($\mu T/\mu R$) which differs by no more ~7.5%. However, since the intra-subject CV for most NTI drugs is smaller than average, the $\mu T/\mu R$ may deviate by a greater degree and the product could still be judged as bioequivalent. For products with an intra-subject CV(%) of ~10% and a sample size of 24 in a 2x2 cross-over study, deviations could approach 15%, even with a 95% CI. The second concern occurs when more than one generic product exists on the market. Although both must be bioequivalence to the reference formulation, they may not be bioequivalent to each other. Therefore, a patient who was switched from one generic to the second generic may absorb a significantly different amount of the drug from the second generic formulation. At least two potential solutions have been proposed. The first would limit the allowable deviation in $\mu T/\mu R$ ratio to 5%. Scaling is the second, where the width of the confidence interval is scaled to the observed intra-subject variability. While these two methods are very different in their approach, for drugs with intra-subject variability close to 10%, the effect is similar. Although limiting the allowable deviation in the $\mu T/\mu R$ ratio is simpler, scaling can be applied to all types of drugs, including variable drugs, and so is more generalizable.

Scott Walker

Sunnybrook and Women's College Health Sciences Centre

Scott Walker is the Coordinator of Research & Quality Control in the Department of Pharmacy at Sunnybrook & Women's College Health Sciences Centre, a University of Toronto affiliated Teaching hospital in Toronto. Scott is also cross-appointed as an Associate Professor in the Faculty of Pharmacy at the University of Toronto where he teaches an undergraduate course in drug analysis. Scott received his undergraduate degree in pharmacy in 1977 from the University of Toronto and then completed a Residency in Hospital Pharmacy before completing his Masters in pharmacy at the University of Toronto. His research interests include drug stability in support of the IV-additive program within Sunnybrook hospital as well as the pharmacokinetics of drugs which has resulted in the evaluation of many drug-drug and drug-food interactions. Scott has more than 80 refereed publications and has been an invited speaker at many conferences. He is a fellow of the Canadian Society of Hospital Pharmacists and has received more than 30 awards from this group in their national awards competitions. Scott has served as a consultant to many pharmaceutical firms and has been the editor of the Canadian Journal of Hospital Pharmacy for the past 10 years.

Bioequivalence Measures and Parameters Criteria for Comparisons, IBE & ABE.

John W. Hubbard, Maureen J. Rawson, Gordon McKay and Kamal K. Midha, PharmaLytics Inc., A Drug Metabolism, Drug Disposition Institute, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

At present, bioequivalence (BE) testing in North America is based on average bioequivalence (ABE) in which the (1-2a) confidence interval on the geometric mean ratio (GMR) of the test and reference formulations is required to fit entirely within preset BE limits. A concern has been expressed that ABE is based on average rather than individual bioavailabilities and does not address the within-subject variabilities of the test (Swt) and reference (Swr) formulations. The concept of Individual bioequivalence (IBE) does, however, address the question of individual BE ratios in a manner that has statistical validity. Moreover, IBE based on replicate designs also assesses the comparability of formulations in terms of pharmaceutical quality such that the test formulation is "penalized" if Swt>Swr or "rewarded" if Swt<Swr. Another factor is the possible presence of subject by formulation interaction (Sd) which tends to make IBE more conservative. One problem, however, is the unpredictability of the aggregate IBE metric composed of means and variance terms when applied to real data rather than simulations. For example, in situations where Swt<<Swr, it is possible for the maximum GMR for BE to rise to unacceptably high levels such that the IBE metric becomes very liberal. However, The FDA Guidance of October 2000 requires that the GMR fall between 80-125%. In this presentation, we shall explore the concept of partial areas under the plasma concentration versus time curve (AUCt) and applicability to ABE and IBE. The latter will be explored in terms of (i) upper bounds of the Hyslop confidence intervals, (ii) Swt, Swr, Sd and (iii), maximum GMR for IBE. These treatments will include the issue of "early exposure" which refers to the partial area of AUC up to the median tmax of the reference formulation.

John Hubbard
University of Saskatchewan

Dr. John W. Hubbard graduated from the School of Pharmacy, University of London, England with a BPharm degree in 1962 after which he spent two years in professional practice as a community pharmacist. In 1964, Hubbard returned to academia as a graduate student under the supervision of Dr. Brian Jacques and, in 1968, was awarded a PhD degree in the College of Non Clinical Medicine, University of London, England. Hubbard then undertook a period of post doctoral studies under the direction of Dr. Ronald T. Coutts in the Faculty of Pharmacy and Pharmaceutical Sciences at the University of Alberta. In 1970, he joined the Faculty of Pharmacy, University of Manitoba as an assistant professor teaching Medicinal Chemistry and Analytical Chemistry and began researching in the area of psychotropic drugs. In 1982, a Development Grant from Medical Research Council of Canada permitted Hubbard to move to the College of Pharmacy, University of Saskatchewan, to join the Drug Metabolism Drug, Disposition Research Group (DMDD) headed by Dr. Kamal K. Midha. The DMDD was subsequently awarded a Program Grant from Medical Research Council of Canada entitled 'Towards the More Efficacious Use of Psychotropic Drugs.' A second area of research interests lies in issues of bioequivalence. Hubbard also acts as a Quality Assurance consultant for PharmaLytics Inc, a Drug Metabolism, Drug Disposition Institute. Hubbard has co-authored more than 120 publications in reviewed journals and also has numerous published abstracts and research presentations.

Measures for the Determination of Bioequivalence: Exposure, Absorption Rate, and Curve Shape

Laszlo Endrenyi, Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

Questions about the principal goal of bioequivalence (BE) studies remain unresolved. It has been suggested that these investigations should serve as either (A) therapeutic surrogates or (B) pharmaceutical quality controls. However, the two goals call for differing study conditions and requirements. Regulatory expectations often reflect this dichotomy. For instance, measures comparing the early phase of concentration (C)-time (t) profiles of two formulations have been interpreted to reflect the contrast (A) of early exposures and (B) of absorption rates. There is agreement that the peak concentration (C_{max}) (A) is useful as an index of peak exposure but (B) is a very insensitive metric and confounds the extent and rate of absorption. After single drug administrations, other measures are very promising. They include partial *AUC* (partial area under the C-t curve) and, especially, an intercept of the ratio of early C *vs*. t or C/t *vs*. t curves. Calculations and computer simulations demonstrate that the latter measures have particularly high sensitivity for the comparison of early C-t profiles and initial absorption rates. With adjustment for total *AUC*, the confounding effect is also eliminated. The intercept procedures are favorable also following repeated drug administrations especially when accumulation is fairly low. Peak-trough fluctuation, $PTF = (C_{max}-C_{min})/C_{ave}$ and, to a lesser extent, *AUC*-fluctuation are somewhat favored with moderately large fluctuations. Still, it is necessary to determine the BE of C_{max} in the steady state, in spite of its insensitivity, in order to assure the safety of a new drug product.

Laszlo Endrenyi
University of Toronto, CSPS

Dr. Endrenyi is Professor of Pharmacology and Biostatistics at the University of Toronto. He has received his B. Eng. in Budapest and his Ph.D. in Toronto. He has served the university in various positions including its Governing Council and as Associate Dean of graduate studies. Externally, he has served on grant review committees and on editorial boards of research journals including the Amer. J. Physiology, J. Pharm. Sci., J. Pharmacokin. Biopharm., and J. Pharm. & Pharm. Sci. He has served on advisory committees including those of HPB and FDA. He has edited a book and written over 140 research papers. His research interests include pharmacokinetics, biostatistics, the design and evaluation of experiments and of clinical trials, and the analysis of bioavailability and bioequivalence. He has consulted with many companies in these areas.

Beyond Bioequivalence!
Canadian & International Issues in Biopharmaceutics & Pharmacokinetics.

Poster Presentations

Posters will be on display in Ballroom A from 8-5 Friday.
Presenters will be available by their posters during the coffee and lunch break.

1. A REVERSED PHASE HPLC ASSAY FOR LOSARTAN AND ITS ACTIVE METABOLITE EXP3174 IN PLASMA.

Pollen Yeung, **Angela Jamieson, Gareth J Smith, P. Timothy Pollak. Pharmacokinetics and Metabolism Laboratory, College of Pharmacy; Department of Medicine (Clinical Pharmacology), Dalhousie University, Halifax, Nova Scotia, Canada.**

Purpose. To establish an HPLC assay for plasma losartan and its active metabolite EXP3174 **Methods.** The HPLC system consisted of a pump (Beckman model 114M), a 250 x 2 mm i.d. C_{18} reversed phase column (Jupiter®, Phenomenex) preceded by a 4 x 4 mm guard column (Licrocart®, EM Merck), a UV detector set at 254 nm (Shimadzu SPD-6AV), and a Beckman integrator (Model 427). The mobile phase was a mixture of 0.01 M ammonium phosphate: acetonitrile: methanol (6:3:1) containing 0.02% sodium azide and 0.04% TEA, with pH adjusted to 3.2. The system was operated isocratically at ambient temperature at a flow rate of 0.3 mL/min. Losartan and its active metabolite EXP3174 were extracted from plasma using C_2 bonded silica gel standard solid phase extraction. **Results:** Recoveries of losartan and EXP3174 from plasma were greater than 60%. Using 0.5 mL of plasma sample, standard curves were linear from 10 to 300 ng/mL ($r^2 = 0.99$). Sensitivity of the assay was < 10 ng/mL. Intra-and inter-assay variations were < 10 and 15%, respectively. The assay has been successful in measuring plasma concentrations of losartan and EXP3174 in patients receiving daily dose of losartan (50 – 100 mg). **Conclusion.** The HPLC assay has adequate sensitivity, reproducibility, and specificity for clinical and preclinical pharmacokinetic studies.

2. EFFECT OF LOSARTAN ON HEART RATE AND BLOOD PRESSURE RESPONSE TO EXERCISE IN RAT.

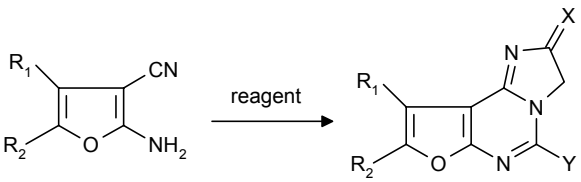
Pollen Yeung, **Agnela Javis and Joe D.Z. Feng. Pharmacokinetics and Metabolism Laboratory, College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada.**

Purpose. To study the Hemodynamic effects of losartan during exercise. **Methods.** Male normotensive SD rats weighing approximately 500g were used for the experiment. Before the study, a polyethylene catheter was implanted into the right carotid artery of each rat under general anaesthesia for hemodynamic recording and blood sample collection. They were allowed to recover (24 h), and randomised into 2 groups (n=6 to 8 each). A blood sample (0.5 mL) was taken from each rat for measurement of plasma catecholamines. One group received losartan (s.c. 10 mg/kg bid for 5 doses), and the other normal saline. Each rat was exercised on a treadmill (7 m/min and 3% slope) for 7 min. BP and HR measurement were recorded throughout the exercise and up to 1 h after the exercise. Plasma concentrations of losartan and its active metabolites EXP3174 were determined by HPLC. **Results:** Exercise increased mean SBP from 106 ± 2.8 to 126 ± 4.4mmHg (19%) and mean HR from 435 ± 12 to 481 ± 13 bpm (11%) in the control rats. In the losartan treated rats, SBP decreased from 109 ± 6.8 to 84 ± 2.2 mmHg before exercise (23%). During exercise, mean SBP increased from 84 ± 2.2 to 102 ± 5.3 mmHg (21%), and HR from 466 ± 4.2 to 504 ± 9.6 bpm (8%). Mean plasma losartan and EXP3174 were 117 ± 37 and 55 ± 23 ug/mL, respectively. **Conclusion.** Losartan attenuates the HR but not the BP response to exercise in normotensive SD rats. (Supported in part by a grant from Dalhousie Pharmacy Endowment Foundation).

3. SYNTHESIS OF NOVEL FURO [3,2-E] IMIDAZO [1,2-C] PYRIMIDINE IN ONE-POT DOUBLE ANNELATION REACTION.

Shaifullah Chowdhury, **Yasuyuki Shibata. National Institute for Environmental Studies, Environmental Chemistry Division, 16-2 Onogawa, Tsukuba, Ibaraki, Japan**

Purposes: With the development of clinically useful anticancer agents, antihypertensive agents, antiviral, antibacterial, antiallergic, antimalarial, antianalgesic and anti-inflammatory drugs, there has recently been remarkable interest in the preparation of annelated pyrimidines as pharmaceutical products. **Methods**: Equimolar amounts of furonitrile and appropriate reagent in dry acetic acid or pyridine was refluxed for an appropriate period of time. After cooling to room temperature, crushed ice was added and the mixture was stirred for 1 h. The separated product was collected by filtration and recrystallized from ethanol to give furo [3,2-*e*] imidazo [1,2-*c*] pyrimidine as brown crystals. The structural establishment was done by ^{1}H-NMR, ^{13}C-NMR and microanalytical data. **Results**: Reaction of various furonitriles with ethyl *N*- [bis (methylthio) methylene] glycinate or ethyl isothiocyanatoacetate or 2-chloroethylisothiocyanate cyclization of a pyrimidine ring and concomitant fusion of an imidazo moiety: thus annelation of an imidazo [1,2-*c*] pyrimido moiety of parent system could be achieved in one-pot reaction in 60-80% yield. By using this double annelation method the new 9 pharmaceutical products furo [3,2-*e*] imidazo [1,2-*c*] pyrimidine derivatives of tricyclic or tetracyclic imidazo moiety were synthesized. **Conclusion**: Reacting various reagents with a wide structural variety of furonitrile substrates gave access to a series of new annelated furopyrimidines in one-pot reactions as depicted below.



R_1R_2 = -$(CH_2)_4$-, MeMe, PhPh; X = O, H_2; Y = SMe, SH

4. A HYDROGEL-ANCHORED PHOSPHOLIPID BILAYER FOR FUNCTIONAL STUDIES OF MEMBRANES AND MEMBRANE TRANSPORT.

C.L. Ng, **Cheng Y.-L., Pennefather P.S., Dept. of Pharmaceutical Sciences, U of Toronto, 19 Russell St., Toronto, Ontario, Canada.**

Purpose: To demonstrate that lipid anchors attached to the surface of the hydrogel promote phospholipid bilayer formation. This hydrogel-anchored phospholipid bilayer should model a cytoskeleton supported cell membrane. Lipobeads® will serve as an artificial cell to allow proper folding and presentation of reconstituted membrane proteins and provide a cell surrogate for studying drug transport. **Method**: The lipid anchor is a newly synthesized, fluorescently labeled, phospholipid acrylamide. Nitro-benzodiazo-labeled phosphatidylethanolamine was reacted with acrylic acid-succinimide in chloroform to form the lipid anchor. The lipid anchor was designed to remain at the oil-water interface and self-orient appropriately, and is cross-linked with the polymer at the hydrogel surface in a one-step process as the gel is forming. **Results:** Scanning laser confocal microscopy confirmed that the covalently attached lipid anchors are on the surface of a poly (n-isopropyl acrylamide) hydrogel and directs the spontaneous organization of a lipid barrier around the bead. **Future Directions:** Experiments are in progress to confirm that this anchored lipid membrane is and artificial bilayer that can support activity of purified and reconstituted drug transport proteins. *Supported by NSERC.*

5. COMPARISON OF MICROPARTICLES PREPARED FROM THE RAPID EXPANSION OF SUPERCRITICAL SOLUTIONS AND BY SPRAY-DRYING.

P.M. Gosselin, **F. X. Lacasse, R. Thibert and J. N. McMullen. Faculty of Pharmacy, University of Montréal, C.P. 6128, succ. centre-ville, Montréal;. Email : gosselp@magellan.umontreal.ca. Pharmaceutical R&D, Merck Frosst Canada & Co, 16711, Trans Canada Hwy, Kirkland, Québec, Canada.**

Purpose. To compare by physical and physico-chemical analysis carbamazepine microparticles prepared from the rapid expansion of supercritical solutions (RESS) and by spray-drying. **Methods**. For both processes, microparticles were produced over a range of different temperatures from 35 to 100°C. For the RESS method, carbon dioxide was used as the solvent at a pressure range of 2000 to 5000 psi. The spray-drying (mini spray-dryer Buchi B-191) method used different organic solvents at atmospheric pressure. Characterization of produced particles included morphology, crystal properties and stabilities, mean particle size and size distribution and thermoanalysis. The influence on particle characteristics of temperature and pressure during both processes has been investigated. The materials were analyzed using scanning electron microscopy (SEM - Jeol JSM-820), image analysis (Grafter Ultimage X-1.41 software), X-ray powder diffraction (XRPD- Siemens D-5000), differential scanning calorimetry (DSC - Seiko RDC-220) and thermogravimetric analysis (TGA - Seiko RDC 220). **Results**. The carbamazepine particles used as initial material had a mean diameter of approximately 100 µm with a size distribution between 20- 400 µm. For both processes, the resulting particles have an average diameter below 3 µm and a narrower size distribution between 1-5 µm. SEM microphotographs, X-ray diffractograms and DSC spectra revealed that the modification of crystal morphology was dependant on the operating conditions of the manufacturing process. **Conclusions.** Significant reduction in mean particle size and size distribution of carbamazepine particles has been observed by RESS and spray-drying method. It has also been demonstrated that the crystalline nature of carbamazepine particles depends on the operating parameters and the process of production. **Acknowledgements.** This work has been presented at the 2000 annual meeting of the American Association of Pharmaceutical Scientists in Indianapolis (Indiana, U.S.A) on November 1, 2000. **Reference.** Gosselin, P. M., Lacasse, F.X., Thibert, R. and McMullen, J.N. Comparison of Microparticles Prepared from the Rapid Expansion of Supercritical Solutions and by Spray-Drying. PharmSci. 2000, 2(4), S-2006.

6. DEXAMETHASONE RELEASE FROM A POLYLACTIC ACID DIP-COATED STENT MODEL.

Tahmer Sharkawi, **Jean Norbert McMullen, Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada.**

Purpose. To compare the in-vitro release kinetics of a restenosis inhibitor from a stent model coated with a polylactic acid polymer film of different formulations. **Methods**. Stainless steel square plates measuring 1cm X 1cm X 0.73 mm were film coated with a polylactic acid polymer formulation containing dexamethasone as a restenosis inhibitor. The molecular weight of the polylactic acid polymers composing the film varied between 25000 and 120000, and film thickness varied from 300 to 400 microns. The coating technique consisted of dipping the stainless steel plates in the polymer solution, which were then subjected to a multi-step drying process. The drug loading was evaluated by dipping the model in tetrahydrofuran where the polymer and drug dissolved, and from this solution the drug content was measured by UV-spectroscopy. The release kinetics were measured by suspending the plates in vials containing 250 ml of phosphate buffer (pH 7,4) secured in a thermostated water bath at 37° C. Aliquots were taken at different intervals for a period up to 3 months and then analyzed for drug content by UV-spectroscopy. **Results**. The release of dexamethasone varied according to the different fractions of high and low molecular weight polymers composing the film. A faster release was observed in the formulations composed of a mixture of the different molecular weight polymer than the films composed of a single molecular weight polymer. **Conclusions**. Release kinetics can be adjusted by the choice of the proper formulation.

7. THE INTERACTION OF AMPHOTERICIN B (AMPB) WITH A SUPPORTED LIPID MONOLAYER; A METHODOLOGY TO STUDY AMPB'S TOXICITY.

Robin Stoodley, **Dan Bizzotto, Kishor M. Wasan. Department of Chemistry, University of British Columbia, and Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.**

Purpose: Amphotericin B (AmpB) is a widely used anti-fungal compound, but its use is limited by its toxicity. AmpB is believed to act by binding (primarily) to ergosterol in a fungal cell's membrane and inducing leakage of the cellular contents. According to the present model of AmpB selective toxicity[1], monomeric AmpB is active against ergosterol-containing membranes, while oligomeric AmpB interacts with sterol-free or cholesterol-containing membranes, resulting in human toxicity. Here the interactions of three forms of AmpB: Abelcet, a lipid complex; Fungizone, solubilized AmpB; and Heat-Treated Fungizone with a Dioleoylphosphatidylcholine (DOPC) monolayer supported on Mercury were examined electrochemically. In addition, a control solution of the solubilizing agent in Fungizone, Sodium Deoxycholate, was studied. **Methods:** Three methods were used to examine AmpB's disrupting influence; the monolayer's potential-induced phase transitions were characterized by measuring the monolayer capacitance. Kinetic studies of one of the phase transitions quantitatively measured AmpB's disruption of the transition, and Thallium Transport experiments probed the porosity of the layer. **Results:** Capacitance study revealed that Fungizone interacted strongly with the monolayer, while Abelcet interacted less strongly, and then significantly only after defects had been created in the layer. Heat-Treated Fungizone showed less influence than Fungizone prior to defect creation, but had a strong influence afterwards. Kinetic study of the second phase transition showed that Fungizone disrupted the phase transition the most, while Abelcet gave the least effect. Thallium transport experiments showed that Fungizone created the most porous monolayer; the other two forms and the deoxycholate all gave approximately equally porous layers. **Conclusions:** These results support a previously proposed mechanism in which AmpB oligomer from Fungizone is able to interact directly with lipid head groups on the surface of a cell membrane, while Heat-Treated Fungizone and Abelcet are not. **Acknowledgements**: *Funding provided from the Canadian Institutes of Health Research.*

[1]S. Hartsel et al. 1999. Activity and Kinetics of Dissociation and Transfer of Amphotericin B from a Novel Delivery Form. AAPS Pharmasci. 1 (4) Article 10.

8. DEGRADATION OF MOMETASONE FUROATE: INFLUENCE OF PH, IONIC STRENGTH, AND SIMULATED BIOLOGICAL FLUIDs.

XW Teng, **Cutler DJ, Davies NM. Faculty of Pharmacy, University of Sydney, NSW 2006, Australia. Email: shirleyt@pharm.usyd.edu.au**

The highly potent anti-inflammatory steroid mometasone furoate* is currently being developed for the treatment of allergic inflammatory disorders and diseases. In vitro, mometasone furoate decomposed into three degradation products in rat and human plasma, and in rat liver, intestine, stomach, lung tissues and human lung tissue without the NADPH-generating system (1). Purpose: This study investigated the degradation kinetics of mometasone furoate in aqueous solutions of different pH and ionic strength, and in simulated biological fluids. Methods: The kinetic studies of mometasone furoate in HCl, NaOH or buffer solutions from pH 2-13 were conducted at 37.0°C shielded from light in a shaking water bath. The ionic strength was adjusted with potassium chloride to μ = 0.60 M. Reactions were initiated by the addition of mometasone furoate to buffer solution equilibrated to 37°C. The initial concentration of mometasone furoate was 2 μg/ml with 0.1 % of methonal. At pre-determined time intervals, samples (0.5 mL) were removed and the reaction was stopped by acidifying the sample with 1M HCl. Samples were extracted with dichloromethane immediately after the addition of ethanolic testosterone 17-acetate (internal standard) and the extracts were kept dry at -20 °C until assayed. The remaining of parent drug and the formation of degradation products were estimated by HPLC using a Beckman C8 column and a mobile phase of methanol and water (6:4, v/v), with UV monitoring at 248 nm and photodiode array detection. The observed decomposition rate constant Kobs was calculated from the linear phase of the profile of mometasone furoate concentration versus time. Results: The degradation of mometasone furoate followed pseudo first-order kinetics, and the degradation rate of mometasone furoate was found to increase with an increase in pH in aqueous solutions. No decomposition was detected in solutions of pH < 4 when mometasone furoate was incubated for 1 wk. Only one decomposition product was observed in buffers of pH 4.30 to 5.32, and three products were detected at pH from 6.73 to 7.98, while four products were found at pH > 8. The pH-log Kobs profile showed a significant pH dependent degradation of mometasone furoate. In addition, there were no significant differences in degradation rate in buffers of pH 7.98 when the ionic strength was adjusted with KBr compared to that adjusted with KCl. At pH 7.38, the degradation of mometasone furoate appeared to decrease with an increase in ionic strength from 0.3 to 2.1M. No degradation was observed when mometasone furoate was incubated in simulated gastric fluid over 7 days, whereas three and four decomposition products were found in simulated intestinal fluid and simulated lung fluid, respectively. Conclusion: Mometasone furoate is stable at pH< 4 but degrades to several decomposition products at higher pH. The pH dependency of these reactions should be considered when formulating or extemporaneously compounding mometsone furoate formulations. *Mometasone furoate was kindly supplied by Schering-Plough Australia Pty Ltd. 1. Teng, X.W., Davies, N.M., Cutler, D.J., Brown, K.F., Clinical and pre-clinical metabolism of mometasone furoate: rat versus human. *J Pharm Pharmceut Sci (Proceedings of the CSPs 3rd annual symposium),* 3:201, 2000

9. PREDICTION OF THE HEPATIC EXTRACTION OF RSD1070 A NOVEL ANTIARRHYTHMIC AGENT USING RAT LIVER MICROSOMES.

Frank Abbott, **Vincent Tong, and Michael J.A. Walker. Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences; Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, British Columbia, Canada.**

Purpose. To predict the hepatic extraction of RSD1070, (±)-trans-[2-morpholinyl-1-(1-naphthaleneethyloxy] cyclohexane mono-hydrochloride using rat liver microsomal incubations. **Methods**. The pharmacokinetics of RSD1070 was examined in rats (n=8) and its metabolism was investigated using pooled hepatic microsomes. The free fraction in plasma and microsomal matrices was determined by equilibrium dialysis. Hepatic extraction was predicted from the scaling-up of the microsomal kinetic data using the well-stirred liver model. **Results**: A Michaelis-Menten model described the consumption of RSD1070 with a K_m of 0.45 µg/mL and V_{max} of 2.81 µg/min/mg protein. Taking the V_{max}/K_m ratio (CL_{int}) as the basis for scaling, the data from the microsomal kinetic studies (75 mL/min/kg) closely approximated the apparent CL_{tot}. Conclusion. RSD1070 is a high hepatic extraction compound (E = 0.94) with a predicted CL_h value that accounted for the CL_{tot} observed in rats. *Previously presented at the 10th North American ISSX meeting (Indianapolis, Indiana) on October 24-28, 2000.*

10. EFFECT OF *ECHINACEA* HERBAL PRODUCTS ON CYTOCHROME P-450 MEDIATED METABOLISM.

Brian Foster, **S. Vandenhoek, C.E. Drouin, J. T. Arnason, And A. Krantis. Therapeutic Products Directorate, Health Canada; Centre For Research In Biopharmaceuticals, University Of Ottawa, Ottawa, Ontario, Canada.**

Purpose Anecdotal information in the literature and from health care professionals revealed that many patients take natural health products in addition to their conventional therapeutic products. The effect of these products on the safety and efficacy of therapeutic products has not been determined. Orally, *Echinacea* is used for treating and preventing the common cold and other upper respiratory infections. *Echinacea* is also used orally as an immunostimulant for fighting a variety of other infections, including urinary tract infections and vaginal candiasis. We had shown previously that an *Echinacea* tincture inhibited the 3A4 mediated-metabolism of a marker substrate. This study was initiated to examine the potential for a wider range of natural products containing *Echinacea* to effect the cytochrome P450 mediated metabolism of marker substrates of other isoforms. **Methods** Aliquots of aqueous extracts from 11 *Echinacea* containing blended teas, and single entity tablets and liquid filled capsules were tested (0.625 - 5.0 mg/mL) in a fluorescence based 96 well assay microplate using Gentest Corp human P450 CYP2C9, CYP2C19, CYP2D6 and CYP3A4 supersomes. The *Echinacea* and Goldenseal tea was also tested against 2C9*2, 2C9*3, 3A5 and 3A7. **Results** All blended teas inhibited the metabolism of the isoforms tested by 52-92%. The single entity tablets and liquid filled capsules had marked differences in their effect on the various isoforms. Some tablets and capsules had no or little inhibitory effect on 2C9 and 2C19 with others having up to a 100% effect. Most capsules affected greater than 60% inhibition of 2D6 and 3A4. The *Echinacea* and Goldenseal tea affected 2C9*2 mediated metabolism to a lower extend than 2C9*1. The multi-component *Echinacea* Special tea was found to express non-competitive inhibition against 3A4. An aqueous aliquot of the *Echinacea* and Goldenseal tea (25 mg/mL) inhibited the 3A4 (58.3%), 3A5 (41.6%) and 3A7 (89.5%) metabolism of the marker substrate. **Conclusions** The commercially available *Echinacea* products studied can inhibit the metabolism of major human P450 isoforms examined in this study. These are crucial isoforms in human drug disposition and the concomitant use of these natural products with conventional therapeutic products may lead to the development of adverse reactions. The potential effect of these products to induce drug metabolism is not known, however, many inhibitors can become inducers after repeated administration.

11. EFFICIENCY TO DETECT PM OF CYP2D6, CYP2C9, AND CYP2C19 BASED ON VOLUNTEERS' PHENOTYPE AND GENOTYPE.

***Eric Masson*, C. Tassé, R. Larouche, B. Girard, and M. LeBel. Anapharm Inc., Montreal, Quebec, Canada.**

PURPOSE: Genetic polymorphism in CYPs has been well recognized for CYP2D6, CYP2C9, and CYP2C19. Since the frequency of poor metabolizers (PM) in Caucasian population is low (< 10%), finding PM in the general population requires large screening effort. We hypothesized that screening efficiency could be improved by screening subjects based on their phenotype obtained from previous participation in clinical studies. **METHOD:** Data from 46 crossover design, bioavailability or bioequivalence studies on drugs affected by CYP2D6, CYP2C9, and/or CYP2C19 was used. Subjects with significantly longer, as well as greater $t_{1/2}$, AUC and metabolic ratios in all study periods were considered potential PM (phenotype). Genotyping was performed in those individuals after obtaining their written consent. Whole blood was collected in EDTA containing tubes for DNA extraction, and genotyped for frequent alleles of CYPs listed above using a validated AS-PCR method and automated fluorometry. **RESULTS:** From the 1171 subjects screened, 111 (9.5%) were potential PM: 72 were for CYP2D6, 34 for CYP2C9, and 41 for CYP2C19. Genotyping confirmed 30.4% of the potential PM as PM homozygotes, 56.4% as PM heterozygotes, and only 15.2% as non PM. **CONCLUSIONS:** Pre-screening volunteers based on their phenotype to identify PM may improve cost, time, and efficiency of screening. *Presented at the ASCPT Annual Meeting, Orlando, Florida, March 6-9, 2001.*

12. A HIGH-THROUGHPUT IN-VITRO CYTOCHROME P450 ASSAY FOR PRECLINICAL DRUG CANDIDATE METABOLISM SCREENING.

***David Kwok*, Dan Sit, Jessica Yeung, Tony Kiang, Thomas K.H. Chang and Frank Abbott. BRI Biopharmaceutical Research Inc.; Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.**

Objective: Human cytochrome P450 enzymes (CYP450) play an essential role in the metabolism of xenobiotics. With the advents of cDNA-expressed human CYP450 enzymes, it has become possible to conduct *in-vitro* high-throughput metabolite screening experiments. The purpose of this study was to develop and validate an *in-vitro* CYP450 enzyme assay supported by high-throughput LC/MS and LC/MS/MS analysis. **Method:** A total of five pathway specific substrates: dextromethorphan, nifedipine, tolbutamide, mephenytoin, and 7-ethoxycoumarin were selected for CYP2D6, CYP3A4, CYP2C9, CYP2C19, and CYP1A1, respectively. Incubations were performed at 37°C using 1.0 mM NADPH for optimization of CYP450 enzyme concentration, substrate concentration and incubation time. The conditions of different solvents, pH, and buffers have been investigated for each substrate. Incubations were carried out using baculovirus expressed cDNA isozymes except for CYP3A4 which has a high activity in pooled human liver microsomes (HLM). The reaction was stopped by the addition of acetonitrile (ACN) containing 12 μM reserpine as internal standard, cooled on ice and centrifuged for 10 minutes. LC/MS/MS analysis of the parent drugs and metabolites were carried out using Micromass Quattro-LC® triple quadrupole instrument in both positive and negative electrospray ionization modes. Chromatographic conditions were developed using a C18 column (Zorbax), 2 X 30 mm, 3μm, using a gradient mobile-phase of 0.1% formic acid and ACN at a flow rate of 0.2ml/min. The total-ion-chromatograms of the five marker substrates, metabolites and the internal standard were monitored by specific multiple-reaction-monitoring (MRM) simultaneously. For metabolite structural characterization of an investigational new drug (IND) candidate, LC/MS full scan data were acquired. **Result:** An optimal incubation time of 30 minutes was selected as a common incubation time for the five assays in support of a high-throughput incubation procedure. Optimal cDNA enzyme concentrations for individual pathways from 10 to 100 nM were observed. Optimal concentrations for each marker substrate were pathway specific, observed between 5 to 100 μM. For screening of an IND, 5 and 20 μM substrate concentrations were observed to be desirable. Km and Vmax for each marker substrate were observed within normal ranges compared to values reported in the literature. The LC/MS/MS and LC/MS assays were shown to be highly specific with an analysis time of less than twelve minutes for all the analytes. **Conclusion:** This study has demonstrated the applicability of using specific cDNA CYP450 substrates in the optimization of *in-vitro* CYP450 assays for pre-clinical metabolite screening supported by rapid and high-throughput LC/MS/MS analysis.

13. DEMONSTRATION OF *IN VITRO* TARGETING BY BIOTINYLATED LONG-CIRCULATING LIPOSOMES TO HUMAN OVARIAN CANCER.

***Zhiming Fan*, SA McQuarrie, MR Suresh, GG Miller, JR Mercer. Address: Faculty of Pharmacy, University of Alberta. Email: fan@ualberta.ca**

Purpose: Ovarian cancer is the most common and deadly malignancies in women. It is difficult to diagnosis it in early phase. We are proposing a novel multistep radioimmunotherapeutic approach to be used as an adjuvant therapy in this group of patients. The object of our project is to use an anti-CA125 bispecific monoclonal antibody (BsMAb) to direct toxic radionuclides encapsulated in the liposomes to the ovarian cancer site. **Methods**: Anti-CA125 MAbs (B43.13 and B27.1) were obtained from tissue culture hybridoma and purification with Protein G column. The specificity and relative affinity of MAb were determined by ELISA. The purity of MAb was determined by SDS-PAGE. Biotinylated antibodies were obtained by labelling antibodies with long-arm biotin. Biotinylated liposomes encapsulated with sulforhodamine B were prepared using a high-pressure extruder. The stability and leakage from the liposomes were determined by Sephadex G50 column chromatography and ultracentrifugation. Preliminary *in vitro* study was performed to demonstrate the multistep targeting of liposomes to ovarian cancer cells. We used the biotin-streptavidin system to attach the biotinylated liposomes via a biotinylated antibody (B27.1) to NIH OVCAR-3 human ovarian cancer cells. Laser scanning confocal microscopy (CLSM) was used to monitor *in vitro* targeting. Biotin uptake mechanism in OVCAR cells is studied by ^{3}H label biotin and enzyme kinetic data will be presented. **Results**: The MAbs B27.1 and B43.13 were successfully purified by Protein G column. SDS-PAGE confirmed its purity. The relative affinity of MAb B27.1 for CA 125 was 1.6 approximately times higher than for MAb B43.13. Liposome stability and leakage tests indicated less than 2% leakage after incubating 4 days at room temperature. CLSM indicated that our immunotargeting approach could successfully direct the biotinylated MAbs to the tumor and that the liposomes specifically bind to the OVCAR cells. **Conclusion:** Biotinylated MAbs do successfully direct liposomes specifically bind to OVCAR cells in vitro.

14. IMPACT OF BIORELEVANT DISSOLUTION MEDIA FOR CLASS I AND CLASS II DRUGS.

***Raimar Loebenberg*. University of Alberta, Dentistry/Pharmacy Centre, Edmonton, Alberta, Canada. rloebenberg@pharmacy.ualberta.ca**

Purpose: The dissolution behavior of high permeability/high solubility drugs (class I, Biopharmaceutical Drug Classification System, BCS) and high permeability/low solubility drugs (class II) was tested in various media. The aim of the study was to investigate whether the use of biorelevant dissolution media (BDM) would be advantageous over the use of standard media for predicting the *in vivo* performance of these drugs. **Methods:** The dissolution tests were performed using USP 24 apparatus 2. Conventional buffers and USP media were compared with two BDMs containing different amounts of lecithin and sodium taurocholate to simulate the fasted state (FaBDM) and the fed state (FeBDM) in the gastrointestinal tract. The dissolution behavior of one metoprolol, two glibenclamide and two carbamazepine formulations were compared using conventional media and BDMs. **Results:** The dissolution of metoprolol (class I drug) was rarely influenced by the nature of the dissolution media. For class II drugs such as glibenclamide and carbamazepine, the dissolution behavior showed significant differences in all media tested. The dissolution results from two different glibenclamide formulations were used as input-function for a computer simulation of the glibenclamide plasma levels using GastroPlus. The simulated plasma levels were compared with those from an *in vivo* bioequivalence study undertaken by the central quality control laboratory of the German pharmacists (CL). An *in vitro/in vivo* correlation (IVIVC) could be established between the dissolution results in FaBDM and the bioequivalence study. Only the dissolution results in the FaBDM were able to discriminate between the two formulations and to predict the oral performance, glibenclamide should be taken in the fasted state. No other media was able to establish an IVIVC. The dissolution behavior of two carbamazepine formulations, a test product and a reference product were compared using the recommended pharmacopeial media (1% sodium lauryl sulphate) and the FeBDM. Carbamazepine should be taken with meals. In the recommended media the test product showed a higher drug release compared to the reference product. In the FeBDM the test product showed a significantly lower drug release compared to the reference product. A comparison with *in vivo* data was not possible because no *in vivo* study was available but the FeBDM results indicate a lower drug release from the test product. The test product was taken form the market because of bioavailability problems which confirms the results in FeBDM. **Conclusions:** BDMs were able to discriminate between clinical relevant formulation differences of two different class II drugs. The use of biorelevant dissolution media seems to be superior compared to conventional media to detect in vitro clinically relevant differences between formulations of class II drugs.

15. ISOTRETINOIN METABOLITES: POSSIBLE CONTRIBUTIONS TO SAFETY AND EFFICACY OF ISOTRETINOIN.

***U. Wiegand,* R. C. Chou, and W. Pirson. F. Hoffmann-La Roche Ltd. Basel, Switzerland**

Isotretinoin (13-cis retinoic acid, Accutane) is a metabolite of vitamin A and also an endogenous compound. The metabolism of isotretinoin in acne patients is fairly complex because several reversible and irreversible metabolic steps are involved. Isotretinoin is metabolized to endogenous all-trans retinoic acid and 9-cis retinoic acid and irreversibly oxidized to 4-oxo 9-cis retinoic acid and 4-oxo 13-cis-retinoic acid, which, in turn, is reversibly transformed to 4-oxo all-trans retinoic acid. In humans all these compounds form glucuronides are subsequently excreted in urine and feces. **Purpose**: Although the overall efficacy and safety of isotretinoin and its metabolites have been determined in clinical trials, the individual contributions of parent compound and each metabolite are not known. We therefore initiated their preclinical characterization with respect to activity most likely relevant for clinical efficacy. *In-vitro* experiments with human hepatocytes were conducted to determine the formation of all isotretinoin metabolites listed above. **Methods**: Minipig sebaceous glands, the limb bud test, hypervitaminosis A effect in mice as well as receptor binding and activation assays were used. Fresh human hepatocytes were collected and prepared at the hospital according to standard procedures. **Results** Isotretinoin metabolites possessed pharmacological actives in experimental models relevant for the antiacne and for the teratogenic effect. The data demonstrate that 4-oxo-13-cis retinoic acid and 4-oxo all-trans retinoic acid may be as active as 13 cis-and all-trans retinoic acid. Receptor binding and activation experiments support these findings (1). The most important result of the hepatocyte study was that the oxidative metabolic pathways and therefore the formation of all oxo-metabolites are dose dependent whereas the isomerization reactions are not. **Conclusions**: Isotretinoin metabolites considerably contribute to the therapeutic effect and teratogenicity of isotretinoin in acne patients. The hepatocyte data suggest that the metabolism in humans may be more complex than initially considered. An additional metabolic regulation mechanism may help to generate the most appropriate retinoid plasma and tissue concentration. Studies in humans are currently being conducted which will give additional information on the importance of isotretinoin metabolites for the overall efficacy and safety of isotretinoin. *Results presented in part as Poster at Society of Investigative Dermatology Meeting in Chicago. May 2000.*

16. COMPARATIVE PHARMACOKINETICS OF OXO-ISOTRETINOIN AND ISOTRETINOIN IN DOGS.

***Ruby Chou,* Brita Morgenroth, Sonja Nick, Zhenmin Liang, Mei Liu, Guenter Gross, Ulf-W. Wiegand. Hoffmann La Roche Grenzacherstr. 124, Basel, Switzerland.**

Purpose Oral isotretinoin (13 cis-retinoic acid, Accutane®/ Roaccutane®) is being used since more than 15 years to treat severe recalcitrant cystic acne. 4-oxo-13 cis-retinoic acid (oxo-isotretinoin) is the major metabolite of objective isotretinoin. It is pharmacologically active in *in vitro* models (ref) and may contribute to the efficacy and safety of isotretinoin. The purpose of this program is to evaluate the pharmacokinetics of oxo-isotretinoin and isotretinoin in male dogs following single intravenous or oral administration. The formation of oxo-isotretinoin from isotretinoin in hepatocyte preparations was also examined. **Methods** Oxo-isotretinoin and isotretinoin were dosed to four male beagle dogs intravenously (2 mg/kg) or orally (4 mg/kg) in a crossover fashion. The dogs were fasted overnight and received 200 g of a special dog food (PAL) 30 min. before the oral dosing. Hepatocytes were prepared from human liver obtained from surgical waste. The samples were analyzed for isotretinoin, oxo-isotretinoin, all-trans-tretinoin, 4-oxo-retinoic acid and 9-cis-retinoic acid using an LC-MS/MS method. Handling of retinoids, dosing solutions, and plasma samples was performed under dimmed light conditions. Dosing solutions were stabilized against oxidation. **Results** Following single iv dosing, the kinetic behavior of isotretinoin and oxo-isotretinoin were similar. Both compounds had low systemic clearances (2.0 vs. 3.7 mL/min/kg) and low volumes of distribution(0.88 vs. 0.65 mL/kg). Both had half-lives about 6.5 and 6.0 hrs, respectively, and the initial plasma concentrations were also similar (Cmax about 6800 and 7800 ng/mL) indicating a similar distribution of the compounds in the central compartment. Following iv and po administration of oxo-isotretinoin in dogs, the trans isomer oxo-retinoic acid was the only detectable metabolite in plasma. Only physiological concentrations of other related retinoids (isotretinoin, retinoic acid, and 9-cis-retinoic acid) were detected, indicating that these retinoids are not formed as metabolites of oxo-isotretinoin in dogs. The formation of oxo-isotretinoin from isotretinoin in human hepatocytes was nonlinear. Oxo-isotretinoin had an oral bioavailability of around 50-60% in dogs. The oral absorption properties of isotretinoin and oxo-isotretinoin were also similar. Following administration of a solution, the oral absorption of isotretinoin and oxo-isotretinoin was comparable, leading to a similar bioavailability as well as similar Cmax (maximum concentration) and Tmax (time needed to achieve maximum concentration) values. Following administration of clinical capsules to dogs, the AUC values of oxo-isotretinoin were similar to those of isotretinoin after dose correction. **Conclusion** We could show, for the first time, that the pharmacokinetic properties and the oral bioavailabilities of isotretinoin and oxo-isotretinoin are similar in dogs. If the dog is a good kinetic model then we can expect that the plasma concentrations of oxo-isotretinoin following oral administration of oxo-isotretinoin in man are similar to those of isotretinoin following oral dosing of isotretinoin in man. **Reference** Wiegand, U-W., Pirson, W., and Bauer, F., Preclinical data indicate role of isotretinoin metabolites for anti-acne effects of isotretinoin.

17. SOLUBILITY AND STABILITY OF COX-2 INHIBITOR ROFECOXIB: EFFECT OF b-CYCLODEXTRIN.

***S. Sattari*, F. Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**

Purpose. Rofecoxib, is a non-steroidal anti-inflammatory drug (NSAID) with selective cyclooxygenase-2 (COX-2) inhibitor properties. Hence, it has a better safety profile than conventional NSAIDs. The drug has low water solubility and based on its chemical structure should undergo photocyclization therefore possesses a light sensitive stability profile. The purpose of this work was to investigate the solubility and stability of rofecoxib in aqueous solution under room conditions and determine the effect of β-cyclodextrin on its solubility and stability. **Methods.** *Solubility test*: Rofecoxib/ β-cyclodextrin mM ratios (n=3) of 1/ 0, 1/1, 1/2, 1/4, 1/6, 1/8, 1/10, 1/12, 1/14, 1/16, 1/ 18, and 1/20 were placed in 20 ml cap screwed vials and pH 2 buffer was added up to 10 mL. The vials were shaken at 200 Hz in a lightproof container for 48 h to achieve the equilibrium. The solutions were filtered into clean glass tubes and the concentration of rofecoxib was measures using HPLC. *Stability test*: The above filtered solutions were kept in the daylight using a 60 w electric bulb and daily samples were collected for 20 days. The temperature was kept at 25C° throughout both experiments. **Results.** The solubility of rofecoxib was 3.2 ± 0.11 mg/L. β-cyclodextrin significantly increased the solubility of rofecoxib to the highest level of 9.1 ± 0.44 mg/L at 1/18 drug/ β-cyclodextrin ratio. Rofecoxib was stable at least for 48 h when protected from light. Under direct light, rofecoxib was degraded in a first order pattern with a 10% degradation time ($T_{10\%}$) of 1.25 ± 0.32 days. Addition of β-cyclodextrin significantly but marginally improved stability of rofecoxib ($T_{10\%}$ = 0.80 ± 0.10 day). **Conclusion.** Rofecoxib is light sensitive. β-cyclodextrin increases the solubility of rofecoxib, but under the condition used, has little protective effect against the drug light sensitivity.

18. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CELECOXIB IN RAT PLASMA.

***Michael Guirguis*, S. Sattari and F. Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**

Purpose. Celecoxib is one of a new generation of non-steroidal anti-inflammatory drugs (NSAIDs). They selectively inhibit cyclooxygenases–2 (COX-2), hence, they are believed to be less likely to trigger gastrointestinal side effects. Our objective was to establish a rapid and sensitive high-performance liquid chromatography method for the determination of celecoxib in rat plasma. **Methods.** Using ibuprofen as internal standard, we extracted celecoxib from 0.2 ml plasma under acidic conditions into isooctane-isopropanol (95:5). After vortex-mixing for 30 sec and high-speed centrifugation for 5 min, the organic layer was evaporated using vacuum centrifugation. The residue was dissolved in mobile phase [acetonitrile-water-acetic acid-triethylamine (47:53:0.1:0.03)] and injected to a reversed-phase chromatographic system consisting of an autoinjector, a variable UV detector set at 254 nm, a computerized integrator, a 10 cm C_{18} analytical column packed with 5μm of reversed-phase particles and a stainless steel filter guard column. The flow rate was 1 mL/min. **Results.** The response was linear within 20-1000 ng/ml range (r=0.99). Extraction efficiency was found to be 70%. Intra- and inter-day variability was <10% with an accuracy of <10%. The run-time was 20 min. **Conclusion.** The assay was suitable for determination of celecoxib pharmacokinetics in rats following single 5 mg/kg doses. This assay was found to be both time- and cost-efficient as compared to the previous reported method. It is readily adoptable for analysis of celecoxib from human plasma. Supported by Canadian Arthritis Network.

19. THE EFFECTS OF ACUTE INFLAMMATION ON CELECOXIB PHARMACOKINETICS.

Michael Guirguis, **F. Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**

Purpose. Celecoxib (CEL) is a relatively new non-steroidal anti-inflammatory drug, thus relatively little is known of its pharmacokinetics. It is known that CEL undergoes liver metabolism by CYP 2C9, and as such there is potential for drug-drug and drug-disease interaction. We set out to determine if a drug-disease interaction exists between celecoxib and mild inflammation, induced my interferon-α. **Methods.** Sprague-dolly rats (300-350g, n=12) where randomly divided in two groups: Control (CEL 5mg/kg PO) and INF-treated (INF+CEL 5mg/kg PO). INF-treated rats were administered INF-α $5x10^6$U IP 12 h and 1h before CEL dosing. The day before CEL administration a silastic catheter was inserted into the right jugular vein for serial blood sampling. On the day of the experiment serial blood samples (200µl/ sample) were then collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 720 min post dose. The catheter was flushed with 200µl of saline after each sample collection. Samples were stored at -20°C until analyzed for CEL using a newly developed HPLC assay. **Results.**

	Control	INF-Treated
AUC (µg h/l)	4635.5 (2212.6)	5434.0 (778.2)
Cmax (µg/l)	941.3 (351.8)	916.8 (230.3)
CL_f (l/h/kg)	1.4 (0.8)	0.9 (0.2)

An examination of the AUC between the two groups showed a trend towards increased AUC in the INF –treated rats but this did not reach significance. As well no significant changes were seen when we examined C_{max} and CL_f. With respect to CL_f a trend towards reduced CL_f was seen. **Conclusions.** The inability of INF treatment to affect CEL pharmacokinetics can point to two possible explanations. Due to the mild nature of INF-induced inflammation, the CYP 2C9 was not sufficiently affected (i.e., reduced CYP 2C9 activity) by it. Secondly CYP2C9 may not be affected by inflammation-induced changes in activity at all. CEL pharmacokinetics is not yet completely characterized and as such further work is needed to determine possible drug-drug and drug-disease interactions of this new and popular drug.

20. THE CHARACTERIZATION OF CELECOXIB PHARMACOKINETICS IN THE RAT.

Michael Guirguis, **F. Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**

Purpose. Celecoxib is a relatively new cyclooxygenase-2 specific agent. Due to is specific binding nature it is believed to lack many of the toxic side effects seen with older non-specific nonsteroidal anti-inflammatory. In this study we set out to characterize the pharmacokinetics and bioavailability of celecoxib using the rat model. **Methods.** Male sprague-dawley rats (250-300g) were anesthetized (pentobarbital 40 mg/kg IP), and a silastic catheter was inserted into the right jugular vein. On the day of the experiment animals were dosed with celecoxib (5 mg/kg IV, PO or IP). Serial blood samples (200µl/ sample) were then collected. Samples were stored at -20°C until which time they were analyzed for celecoxib using HPLC. **Results**.

	T_{max} (min)	C_{max} (µg/L)	$T_{½}$ (hr)	AUC (µg*hr/L)	Vd L/kg)	Cl L/hr/kg)
IV (n=8)	1 (0)	18201 (31531)	3 (1)	9265 (1800)	2 (0.6)	0.6 (0.1)
IP (n=3)	17 (13)	1236 (195)	5 (2)	6636 (1719)	6 (0.5)	0.8 (0.2)
PO (n=6)	98 (75)	941 (352)	5	4640 (2210)	5 (2)	0.4 (0.2)

Data presented as mean (standard deviation)

The bioavailability constants were found to be F= 0.59, F_{liver} = 0.7 and F_{gut} =0.8. **Conclusions.** The rat model is appropriate for examination of celecoxib pharmacokinetics. Celecoxib can be classified as medium to low extraction drug that undergoes limited first pass metabolism. Celecoxib undergoes limited gut effects, and the majority of first pass effects are associated with liver metabolism.

21. THE EFFECTS OF NSAID THERAPY ON THE POTENCY OF ANTIHYPERTENSIVE AGENTS.

Michael Guirguis, **F. Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**

Purpose. To use a validated animal model and determine if 1) nonsteroidal anti-inflammatory drugs (NSAIDS) affect the potency of anti-hypertensive agents (i.e., propranolol) and 2) if this is related to their well-known gastrointestinal (GI) side effect associated with inhibition of cyclooxygenase 1. **Methods.** Flurbiprofen (FL) was used to induce GI side effects, metronidazole (MZ), an NO donor effective in preventing NSAID-induced GI side effect, was co-administered to reverse the effect of FL. Propranolol (PROP), a nonselective β-adrenergic receptor antagonist, was used to test the effect of FLU on anti-hypertensive agents. Male Sprague Dawley rats (n=30) were divided in to four groups, Flu (2.5 mg/kg *bid*), MZ (50mg/kg *bid*), Flu+MZ, and placebo. The rats were dosed for four days. In the FL+MZ group, MZ was dosed 1 h before the FL dose. A modified lead I ECG was used to record the PR and RR intervals. PR-intervals are surrogate markers of β-adrenergic function, which is affected by inflammation. On day 4, one hour post FL dosing, PROP (25mg/kg) was administered orally and the response was monitored for 180 min. Differences in ECG intervals were obtained by calculating average % differences from pretreatment baselines. **Results.**

	Flu	Flu+MZ	MZ	Placebo
Max % change in PR interval	5 (6)	24 (7)	27 (9)	26 (14)
Max % change H.R.	11 (12)	3 (15)	12 (9)	17 (20)

Data presented in means with standard deviation in bracts

FL pre-treatment resulted in a significant reduction in the potency of PROP (p= 0.01) as compared to placebo. Response to PROP was normalized with the addition of MZ. MZ mono-therapy did not affect PROP response as compared to placebo. **Conclusions.** FL therapy alters the body's response to PROP. The reversal of this response with MZ therapy points to the involvement of GI side effects as the potential cause of these alterations in PROP response.

22. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF GLUCOSAMINE IN RAT PLASMA.

Ali Aghazadeh, **Saeed Sattari, Franco Pasutto and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**
fjamali@pharmacy.ualberta.ca

Purpose. Glucosamine has recently received a great deal of attention as a treatment for degenerative joint disease. It is a natural component of glycoproteins found in connective tissue and articular cartilage. Current assays typically use radiolabled glucosamine and cannot differentiate the parent compound from its metabolites and/or degradation products. The only reported HPLC assay is difficult to reproduce. The objective of this study was to develop an efficient, sensitive, and simple method for determination of glucosamine in rat plasma. **Method.** Using galactosamine as internal standard, 0.1 mL of blank rat plasma was spiked with glucosamine standard solutions to final concentrations of 0.5 to 20 mg/mL. Plasma proteins were precipitated by adding 0.4 mL of acetonitrile, vortex-mixing for one min, and centrifuging at 2500 g for 3 min. The upper layer was transferred to a clean test tube and 0.2 mL of methanol was added to facilitate evaporation, which was conducted at 40 °C under vacuum. To the dried residue, 0.2 mL of 100 mg/mL 1-naphthyl isothiocyanate, dissolved in methanol- acetonitrile-triethylamine (1:1:0.3), was added and kept at room temperature for 20 min to allow the derivatization reaction to complete. The reaction was quenched by adding 0.4 mL of 1.5% acetic acid. The excess derivatizing reagent and its degradation products were extracted with 1.0 mL chloroform by vortex-mixing for one min and centrifuging at 2500 g for 1 min. The aqueous layer was transferred to a conditioned styrene divenylbenzene anion exchange column containing the quaternary ammonium functional group. The column was washed with 0.5 mL of methanol-water-acetic acid (2:1:1) and 0.1 mL of the eluted solution was injected into an HPLC system consisting of an autoinjector, variable UV spectrophotometer detector set at 254 nm, and an integrator. The mobile phase consisted of acetonitrile-water-acetic acid-triethylamine (4.5: 95.5: 0.1: 0.05) and was pumped with a flow rate of 0.9 mL/ min at 41°C. A 10 cm ′ 4.6 I.D. 5 mm C18 reversed phase analytical column was used for analysis. **Results.** Excellent linearity (r=0.99) was obtained within the 0.5 to 20 mg/mL range. The detection limit was 500 ng/mL and the CV was between 6 to 15% for high and low concentrations. **Conclusion.** The assay was applicable for the determination of glucosamine bioavailability and pharmacokinetics in the rat. This method is convenient and sensitive and may also be used for the determination of glucosamine in human plasma and pharmaceutical dosage forms.

23. PHARMACOKINETIC STUDY OF GLUCOSAMINE IN THE RAT.

Ali Aghazadeh, **Saeed Sattari, Franco Pasutto and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**
fjamali@pharmacy.ualberta.ca

Purpose. Glucosamine has recently received a great deal of attention as a potential agent in the treatment of degenerative joint disease. The purpose of this work was to determine the absolute bioavailability and pharmacokinetics of glucosamine after single dose administration. **Method.** Five male Sprague Dawley rats were cannulated (right jagular vein) and, after overnight recovery, received 350 mg/kg i.v. or oral glucosamine solution in a randomized crossover design study with a wash out period of 2 days. Plasma samples were obtained up to 8 h and glucosamine concentrations were quantified using a novel reversed phase HPLC method developed in our laboratory. Pharmacokinetic parameters were calculated using a noncompartmental model (WinNonlin version 3.1). **Results.** Mean ± standard deviations (in brackets) of pharmacokinetic indices are depicted in the table below. The absolute bioavailability was 15% (± 0.1).

	C_{max} (µg/mL)	T_{max} (min)	$t_{1/2}$ (h)	V_d (L/kg)	AUC_{0-t} (µg.h/mL)	AUC_{0-8} (µ g.h/mL)	Cl (L.kg/h)
IV			1.4 (o.8)	2.8 (0.9)	149 (53)	151 (54)	1.7 (0.9)
PO	24.8 (15)	13 (10.4)	3.1 (1.7)	-	18.0 (11.2)	20.0 (9.7)	-

Conclusion. Glucosamine was rapidly absorbed, highly distributed and highly cleared. The observed low oral bioavailability is likely due to first pass metabolism.

24. REDUCED METABOLIC CHIRAL INVERSION OF R-IBUPROFEN IN STRESSED RATS.

S. Ling, **F. Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.**

Purpose. In rats and humans, the main clearance pathway of R-ibuprofen is its chiral inversion to S-ibuprofen. In patients with dental surgery pain, the stereoselective disposition of ibuprofen in plasma is reversed, hence the concentration of the active S enantiomer is lower than that of the R enantiomer, due perhaps, to the stress of dental pain. In this study, we tried to determine whether stress alone, in the absence of pain and tissue injury, alters the metabolic chiral inversion of ibuprofen enantiomers in the rat similar to what has been observed in humans with moderate to severe pain. **Methods.** Male Sprague-Dawley rats (250-300g) were catheterised in the right jugular vein while anaesthetized (pentobarbital 40 mg/kg ip). Three daily oral doses of 20 mg/kg R-ibuprofen (suspended in 1%CMC) were administered to Control and Stressed (n=4-6 rats/group) rats at 1100 h and serial blood samples were collected. On the second day, prior to drug dosing, Stressed rats received 2 h (0800-1100 h) of stress by being restraint in restraining tubes. Plasma concentrations of R- and S-ibuprofen were determined by a stereospecific HPLC method. **Results.** AUC_S after administration of R and AUC_S/AUC_R ratio, which were used as measures of metabolic chiral inversion, were significantly reduced by stress.

	Control		Stressed	
	AUC S:R	AUC_S	AUC S:R	AUC_S
Day 2	2.37(0.54)	1900(600)[1]	3300(1000)	1.39(0.20)[1]
Day 3	2.04(0.16)	2700(1000)	3500(1100)	1.79(0.13)[1,2]

[1]Different from Control; [2]Different from Day 2; (p<0.05)

No other pharmacokinetic indices showed significant differences within or between groups of rats. **Conclusions**. Stress reduces chiral inversion of R-ibuprofen to its antipode indicating reduced hepatic metabolic activity. *Supported by MRC 983587.*

25. PHARMACOKINETICS OF LOSARTAN AND EXP-3174 IN RHEUMATOID ARTHRITIS PATIENTS.

Noriko Daneshtalab, **Richard Z. Lewanczuk, Anthony S. Russell, and Fakhreddin Jamali.**
Faculties of Pharmacy & Pharmaceutical Sciences and Medicine, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Losartan (LOS) is an angiotensin II receptor antagonist. It is metabolized by the hepatic cytochrome P450 to a more active compound, EXP-3174 (EXP). Inflammatory conditions reduce hepatic clearance of many cardiovascular drugs depending upon the severity of the disease. The metabolism of LOS to EXP may also be affected by inflammation. We studied the effect of the severity of rheumatoid arthritis (RA) on the pharmacokinetics (PK) of LOS and its active metabolite. **Methods:** A total of 26 patients aged 18-72 with RA were recruited into the study. They included 14 patients with active arthritis (ARA) and 12 with arthritis in remission (RRA). Subjects took single oral dose of 100 mg LOS tablets with 240 ml of distilled water. Blood samples were collected immediately prior to drug administration (time 0) and at 15, 30, 45 min, 1, 2, 3, 4, 5, 8 and 12 h. The plasma was collected and stored at -80°C until analyzed. LOS and EXP were measured together using a validated reverse phase HPLC method after liquid-liquid extraction. PK of LOS and EXP were delineated by noncompartmental analysis. **Results:** The following arithmetic mean and standard deviation PK parameters were observed:

Parameter	LOS		Healthy	EXP		Healthy
	ARA	**RRA**	subject	**ARA**	**RRA**	subject
AUC $_{0-\infty}$, mg.hr/L	0.9 (0.4)	1.0 (0.44)	1.1	1.7 (0.6)	2.4 (1.1)	6.0 (1.2)
$t_{½}$, hr	1.8 (1.4)	1.8 (0.9)	1.5 (0.4)	3.6 (1.2)	5.0 (3.1)	4.2 (0.5)
C_{max}, mg/L	0.5 (0.31)	0.5 (0.2)	0.8 (0.3)	0.3 (0.1)	0.4 (0.2)	0.6-1.2
t_{max}, hr	1 (0.79)	0.9 (0.5)	0.7 (0.3)	3.4 (1)	2.8 (1.1)	2.0 (0.6)
Cl/F, L/hr Arithmetic	142.7 (84)	126.8 (62)	N/A	——	——	——
Harmonic	108	104	93	——	——	——

Disease severity does not seem to have an effect on the PK of either LOS or EXP. However, there appears to be a significant difference in the PK indices of RA patients, especially regarding the AUC of EXP, as compared to those reported in literature for subjects with no inflammatory conditions. **Conclusion:** There is no significant difference in the PK of LOS and EXP between ARA and RRA. As compared with the literature values, the AUC of EXP is substantially reduced by inflammation. This may be explained by the fact that metabolic conversion of LOS to EXP depends on hepatic metabolism which is known to be diminished by inflammatory conditions. This may be of clinical importance considering that the antihypertensive effect of LOS stems mainly from EXP. (Supported by Novartis Switzerland).

26. PHARMACOKINETICS OF VALSARTAN IN RHEUMATOID ARTHRITIS PATIENTS.

Noriko Daneshtalab, **Richard Z. Lewanczuk Anthony S. Russell, and Fakhreddin Jamali.**
Faculties of Pharmacy & Pharmaceutical Sciences and Medicine, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Inflammatory conditions reduce clearance of many cardiovascular drugs depending upon the severity of the disease. We studied the effect of the severity of rheumatoid arthritis (RA), as an example of an inflammatory condition, on the pharmacokinetics (PK) of a non-cytochrome P450 metabolized orally active, non-peptide angiotensin II receptor antagonist valsartan. **Methods:** A total of 23 male or female patients aged 18-72 with RA were recruited into the study. They included 14 patients with active arthritis and 9 with arthritis in remission. Subjects took single oral doses of 160 mg valsartan tablets with 240 ml of distilled water. Blood samples were collected immediately prior to drug administration (time 0) and at 15, 30, 45 min, 1, 2, 3, 4, 5, 8, and 12 h. The samples were spun down and the plasma was collected and stored at -80°C until analyzed. Valsartan was measured using a validated reverse phase HPLC method after liquid-liquid extraction. Pharmacokinetics of valsartan was delineated by noncompartmental analysis. **Results:** The following arithmetic mean±SD PK parameters were observed:

Parameter	**Active RA n=14**	**Remission RA n=9**	**Healthy Subjects (160 mg)***
AUC $_{0-\infty}$, mg.hr/L	14.9 ± 8.8	18.11 ± 16.3	21.2 ± 6.5
$t_{½}$, hr	3.2 ± 0.9	5.1 ± 3.8	5. 8 ± 2.4
C_{max}, mg/L	2.8 ± 1.7	2.7 ± 1.8	3.3 ± 1.0
t_{max}, hr	2.8 ± 0.7	2.8 ± 0.7	1.3 - 5.2
Cl/F, L/hr			
Arithmetic	14.8 ± 7.9	14.7 ± 9.0	Not available
Harmonic	10.8	8.8	7.5

Disease severity had no significant effect on PK of valsartan. In addition, the PK indices calculated in RA patients appear to be close to that reported in the literature for subjects with no inflammatory conditions. **Conclusion:** There is no significant difference in the PK of valsartan among active RA, RA in remission, and subject without inflammatory conditions. This may be explained by the fact that valsartan clearance does not depend on hepatic metabolism that is known to be diminished by inflammatory conditions. This may be of clinical importance considering the dependency of other commonly used cardiovascular drugs on hepatic clearance. (Supported by Novartis Switzerland).

27. ANTI-TNF INFLIXIMAB PREVENTS THE EFFECTS OF INFLAMMATION ON THE PHARMACODYNAMICS OF ATENOLOL IN RATS.

Kassem Abouchehade, **Anthony S. Russell, Fakhreddin Jamali. Faculty of Pharmacy & Pharmaceutical Sciences; Faculty of Medicine, Department of Rheumatology; University of Alberta, Edmonton, Alberta, Canada.**

Purpose. Inflammation down-regulates β-adrenergic receptors. We determined the effect of acute inflammation on response to the β-adrenergic antagonist, atenolol in the presence and absence of reduced tumour necrosis factor (TNF). Infliximab is an anti-TNF drug. **Methods.** Adult Sprague-Dawley rats were cannulated for blood sampling. ECG electrodes were attached for PR interval and heart rate measurements. Rats were divided into three groups (n=6/group): Control, Inflamed (IFNα_{2a}, *sc* 12 h and 3 h pre-atenolol) and Inflamed+Infliximab (3 mg/kg *iv* 12 h pre-atenolol). Prior to and 0-6 h following 5 mg/kg, *i.v.* atenolol, ECG was reported and blood samples collected. Plasma samples were assayed for nitrite (nitric oxide metabolite) and atenolol plasma levels by the Griess and an HPLC method, respectively. Statistical analysis performed using unpaired Student's *t*-test and ANOVA. **Results.** Plasma nitrite and TNFα levels were elevated 3-folds and 4-folds in inflamed rats respectively ($p<0.01$), an indication of nitric oxide and TNFα over-production. Atenolol caused significant prolongation of PR intervals in all rats peaking after approximately 4 h. However, the PR interval prolongation to atenolol was significantly less in inflamed rats (9±4 %) as compared to controls (19±7%) confirming receptor down-regulation by inflammation. Infliximab blocked the diminishing effect of inflammation on atenolol action. Atenolol prolonged PR interval in the Inflamed+infliximab group by 23±7%, which was not significantly different from controls. Inflammation had no effects on atenolol pharmacokinetics (Control *vs.* Inflamed: AUC: 147±30 *vs.* 139±10 $\mu g.mL^{-1}.h$; $t_{1/2}$: 2.9 ± 1.2 *vs.* 2.8 ± 0.8 h, respectively). There was no significant difference among the groups with respect to the heart rate post-atenolol dose. **Conclusion.** Inflammation caused reduced response to atenolol. This was not caused by altered drug pharmacokinetics. Anti-TNF treatment prevented the induction of inflammation, preserved normal healthy conditions in rats and preserved normal responses to atenolol. The reduced response to atenolol may be related to increased expression of the pro-inflammatory mediators nitric oxide and TNFα. (*Supported by* MRC 983587).

28. THERAPEUTIC INEQUIVALENCE OF ORALLY ADMINISTERED CHLORTETRACYCLINE AND OXYTETRACYCLINE IN PIGS.

Jérôme R.E. del Castillo, **Jean-Guy Besner, Université de Montréal, Faculté de pharmacie, Montréal, Quebec, Canada.**

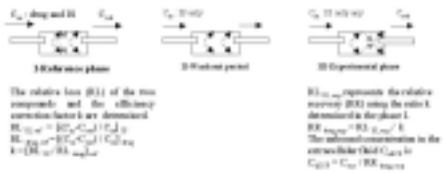
Purpose: With nearly 50 years of use, the tetracyclines remain the most heavily prescribed antibiotics in veterinary medicine, particularly in pigs. Chlortetracycline (CTC) and oxytetracycline (OTC) are considered to be therapeutic equivalents when administered in feed at equal doses but this has never been proven experimentally. **Methods:** In this study, CTC and OTC have been administered intravenously and in feed to 5-week-old pigs (n=34/drug), which were afterwards put on a dosing regimen at 12 h intervals for 6 days (dose=22, 44, 66 or 88 mg/kg of drug hydrochloride; n>8/dose/drug). Non-compartmental pharmacokinetic analysis was applied on individual sets of plasma concentrations associated with single i.v. and in-feed dosages, and with the drug regimen day 4 morning dosage. On day 5 of the therapeutic regimen, medicated and control (n=36) pigs were inoculated intranasally with an *A. pleuropneumoniae* suspension (MIC=2 mg/L for both drugs), and clinical signs were monitored for 22 h after infection, after which pigs received euthanasia to determine the extent of lung lesions and drug concentration in lung tissue. Plasma and tissue concentrations of drug were determined with validated HPLC methods. **Results:** CTC and OTC had significantly different clearances (0.47 vs. 0.38 L/h/kg, resp.), oral bioavailabilities (28% vs. 5%, resp.), and volumes of distributions (3.81 vs. 2.73 L/kg, resp.). CTC average plasma concentration at steady state (Cavg) was 4-fold higher than that of OTC when administered at equal in-feed doses. At necropsy, lung concentrations of both drugs were approximately 10% greater than their respective plasma concentrations. Median clinical scores following infection in the CTC, OTC and control group were respectively 1, 10.25 and 9.5 ($P<0.0001$). In addition, the median extent of lesions in these groups accounted respectively for 1.3%, 12.7% and 21.1% of lungs ($P<0.0001$). Clinical score and the relative extent of lung lesions could be predicted as a function of plasma concentration with the inhibitory Hill model. In both cases, transition between baseline and maximal prophylactic effect occurred between 0.25 and 1.25 mg/L, with IC_{50} were approximately 0.75 mg/L. **Conclusion:** These results show that feed-administered CTC and OTC are incompletely absorbed in pigs. Their pharmacokinetic properties are significantly different, and so are their Cavg and their prophylactic effects. Therefore, feed-administered CTC and OTC should not be considered to possess neither the same pharmacokinetic behaviour nor the same prophylactic efficacies in pigs.

29. DETERMINATION BY MICRODIALYSIS OF ROCURONIUM CONCENTRATION IN THE MUSCLE BIOPHASE IN ANESTHETIZED DOGS.

***Samia Ezzine*, Nobuharu Yamaguchi, France Varin and. François Donati. Faculté de pharmacie; Département d'anesthésie, Centre Hospitalier, Université de Montréal, Montréal, Quebec, Canada.**

Purpose: According to their physicochemical and pharmacokinetic characteristics, some neuromuscular blocking agents (NMBAs) may undergo chemical or enzymatic hydrolysis. Measurement of drug concentration in the biophase (unbound concentration in the extracellular fluid) should provide an answer to that assumption. The aim of this study was to develop a microdialysis technique that allowed quantification of NMBAs concentrations in the muscle tissue of anesthetized dogs. **Methods**: The reverse microdialysis or retrodialysis approach was performed in eight anesthetized dogs. Rocuronium, was chosen as the study drug because it is eliminated exclusively by organ dependent pathway. Vecuronium was used as the internal standard (IS) for rocuronium. The flow rate was 2µl/min. Microdialysis probes were inserted in the gastrocnemius muscle and calibrated *in vivo* as shown in the figure below (reference phase). Carotid artery and femoral vein were cannulated for blood sampling. A baseline blood sample was drawn for protein binding determination. One hour after starting IV rocuronium perfusion (wash out period), three microdialysis samples were collected during a 2 hour period (experimental phase). In the middle of each 40 minute sampling interval, arterial and venous blood samples were drawn for steady-state confirmation. Microdialysis samples and the decanted plasma were frozen at -70°C until HPLC analysis.

Fig: In vivo microdialysis study design



Results: Preliminary experiments for the *in vitro* calibration (n=3) showed that the average probe efficiency RR of rocuronium was 50% ± 19% and the average RL of vecuronium was 48 ± 21. Recovery values, efficiency correction factor k as well as extracellular concentrations *in vivo* are represented in the table below as mean ± SD.

Table: In vivo microdialysis results

Reference phase (n=5)			Experimental phase (n=5)	
$RL_{drug, ref}$ %	$RL_{IS, ref}$%	k	$RL_{IS, exp}$%	$RR_{drug, exp}$% $C_{uECF}/Cpss_u$
41 ± 20	37 ± 19	0.9 ± 0.5	42 ± 24	43 ± 9 94 ± 11

Discussion: Our study shows that rocuronium unbound extracellular muscle concentrations are in good agreement with steady-state plasma concentrations corrected for the protein unbound fraction; C_{uECF} / $Cpss_u$ was 94 %. As rocuronium is not likely to undergo peripheral elimination, these results proved that the retrodialysis is a reliable technique for assessing biophase concentrations in the dog. The advantage of combining a reference phase prior to the experimental phase (combined retrodialysis) enables to assess changes in probe efficiency over time and to correct the C_{uECF} for the ratio efficiency correction factor in each dog. Using this technique, we will be able to quantify extracellular muscle tissue concentrations of NMBAs and provide an answer to what extent some NMBAs undergo peripheral elimination. These results should foster the development of new pharmacokinetic models.

30. HEAT TREATMENT OF AMPHOTERICIN B MODIFIES ITS SERUM PHARMACOKINETICS, TISSUE DISTRIBUTION AND RENAL TOXICITY FOLLOWING A SINGLE INTRAVENOUS DOSE TO RABBITS.

***E. Kwong*, M. Ramaswamy, S. C. Hartsel, K. M. Wasan, E. A. Bauer. University of British Columbia, Vancouver, British Columbia, Canada; University of Wisconsin-Eau Claire, Eau Claire, Wisconsin, USA.**

Purpose: The purpose of this investigation was to determine the serum pharmacokinetics, tissue distribution and renal toxicity of amphotericin B (AmpB) following a single dose of Fungizone (FZ) and a heat-treated form of FZ (HFZ) to rabbits. **Methods:** FZ solutions were heated at 70°C for 20 minutes to produce HFZ. A single intravenous dose of FZ or HFZ at 1mg/kg was administered to New Zealand white female rabbits. Blood samples were obtained before drug administration and serially thereafter. After the final blood sample, each rabbit was humanely sacrificed and the right kidney, spleen, lung, liver, and heart were harvested for AmpB analysis. Serum creatinine levels were measured before and 10 hours after drug administration. AmpB concentration in the serum and tissues were analyzed using HPLC. **Results:** FZ administration to rabbits resulted in a greater than 50% increase in serum creatinine concentrations compared to baseline. However, HFZ administration resulted in no difference in serum creatinine concentrations compared to baseline. AmpB AUC after HFZ administration was significantly lower compared with the AmpB AUC in rabbits administered FZ [HFZ, 3.3 +/- 0.4 µg h/ml (n=3) *vs*. FZ, 11.3 +/- 2.5 µg h/ml (n=6)]. However, AmpB systemic CL was significantly greater in rabbits administered HFZ compared to rabbits administered FZ [HFZ, 303 +/- 39 ml/h/kg (n=3) *vs*. FZ, 88.9 +/- 19.4 ml/h/kg (n=6)] without any differences in V_{ss}. Only liver concentrations of AmpB were significantly lower in rabbits administered FZ than in rabbits administered HFZ [FZ, 3.1 +/- 0.7 µg AmpB/g tissue (n=6) *vs*. HFZ, 5.0 +/- 1.6 µg AmpB/g tissue (n=3)]. **Conclusions:** These finding suggest that the pharmacokinetics, tissue distribution and renal toxicity of AmpB are different following administration of HFZ compared with FZ to rabbits. **Acknowledgements.** Canadian Institutes of Health Research (CIHR; #MT-14484 to KMW).

31. A NEW NON-INSTANT MIXING PHARMACOKINETIC MODEL TO REPRESENT ORAL ADMINISTRATION OF CYCLOSPORIN (NEORAL).

Andy Nong, **Laurent Claret. Faculté de Pharmacie, Université de Montréal, Montréal, Quebec, Canada.**

PURPOSE: Pharmacokinetic and mathematical models were developed to describe the absorption, distribution, metabolism and elimination processes in an organism. Cyclosporin A is a potent immunosuppressive agent used to prevent allogenic transplant rejection and to treat some autoimmune diseases. Pharmacokinetic profiles of cyclosporin are often described by deterministic compartmental models. However, none of these models are sufficiently precise to calculate an optimal drug regimen. We believe that the pharmacokinetic processes of cyclosporin are non-homogenous and can not be properly represented by these classical models. **METHODS**: In this study, we introduced a stochastic compartmental model to represent cyclosporin data. Many stochastic models were developed based upon the non homogenous nature of the pharmacokinetic process of cyclosporin and the non-instant mixing effect observed during its absorption. Based on real data observed after oral administration of cyclosporin (Neoral®, Novartis) on 10 patients, we compared the fit of all models with the root mean squared estimator (RMSE), Akaike's information criteria (AIC), and Fisher's correlation-covariance matrix. **RESULTS**: The stochastic one compartment model with a non-instant mixing depot compartment has the lowest criterion (RMSE=0.1667, AIC=114.42) compared to the best deterministic compartmental model with lag time (RMSE=0.2784, AIC=126.93). The stochastic model also has a lower residual error on its estimated parameters (CV of 5-15%) than the deterministic models (CV of 10 - 100%). **CONCLUSION**: The non-instant mixing stochastic model is a better description of the variable pharmacokinetic process of cyclosporin. The use of a stochastic compartmental model can reduce the number of parameters and represent a system with a more realistic point of view.

32. BIOEQUIVALENCE DETERMINATION IN THE ASSESSMENT OF THERAPEUTIC EQUIVALENCE OF WARFARIN PRODUCTS.

Yu Chung Tsang, **Jiuhong Zha, and Michael Spino. Apotex Inc., Weston, Ontario, Canada.**

Purpose. Several generic warfarin products have been designated as bioequivalent to the brand, Coumadin, in the US and Canada. All used bioequivalence (BE) studies to demonstrate therapeutic equivalence and a few, also conducted confirmatory clinical efficacy studies. The question of the relevance of additional clinical testing to demonstrate therapeutic equivalence has been raised. The purpose of this study was to examine the appropriateness of using pharmacokinetic (PK) measures for the evaluation of therapeutic equivalence between warfarin products. **Methods.** From the results of a published BE study in which Coumadin 5 mg tablets were administered to the same subjects twice (Yacobi et al, J Clin Pharmacol 2000; 40:826-835), the mean $AUC_{0\text{-}inf}$ and its intra-subject variance for Coumadin were obtained. Using the intra-subject variance as the source of variation, 1000 individual $AUC_{0\text{-}inf}$ values were randomly generated around the mean $AUC_{0\text{-}inf}$. Based on the PK/pharmacodynamic (PD) model proposed by Murray et al (Ther Drug Monit 1987; 9:1-5) to describe the response to warfarin, the corresponding prothrombin complex activity (PCA) and the prothrombin time ratio (PTR) were calculated for each simulated $AUC_{0\text{-}inf}$. The INR value was then determined for each PTR ($INR = PTR^{ISI}$) with an ISI value that will yield an average INR value of about 2.5 for the simulation data. The variances of PCA, PTR and INR were then estimated in order to determine the impact of random variation of $AUC_{0\text{-}inf}$ on the variability of each PD variable. Similarly, using the intra-subject variance of INR for Coumadin obtained from a published study of patients with atrial fibrillation (Neutel et al, Cardiovasc Rev Rep 1998; 19:49-59), 1000 INR values were randomly generated. The PCA value was then calculated for each randomly generated INR; the total variance of the calculated PCA values was also determined. The variance of PCA due to non-PK factors was estimated by the difference between this total variance and the variance caused by variable $AUC_{0\text{-}inf}$. **Results.** From the literature data, the intra-subject cv of 7.3% and 17.9% were obtained for $AUC_{0\text{-}inf}$ and INR respectively. Based on the simulation data, the intra-subject cv of PCA due to variable $AUC_{0\text{-}inf}$ and non-PK factors were estimated to be 6.6% and 13.4% respectively, indicating a much higher variability of PD response due to non-PK than to PK factors. A similar degree of difference in intra-subject cv between PK and non-PK factors was also observed for INR. The spread of simulation data due to variable $AUC_{0\text{-}inf}$ was completely engulfed within that caused by non-PK factors for each PD variable. **Conclusion.** BE determination is an appropriate measure of difference between warfarin products due to the much lower variability of PK measures than that of PD measures. Thus it is reasonable to conclude that generic warfarin, which is bioequivalent to Coumadin will be therapeutically equivalent as well. Furthermore, the greater variation of INR than AUCs decreases the discriminating abilities of clinical efficacy studies in the assessment of therapeutic equivalence of warfarin products. This observation is predictable because INR results are a function of both the PK data of a formulation as well as non-drug related factors that influence this clinical surrogate.

33. STEREOSELECTIVE PHARMACOKINETICS AND ABSOLUTE BIOAVAILABILITY OF DOXEPIN IN HEALTHY VOLUNTEERS.

J.-H. Yan, **J. W. Hubbard, G. McKay, E. D. Korchinski, and K. K. Midha. College of Pharmacy and Nutrition, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.**

Purpose. To define for the first time, the stereoselective pharmacokinetics of doxepin after single intravenous doses in healthy volunteers, and to investigate the influence of route of administration on the ratio distortion of geometric isomers of N-desmethyldoxepin. **Methods.** It was a randomized, two-way crossover design in which 12 healthy male volunteers were given single doses of commercial doxepin intravenously and orally on two occasions separated by two weeks. Serial plasma samples and total urine (12 hour segments) were collected through 120 hours post dosing. Plasma and urine samples were analyzed by validated, stereoselective HPLC procedures for doxepin and N-desmethyldoxepin cis- and trans-isomers. Plasma concentration versus time data of doxepin isomers was subject to noncompartmental and/or compartmental pharmacokinetic analyses. **Results.** The mean intravenous data of doxepin isomers fit a two compartment model (iv bolus, no lag time, 1st order elimination). The mean absolute oral bioavailability was 29% for both doxepin isomers, which is consistent with earlier estimates based on Gibaldi's method for calculation of hepatic availability. After either intravenous or oral administration, plasma concentrations and urinary recoveries of trans-doxepin were higher than that of cis-doxepin such that the cis:trans ratio was approximately the same as that in the administered dosage form (16:84), whereas plasma AUCs and urinary recoveries of cis-N-desmethyldoxepin tended to be much higher than those of its geometric antipode. **Conclusion.** The observed oral bioavailability (29%) seemed to result largely from metabolism in hepatic cells. Both isomers of doxepin were extensively eliminated by metabolism such that renal clearances were very low after administration by either route. There was no apparent influence of route of administration on the observed ratio distortion of N-desmethyldoxepin isomers. *This work was presented as a poster at the AAPS annual meeting on Nov 01, 2000.*

34. SPATIAL EFFECTS IN MODELING PHARMACOKINETICS OF RAPID ACTION DRUGS.

Patrick Lafrance, **Vincent Lemaire, France Varin, François Donati, Jacques Bélair, Fahima Nekka. Département de Mathématiques et de Statistiques; Centre de Recherches Mathématiques; Faculté de Pharmacie ; Département d'anesthésiologie, Université de Montréal, Montréal, Québec, Canada.**

Purpose Neuromuscular blocking agents are rapid action drugs that are used in anaesthesia during surgery. Employed to insure suitable conditions for intubations, their maximal effect should occur as rapidly as possible after intravenous administration, typically 60 to 90 seconds. With such rapid action, predictions of early plasma concentrations following administration are essential to evaluate accurately pharmacokinetic and pharmacodynamic parameters. Conventional pharmacokinetic models, however, cannot provide satisfactory results for this prediction of the early phase. Compartmental models are unable to appropriately describe the very fast initial rise in the concentration curve of these rapidly acting drugs, because of non uniform mixing in the compartment. **Methods** One remedial approach is to split the compartment playing the role of plasma in two parts, and to try to fit the ensuing model piecewise. Although an acceptable statistical fit can be reached by this procedure, making predictions in continuous time is unwieldy and complex. We will present an alternate approach which incorporates a more physiological treatment of the initial, high velocity transients in the drug concentration. The model we propose can be used to describe the plasma concentration of the drug from the initial moment of injection of the bolus all the way to its elimination down to detectable levels. **Results** This model takes into account the initial physiological and spatial heterogeneity of the drug concentration in the plasma. It takes the form of a diffusion equation on a circular domain, with a time-dependent leakage term. Its solution is then determined, analytically, and a comparison is made, by adjusting some of the parameters in the model with clinical data previously published on two particular agents, vecuronium and doxacurium. **Conclusion** Our model provides a better description of the time course of concentration profiles of fast-acting agents, and could therefore be used to better understand their pharmacokinetic and pharmacodynamic characteristics.

35. IN VITRO-IN VIVO CORRELATION OF THE ELIMINATION OF SUCCINYLCHOLINE IN ANESTHETIZED PATIENTS.

***J. Roy*, F. Varin, D. Boismenu, and F. Donati.**
Faculté de Pharmacie, Université de Montréal; The Mass Spectrometry Unit, McGill University; Département d'anesthésie et de réanimation, Centre Hospitalier de l' Université de Montréal, Pavillon Hôtel-Dieu; Montréal, Québec, Canada.

Purpose: Succinylcholine (SUX) is a short-acting depolarizing neuromuscular blocking agent widely used in anaesthesia since 1951, but its pharmacokinetics are poorly documented. SUX is hydrolysed by plasma cholinesterase and its metabolism is subject to genetic variants that may lead to prolongation of muscle paralysis with apnoea. The purpose of this study was first to develop an assay for SUX with an adequate sensitivity and to compare its *in vivo* elimination half-life with that obtained for its *in vitro* degradation in plasma. **Methods**: Following a written and informed consent, 6 ASA I-II patients (range: 36-56 yrs) participated in the study. Anaesthesia was induced with remifentanil (0.5-1 μg/kg) and propofol (2-3 mg/kg) and maintained with propofol 100-200 μg/kg/min, remifentanil and O_2. Once a stable state of anaesthesia was established, a 48 ml of blood was drawn from each patient for the *in vitro* incubations and then, a 1 mg/kg bolus dose of SUX Chloride administered over two seconds. Arterial blood samples were collected every five seconds for the first two minutes, and at every minute for eight minutes. Within four hours of plasma collection, *in vitro* incubations were carried out at a concentration of 2-3 μg/ml. Samples were collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes. Quantification of the plasma concentrations of SUX was carried out using a validated mass spectrometric assay (1). An extraction method compatible with direct infusion inlet was developed with an analysis cycle time of 7-8 min. SUX was extracted from human plasma on C-1 solid-phase cartridges and analyzed using positive ion electrospray tandem mass spectrometry. SUX plasma concentrations were determined by a stable isotope dilution assay using hexadeuterosuccinylcholine diiodide (SUXd6) as the internal standard. Calibrations curves were linear from 25-4000 ng/ml. For intraday precision, CV were ≤ 6% and accuracy ranged from 98 to 103 %. For interday precision, CV were ≤ 10% and accuracy ranged from 90 to 102%. **Results**: Non compartmental PK analysis was carried out for the determination of the *in vitro* and *in vivo* parameters, as presented in the table below.

In vivo parameters	Mean ± SD (CV %)	In vitro parameter	Mean ± SD (CV %)
Kel (min^{-1})	0.97 ± 0.30 (31)	K in vitro (min^{-1})	1.07 ± 0.49 (46)
Cl (L/kg/min)		0.037 ± 0.008 (21)	
Vss (L/kg)	0.033 ± 0.003 (9)		

There is a linear correlation (r^2=0.94) between the K *in vitro* and the Kel for the six patients studied. **Conclusion**: The strong correlation between the K *in vitro* and the Kel for SUX suggest that the *in vitro* plasma degradation rate is a good predictor of the role of plasma cholinesterases on the overall *in vivo* rate of elimination of SUX. **References**:1-Roy JJ, Boismenu D, Gao H, Mamer OA, Varin F. Analytical Biochemistry 2001;290(2):238-45.

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