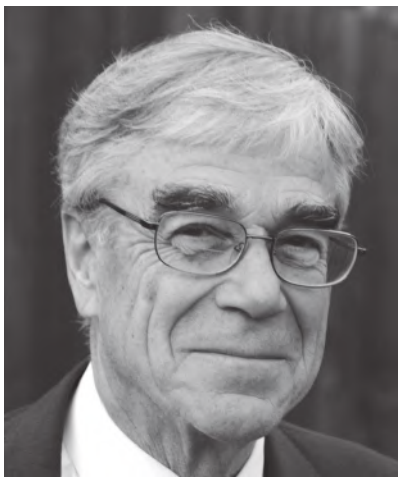
New Zealand Institute of Chemistry

supporting chemical sciences

April News

NEWS

New Zealand Canterbury and Otago graduate, Em. Prof. *Robin* ***Clark***, CNZM, FRS. Hon. FRSNZ, who has been at University College London for many years, was the inaugural recipient of the biennial Franklin-Lavoisier Prize of the Maison de la Chimie (Paris) and the Chemical Heritage Foundation (Philadelphia). The prize was presented in late January in Paris, where he addressed a special meeting of the Maison on *Spectroscopy in Art and Science*. The name of the award is taken from Benjamin Franklin (American statesman, inventor and scientist) and Antoine Lavoisier (French scientist regarded as the father of modern chemistry).



Robin's research is in inorganic chemistry and spectroscopy; more recently on metal-metal bonded complexes, mixed-valence chemistry, infrared, Raman and resonance Raman spectroscopy, matrix isolation spectroscopy, spectroelectrochemistry, and pigment studies mainly by Raman microscopy. This has led to the publication of more than 500 scientific papers, 3 books, and 36 edited books. Robin has held visiting professorships in 11 countries and has lectured at over 350 universities and institutions in 36 countries throughout the world. He has served on many national committees, including the councils of the Royal Society, the Royal Institution of Great Britain, University College, London, and the Senate of the University of London. He has chaired the Steering Committee of the International Conferences on Raman Spectroscopy. He visited various centres, including the Chemistry and the MacDiarmid Institute in Wellington in February.

NZIC AWARDS

Nominations for the following 2009 awards are now sought:

Easterfield Award,

Fonterra Prize for Applied and Industrial Chemistry,

Maurice Wilkins Prize for Chemical Research,

ABA Books Denis Hogan Chemical Education Award.

The closing date with the NZIC Secretariat is 30 June 2009. Details and method of nomination/application can be found at: www.nzic.org.nz

NZIC Membership

Council congratulates Dr *Jadranka* ***Travas-Sejdic*** of the Auckland Branch who was elected to Fellowship at the February Council meeting.

New Members:

Rinaldo ***Azzara***, *Devon* ***Britow***, *Bernard C*. ***Kimble***, *Johannes* ***Reynisson***, *Vijayalekshmi* ***Sarojini*** and *Ken* ***Taylor*** join the Auckland Branch; *Kate* ***Palmano*** to Manawatu and *Aidan G*. ***Young*** to Wellington; *Nigel I*. ***Joyce***, *Andre* ***Lamarque***, *Shazia* ***Zaman***, and *Neroli* ***Ayling*** join the Canterbury Branch; and *Joseph* ***Lane*** and *Jacob* ***Shepherd*** have joined the Otago Branch.

New Student Members:

Louise ***Stubbing*** (AKL), *David I*. ***Weller*** (WEL) *Humphrey* ***Feltham***, *Samuel* ***Lind*** and *Syahidah A*. ***Muhammad*** (OTA).

NZIC News from Council

The first royalties from NZIC's partnership in the journal *Physical Chemistry Chemical Physics* were received by the Secretariat just before last Christmas for the 2008 year; five NZ-authored manuscripts appeared. Approved Branch grants for 2009 are: Auckland $2000, Waikato $3000, Manawatu $3000, Wellington $3600, Canterbury $3000, Otago $3000, Chem. Educ. Group $4000. Members will have noticed from their recent annual accounts that subscriptions for 2009 remain unchanged; please make early payment.

Council has been concerned about moves of the New Zealand Innovation Centre in Auckland to use the acronym NZIC and the potential confusion that this would create. Discussions are taking place and alternatives for the Auckland Centre have been offered, *e.g.* NZCI.

Dr *Jan* ***Wikaira*** has negotiated with Radio NZ to broadcast *casual chemistry conversations* of five minute's duration. Thus, there is a need for chemistry communicators to become involved, as well as ideas for these and longer sessions which promote chemistry innovation. Contact Jan at: *jan.wikaira@canterbury.ac.nz*

The Waikato Branch has accepted Council's invitation to host the 2010/11 conference. The organizing committee will be convened by *Michèle* ***Prinsep*** and a 2011 date is likely.

New Zealand is Different

Following the article by Brian Easton in *The Listener* (**2009**, *Jan 24-30*, 54), which was effectively a review of NZIC's book *New Zealand is Different*, Council is keen to follow up the renewed interest this has created. It has adopted production of ***Volume 2*** as a project for the 2011 International Year of Chemistry; chapter authors are sought – contact the Secretariat.

Chemical Education Specialist Group

The NZIC Chemical Education Specialist Group, under the guardianship of Dr *Suzanne* ***Boniface*** (Wellington), now actively seeks those NZIC members with interests in chemical education to join the group. Please send your name and e-mail details to the NZIC Office: *rendle@xtra.co.nz* In addition, chemistry educators who are not members of the Institute may also join the specialist group and we encourage members to use their influence with their teacher colleagues to get them to join.

BRANCH NEWS

AUCKLAND

The Branch AGM was held on December 9 last, a little later than usual. The speaker, Prof *Andrew* ***Waterhouse*** (UC-Davis) was recently appointed Honorary Professor with the University's Wine Science programme and he addressed us on *Understanding wine oxidation*.

The Branch had two meetings in the same week of February. Firstly on Feb 18, Prof *Julie* ***MacPherson*** (Warwick, UK), a leading expert in applications of miniature electrodes, gave a very interesting talk on *High resolution electrochemical imaging: building electrodes into atomic force microscopy (AFM) tips*. Then on Feb 20, Prof *Martin* ***Banwell*** gave his RSC-RACI-NZIC Australasian Lecturer entitled *A little bit of strain can be good for you: gem-dihalogenocyclopropanes as building blocks for chemical synthesis*. Both talks were very well attended, and the audiences were complementary as well as complimentary.

University of Auckland- Chemistry

The Chemistry Department celebrated 125 years on 16 December 2008. A large number of former staff returned to mark the event.

Two staff members, *Jadranka* ***Travas-Sejdik*** and *Paul* ***Kilmartin***, were promoted to A/Prof, while *Laura* ***Nicolau*** and *David* ***Barker*** were promoted to Senior Lecturer positions. Dr. *Cather* ***Simpson*** has been appointed as Director of the Laser Laboratory, a joint appointment between Chemistry, Physics, and the Science Faculty. Finally, Dr. *Johannes* ***Reynisson*** has joined us from Iceland *via* the UK. Dr. Reynisson holds a joint appointment between Chemistry and the Auckland Bioengineering Institute as a lecturer of computational chemistry and molecular modelling.

Prof *Ekkehardt* ***Hahn*** (Munster) gave a wonderful chemical demonstration lecture entitled *Natural Chemistry - Reactions with Air, Mineral Water and Orange Juice* last December. His lecture was sponsored by the NZ-German Science Circle. Dr *Don* ***Eigler*** visited on February 17 and spoke on The Small Frontier focussing on the application of Scanning Tunnelling Microscopes to manipulate small structures, which he first demonstrated at IBM with the case of individual xenon atoms in 1989.

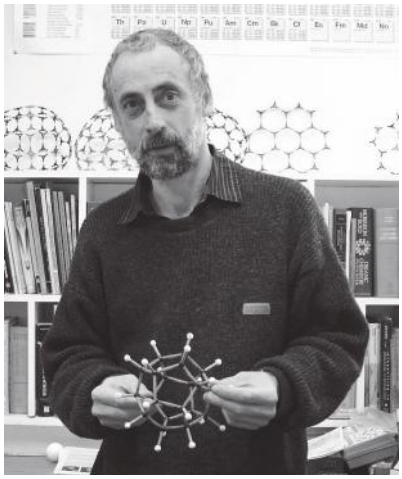
PhD student *Andrew* ***Dalebrook*** won a best poster award at the IC08 RACI/NZIC Inorganic Chemistry Conference in Christchurch for *Syntheses and reactions of iridabenzenes with sulfur functions*. Winners of the Department's 2nd-year PhD student poster competition last December were *Cary* ***Lam*** (1st), *Raoul* ***Peltier*** (2nd) and *Danae* ***Larsen*** (3rd).

CANTERBURY

Michael ***Edmonds*** (CPIT) is the new Chairperson of the Canterbury Branch with *Paul* ***Kruger*** as Secretary and *Bill* ***Swallow*** as Treasurer.

University of Canterbury

Prof *Peter* ***Steel*** was the recipient of the 2008 University Research Medal, UC's highest recognition of outstanding research. Over the last 20 years Peter has made seminal contributions in several areas of chemistry including organic chemistry, co-ordination chemistry, organometallic chemistry, and X-ray crystallography. In the past decade he has been a pioneer of metallosupramolecular chemistry. The 2008 Applied Biosystems Award, the premier prize of the NZ Society of Biochemistry and Molecular Biology, was awarded to *Emily* ***Parker*** at the NZIC joint conference last December (see separate report). She was also successful in gaining Bright Ideas funding to support a project for new anti-TB drugs. As well as being promoted to Professor, *Alison* ***Downard*** has been awarded $15,000 funding from the Dumont d'Urville NZ/France S & T Support Programme. This will allow Alison and PhD student *Andrew* ***Gross*** to visit the Université Josef Fourier (Grenoble) to establish a collaborative project with *Jean-Claude* ***Moutet*** and *Pierre* ***Labbe***, entitled *Electrochemical quartz crystal microbalance with dissipation monitoring as a new tool for studying nanostructured carbon surfaces*. Researchers from the French team will also make visits to UC. Other promotions were *Owen* ***Curnow*** and *Paul* ***Kruger*** to Associate Professor and *Jan* ***Wikaira*** to Senior Lecturer. Paul Kruger, featured on Nature's chemistry blog, *The Sceptical Chymist*, as part of a short interview feature called *Reactions*: (see: http://blogs.nature.com/thescepticalchymist/2008/11/reactions_paul_kruger.html).



Prof Peter Steel

Recent student successes include *Jan-Yves* ***Ruzicka*** and *Thomas* ***Lechte*** who have won UC Doctoral Scholarships. *Francine* ***Smith*** was awarded the 2009 Environment Canterbury Resource Management Postgraduate Scholarship for research on cyanobacteria. Supervisor *Sally* ***Gaw*** has received a grant of $10,000 from the Brian Mason Trust and the work is being undertaken in collaboration with *Faradina* ***Merican***, a PhD student in Biology and a large, cross-disciplinary supervisory group. *Claire* ***Marshall***, also working with Sally, won the 2009 Sadie Balkind Scholarship from the Federation of Graduate Women. *Phil* ***Emnet***, a UC University Prize awardee, and *Kat* ***Dewey***, a UC Masters Scholar are two other members of this environmental chemistry group.

The Department of Chemistry awards for 2008 were: NZIC Chemistry Prize (200 level): *Michael* ***O'Donnell***, Haydon Prize in Chemistry (300 level): *Caleb* ***Allpress***, The Jack Fergusson Prize (300-level labs): *Claire* ***Marshall***, The C E Fenwick Prize (400-level demonstrator): *Katrina* ***Dewey***, C E Fenwick Prizes in Chemistry (400 level): *Philipp* ***Emnet***, Cuth J Wilkins Prize (MSc thesis): *Hemi* ***Cumming***, Dr Gregory S.C. Hii Prize (Organic PhD): *David* ***Tran***, Ralph H. Earle Jnr Seminar Prize (PhD): *James* ***Bull*** and *David* ***Garrett***.

Ruomeng ***Wang***, *Henry* ***Toombs-Ruane***, *Jayne* ***Gulbransen***, and *Chris* ***Hawes*** have been offered College of Science Scholarships and *Solomon Wasseyehun* ***Kelemu*** has taken up a scholarship at ANU.

Recent MSc completions include *Louise* ***Crawley*** (Hons. – Environmental Sci.) for research on *Applications of non-dispersive infrared (NDIR) spectroscopy to the measurement of atmospheric trace gases* (supervised by *Peter* ***Harland*** and *Majed* ***Alghamdi***).

A Retirement …

A function to mark Prof *John* ***Blunt***'s retirement was held in early February. John becomes an Emeritus Professor in the Department and so we expect to see plenty of him. Murray Munro, John's long-time friend and research collaborator, ran the function with input from many others. Accolades from around the world formed the basis of a remarkable slide show. Amongst the attendees were the first three students, *Ian* ***Miller***, John and *Colin* ***Freeman*** granted direct entry into year 2 of the Canterbury BSc Hons programme in 1961. John and Colin have forged very successful academic careers as members of this department, while Ian was a Group Leader at DSIR before establishing the independent company Carina Chemical Laboratories Ltd.

….and a Welcome

Ian ***Shaw***, our first PVC (Science), has taken a position as Professor of Toxicology in the Chemistry Department. As well as teaching in our environmental science, biochemistry, and general chemistry programmes, Ian will be continuing his research in to the effects of estrogen mimics on growth and development, particularly foetal development, after exposure *in utero*.

John Blunt with Murray Munro at John's farewell.

L-R: Ian Miller, John Blunt and Colin Freeman (courtesy of Sunita Chamyuang)

Recent visitors have included Prof. *Bohari M*. ***Yamin*** (Universiti Kebangsaan, Malaysia) who visited the X-ray lab. Dr *Alexander (Sandy)* ***Briggs*** (University of Victoria, BC) is on three months study leave. Prof *Alison* ***Smith*** (Plant Biochemistry, Cambridge, UK) is a visiting Erskine Fellow. The supervisors of *Carolina* ***Santiago***, Dr *Jacinta* ***Santhanam*** and A/Prof *Jalifah* ***Latif***, have arrived from the School of Pharmacy of UKM, Kuala Lumpur. Paul Kruger hosted two third-year graduate-student visitors, *Tayo* ***Ikotun*** and *Nerissa* ***Viola-Villegas*** from Prof *Rob* ***Doyle***'s lab in Syracuse University. *Maria* ***Johansen***, *Carolina* ***Santiago*** and *Siti Alwani* ***Ariffin*** are working with Murray Munro and John Blunt in the Marine Group for about two months. Maria is a 2nd year PhD student at the Centre for Microbial Biotechnology at the Technical University in Denmark. Siti is a student in Otago's Department of Medicine in Wellington, and Carolina is from the National University of Malaysia where she is currently pursuing an MSc in Health Science. *Ben* ***Flavel***, a recently PhD graduate from Flinders University is here for six months working with *Alison* ***Downard***.

Intermediate Science Day was a hugely successful event run as part of the Rutherford Centenary celebrations. Nearly 500 pupils had such a good time that it is hoped to make this an annual event expanding to accommodate the many more primary and intermediate school pupils in Christchurch. The day started off with four short lectures (***Kerry Swanson***: *How big is small?*, *Chris* ***Fitchett***: *The chemistry of colour*, *Simon* ***Kingham***: *Exploring the digital world*, and *Jenni* ***Adams***: ***What makes the world go round?*** followed by a series of activities. In Chemistry, pupils made slime, investigated pH, tested their measuring skills and saw some exciting demonstrations by postgraduate students and staff.

ESR

The KSC Breath Alcohol Laboratory has successfully accomplished the AS-CLD/LAB *International* assessment, in compliance with the ISO/IEC17025 standard. It becomes an accredited Calibration Laboratory.

The paper *Decision analysis trees for identification of faecal sources in water* (M. L. Devane, B. Gilpin, D.Wood, B. Robson and F. Nourozi) describes a toolbox of tests for investigating faecal pollution for excess *Escherichia coli* in waterways. It won the Hynds Paper of the Year award at the 2008 NZ Water and Waste Conference in September last year.

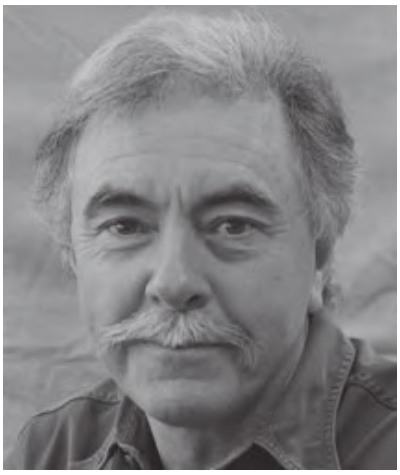
MANAWATU

***Beer Tasting at the* Brewer's Apprentice**

The Manawatu Branch began the year with a social gathering at the local Monteith's Concept Bar, *Brewer's Apprentice*, to sample Greymouth's finest. Monteith's has a range of six beers available all year round and one seasonal offering, all of which we got to taste. We learnt about the characteristics of each beer and then discovered how the taste of each was enhanced by the complimentary nibbles - golden beer battered fish, lime salsa, and rich chocolate pudding - that were provided. Beer tasting, it seems, is as complex as wine tasting. The night was enjoyed by all our members who attended.

Prof *Peter* ***Derrick*** (Head, Institute of Fundamental Sciences), was awarded the 2009 Morrison Medal in Sydney by the Australian and New Zealand Society for Mass Spectrometry. The Mor-

rison Medal honours contributions to the development of mass spectrometry in Australia made by Prof *Jim* ***Morrison*** and is awarded in recognition of significant achievements in an area of mass spectrometry.



Professor Peter Derrick

Congratulations to *Jaime* ***Withers***, *David* ***Martin*** and *Nessha* ***Wise*** who were all awarded Massey Doctoral Scholarships for PhD study, and to *Hillary* ***Corkran*** for being awarded a VC Doctoral Scholarship. Students awarded Summer Scholarships were Jaime Withers and *Kerry* ***Betz-Stablein*** (with *Filichev* ***Vyacheslav***), *Shane* ***Chapman*** (*Gareth* ***Rowlands***) *Janice* ***Moody*** (*Shane* ***Telfer***), *Nick* ***Bent*** (*Mark* ***Waterland***), *Christopher* ***Lepper*** (*Pat* ***Edwards***), and *Daniel* ***Cummins*** (*David* ***Harding***). Filichev Vyacheslav received an early career research medal in late 2008 for his work on chemical modification of DNA with direct attachment of organic reporter molecules.

Benny ***Theng*** has recently returned from three months in Chile where he was a visiting professor at Universidad de la Frontera (UFRO) in Temuco giving a series of lectures to staff and students in the Chemistry and Natural Resources Departments. His main task, however, was to help PhD students with writing manuscripts for publication in international journals. In addition to completing a thesis, these students are required to have at least one paper published, and another submitted, before being admitted to the degree. Benny also gave an oral paper, and chaired a session, at the 5th International Symposium on Mineral-Organic-Microorganism Interactions (ISMOM-2008) in nearby Pucon last November, and accepted the invitation to join the editorial board of the *Journal of Soil Science and Plant Nutrition* (Chile).

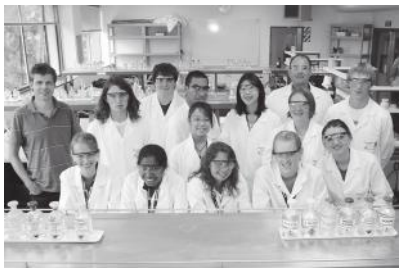
Mid-February saw the Manawatu Branch host Prof *Martin* ***Banwell*** (ANU) on his RSC-NZIC-RACI Australasian lecture tour. His talk *Chemoenzymatic Methods for the Assembly of Biologically Active Natural Products* was well-attended, attracting not only the usual chemistry suspects but also biochemists and molecular biologists.

Mark Waterland, Shane Telfer and *Ashton* ***Partridge*** were part of a MoRST delegation that visited laboratories in Japan at the end of January. These included the National Institute for Materials Science (NIMS) in Tsukuba where they gave presentations at a two-day workshop on nanotechnology. NIMS is a research institution that plays a similar role to the CRI's in NZ but without commercial activity. The visit included a tour of the very impressive facilities, which illustrated rather graphically the level of investment in Japanese science. The delegation also visited the Tokyo Institute of Technology and met with a number of staff. TIT has a large number of chemists on their staff but no formal Chemistry Department - the chemists are dispersed across science and engineering faculty according to their research interests. Mark Waterland and Ashton Partridge met with a Korean delegation at IRL in early February. The Korean delegation was in NZ as part of a continuing programme of visits coordinated by FRST.

In early February, Prof *Jurgen* ***Grotemeyer*** (Physical Chemistry, Christian Albrechts University, Kiel) gave an enlightening talk about *mass spectroscopy with light*. Also on the same day, Prof *David* ***Knight*** (Cardiff University) gave a thought provoking presentation entitled *Frisky Protons with a Silver Lining: New Methods in Heterocyclic Synthesis*.

Nobel Laureate Prof Sir *Harold* ***Kroto*** (Florida State University) was kept very busy when he visited Massey University on February 16. He had breakfast with some local high school students, and was a panel member for a Research Forum along with Profs *Richard* ***Blaikie***, *Jeff* ***Tallon*** and *Peter* ***Schwerdtfeger*** with Peter Derrick as Chairman. Questions involved the possible repackaging of traditional science papers and humanitarian issues. He then attended an informal luncheon with the postdoctoral fellows, postgraduates and summer students. In the afternoon he delivered the Sir Neil Waters Distinguished Lecture Series (Chemistry) on *Science, Society and Sustainability*. His lecture attracted a large mixed audience and covered the three themes in a relaxed and interesting way. There was much food for thought for the younger members of the audience, and at the end he showed a video clip of children balancing a model of his favourite C60 molecule on their heads. As an aside, he advocated the use of photographs of scientists taken after they had made their discoveries, not when they were old!

A dozen senior high school students from around NZ converged on Massey for the inaugural NanoCamp mid-January. The event, funded by the MacDiarmid Institute, involved participants making OLEDs, quantum dots and conducting polymers, and they got *hands-on* with optical tweezers and AFM. The students also composed a NanoCamp rap and amused locals by wearing their lab coats out to dinner.



The NanoCamp Gang

In March, the Centre for Separation Science welcomed back *Olekile* ***Tibe*** to start his PhD degree. Olekile gained an MSc with us in 2003 and he is now to study the chemistry related to plants from his native Botswana. It is interesting to note we are also talking to a young lady from Malaysia who seeks to join us for a programme of study on some Malaysian plants. *Rachel* ***White*** is currently preparing to present her two-year study at the 21st American Peptide Symposium: *Breaking Away* in June in the US.

*Islah-****u-Din*** commenced his PhD studies at IRL working on Hybrid Organic-Inorganic materials with Prof *Jeff*

Tallon. Islah will be co-supervised by Mark Waterland and Shane Telfer at Massey University.

Trevor ***Kitson***, *Adrian* ***Jull*** and Mark Waterland attended a meeting of 100-level chemistry coordinators, organised by Dave Warren from Otago, in Wellington on February 17. Among other things, the meeting discussed the future of the Chemical Education group, and in particular how to encourage the participation of high school chemistry teachers as members.

The Plieger group is expecting two visiting MSc students, *Fabien* ***Beiloret*** and *Pierre-etienne* ***Brethenoux***, from Poitiers University to arrive in April. *Quintin* ***Knapp*** has just submitted his MSc thesis on spectroscopic studies of anion encapsulating helicates and *Karl* ***Shaffer*** is planning a trip to LANL in the US to finish his PhD studies on beryllium binders.

OTAGO

University Chemistry Department

The Branch congratulates *Henrik* ***Kjaergaard***, *Keith* ***Gordon***, and *Lyall* ***Hanton*** on their elevation to Professor. Henrik was awarded the NZIC's Maurice Wilkins Center Prize for his significant contribution to developing and using theoretical chemistry in studying atmospheric processes (see p. 85).

Sally ***Brooker*** and Keith Gordon heaved a sigh of tiredness and relief as the 4th MacDiarmid Institute for Advanced Materials and Nanotechnology conference (AMN-4) in February came to an end. The co-chairs organized a highly successful meeting, with over 300 delegates from seventeen countries that includes ~100 students. Prof Sir *Harold* ***Kroto***, an Otago James and Jean Davis Prestige Visitor, gave an energetic and entertaining opening plenary lecture and he followed this with a similarly gripping mid-week public lecture.

Brookers Bunch have welcomed newcomer *Rajni* ***Sanyal*** for her Honours project. Having completed a BSc (1st class Hons) and an MSc (distinction), respectively, *Matthew* ***Cowan*** and *Humphrey* ***Feltham***, have returned as PhD candidates. *Laszlo* ***Mercs*** (Switzerland) is to start a postdoctoral fellowship in May, and *Jon* ***Kitchen***, having submitted his PhD thesis prior to IC08 last year within three years of starting, has returned to work in the group as a MacDiarmid postdoctoral for a few months; he will transfer to a Marsden-funded position later in the year. Jon was awarded the Stranks Prize for the best student lecture at IC08 and also gave a great lecture at AMN-4. *Julia* ***Rinck*** (Karlsruhe) was with us over the summer months and achieved much during her stay. Subject to visa requirements, *Matin* ***Momeni*** is expected to join the group mid-year for PhD study jointly supervised by Sally and *James* ***Crowley***.

The Marine and Freshwater Chemistry Group welcomed *Eike* ***Breitbarth*** and *Linn* ***Hoffmann***, (Gothenburg, Sweden) to postdoctoral posts. Eike is studying changes in the speciation and bioavailability of trace metals that occur with ocean acidification; Linn is investigating the effects of the trace metals on phytoplankton. Three members of the group were the NZ signatories of the Monaco Declaration released in February. It highlights the severe damages expected with continuing ocean acidification. *Kim* ***Currie*** (NIWA), *Christina* ***McGraw*** and Eike Breitbarth joined 152 other scientists from 26 countries in calling for immediate action by policymakers to reduce CO_2 emissions in order to prevent widespread damage to the marine ecosystem.

Dr *Jo* ***Lane*** (2008 PhD with *Henrik* ***Kjaergaard***) has accepted a Lectureship in Physical and Theoretical Chemistry at the University of Waikato effective from August.

WAIKATO

University of Waikato

Derek ***Smith*** retired from the Chemistry Department recently after 36 years of service. We wish him all the best for his retirement. A detailed account of his time at Waikato will appear in the July issue.

Two visitors to the Department have given seminars recently. Profs *David* ***Knight*** (Cardiff) and *Martin* ***Banwell*** (ANU) gave interesting lectures entitled *Frisky protons with a silver lining: New methods in heterocyclic synthesis* and *Chemoenzymatic methods for the assembly of biologically active natural products*, respectively, the latter in his capacity as the RSC-RACI-NZIC Australasian chemistry.

NIWA

Micha ***Rijkenberg*** participated in the FeCycle/Springbloom research cruise on the RV Tangaroa in September/October last year. The overarching goal of the cruise was to examine factors that determine plankton growth and carbon export in NZ waters during the spring bloom. Due to its low solubility in seawater, iron forms an important factor that may limit, and thus control, plankton growth and subsequent carbon export. However, the photoreduction of iron enhances its bioavailability and residence time in the euphotic zone. During the FeCycle/Springbloom cruise Micha investigated the susceptibility of iron for photo-reduction.

Hilke ***Giles*** participated in a research trip to the Taylor Valley (Antarctica) last October/November, which was a collaborative event between the NIWA Antarctic Aquatic Ecosystems project and the US Long Term Ecological Research (LTER) Programme. The research performed during this trip was designed to refine the understanding of factors that control the productivity of the benthic microbial communities in Lake Hoare (dominant primary production and biodiversity elements within the system) and how they respond to changing environmental conditions. To this end, high resolution measurements of O_2 evolution within microbial mats were made over a range of light intensities (using an O_2 micro-profiling system), to allow light *vs*. primary production relationships to be mathematically modelled. In addition, an experiment was installed into the lake that artificially modifies the light received by microbial mats. This is to run for two years until a return trip determines how this has affected mat characteristics. In this way a better understanding and easier prediction of the sensitivity of these locally important ecosystems to changing growth conditions, *e.g.* thinning or thickening of ice cover in response to climatic variability. Additionally, an understanding of the historical record of growth retained in the sediments underlying the microbial mats will enlighten our knowledge of recent system variability.

Michael ***Stewart*** and *Craig* ***Depree*** have been working on natural antifouling compounds and their paper *Antifouling Sesterterpenes from the New Zealand Marine Sponge Semitaspongia bactriana* has been accepted for publication in *Natural Product Communications*.

WELLINGTON

The February meeting of the Branch saw a good-sized audience hear Prof. *Martin* ***Banwell*** (Director, Research School of Chemistry, ANU) deliver the 2008 RSC-RACI-NZIC lecture with the abbreviated title: *A little bit of strain can be good for you*. The lecture provided a survey of much of Martin's highly successful small ring chemistry involving gem-dihalocyclopropanes that had its origin in his VUW PhD studies. In March Dr *Tim* ***Coutts*** (National Renewable Energy Laboratory, Colorado), one of the AMN-4 plenary lecturers, visited from Canterbury University where he was on leave. He spoke on the science and properties of transparent conducting oxide films in which he discussed some of the materials and characterization techniques used at NREL, *the relationship between the electrical and optical properties of the films*, and the method of four coefficients used to derive the effective mass, relaxation time, Fermi energy, and scattering parameter. The method, applied to films of CdO, ZnO, and In_2O_3, provides fundamental properties and shows where improvements in the materials may and may not be possible.

A BBQ function for the 2009 student intake was held on March 9 and new and existing student members and a number of staff attended.

Victoria University

Dr *Rob* ***Keyzers*** has been appointed to a lectureship in organic chemistry and will take up his appointment mid-year. *Mina* ***Razzak***, a BSc (Hons.) graduate of VUW, has spent the summer in the ***Northcote*** laboratory as part of her Cambridge-VUW PhD programme jointly supervised by *Ian* ***Patterson*** and *Peter* ***Northcote*** and presented some of her results under the title: *Total Synthesis of saliniketals A and B*. *Chris* ***Munro***, working with *John* ***Spencer***, completed his masters degree over the summer period.

Prof. *Robin* ***Clark*** (UC – London), inaugural recipient of the Franklin-Lavoisier Prize visited on Feb 12 and gave a lecture entitled: *Raman Microscopy, Pigments and the Arts/Science Interface* to an audience that included members of the Te Papa art and heritage preservation staffs.

PhD candidate *Ying (Sherry)* ***Xu*** was a winner of the student poster awards at the recent AMN-4 conference in Dunedin for her presentation *Solution phase synthesis of SnS nanoparticles*.

In January Prof *Ken* ***MacKenzie*** attended the 33rd Annual Conference on Advanced Ceramic Materials at Daytona Beach (Florida) where he gave a plenary paper. At AMN-4 in Dunedin he presented an invited paper. Three of his postgraduates have successfully completed their studies: *Nils* ***Rahner*** (MSc - inorganic polymers as potential bioactive materials), *Jonathan* ***Tailby*** [BSc (Hons.) - properties of portland cement-geopolymer composites], and *Bryan* ***O'Leary*** [(BSciTech (Hons.) - sialon ceramics fabricated by carbothermal reduction and nitridation of inorganic polymer precursors].

Note added by editor: February saw Ken's 300th international journal publication - something of a milestone for a New Zealander! – Congratulations Ken.

Dates of Note

On April 15 in 1770, *Joseph* ***Priestley*** made the first mention, in English, that a piece of a rubber substance could erase marks from black-lead pencils. *Joseph* ***Black***, the British chemist and physicist who experimented with *fixed air* (CO_2), discovered bicarbonates and identified latent heat, was born on April 16, 1728.

Wilhelm ***Körner***, the German organic chemist who, in 1874, established how to determine the positions of the substituents on di- and tri-substituted benzenes by counting product or source isomers (five years before the van't Hoff-Le Bel hypothesis of tetrahedral carbon) was born on 20 Apr 1839, 60 years ahead of the death of *Charles* ***Friedel*** of Friedel-Crafts fame.

Paul ***Karrer***, the Swiss chemist who investigated the constitution of carotenoids, flavins, and vitamins A and B_2, and 1937 Nobel Prize Laureate (with Haworth) was born on 21 Apr 1889 and died on Jun 18, 1971. *Donald J*. ***Cram***, 1987 Nobel laureate in chemistry (with Pedersen and Lehn) for host-guest work, was born on 22 April 1919 and died on 17 Jun 2001.

April 26 marks the 55th anniversary of beginning of mass testing of the Salk polio vaccine involving about 1.8 million children.

Alexander William ***Williamson***, of Williamson etherification fame, was born on 1 May 1824 and died on May 6 1904. On May 1 in 1889, Bayer introduced aspirin in powder form to Germany. May 2 marks the 30th anniversary of the death of *Giulio* ***Natta*** (Ziegler-Natta polymerization catalysts) and the 75th of *Sergey Vasilyevich* ***Lebedev***, the Russian chemist who produced synthetic rubber from butadiene in 1910 and gave the Soviet Union the largest synthetic rubber industry in the world by 1940.

May 6 marks the 150th anniversary of the death of *Alexander von* ***Humboldt***, the noted German natural scientist. On May 12, 125 years ago ***Hilaire***, *Count of Chardonnet de Grange*, informed the Academe Francais that he had made artificial silk (rayon) from cellulose. He displayed his product at the Paris Exposition of 1891 where it was known as *Chardonnet silk*. *Pierre* ***Curie***, co-winner of the 1903 Nobel Prize for Physics was born 150 years ago on May 15.

William ***Nicholson***, the English chemist who discovered the electrolysis of water on 2 May 1800, died on May 21, 1815, and the day marks the 75th birthday of Swedish biochemist *Bengt Ingemar* ***Samuelsson*** who was co-recipient (with Bergström and Vane) of the 1982 Nobel Prize for Physiology or Medicine; the three isolated, identified, and analysed numerous prostaglandins.

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Nanotechnology in Good Health?*

Hilda Coulsey and Alan Smith

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The business environment for large pharmaceutical companies is changing. Profits from blockbuster drugs are under threat due to expiration of patents. Healthcare will change because of the ever increasing cost to develop drugs. The only way forward, to become more efficient in cost and patient care, will be through nanotechnology.

For those not familiar with nanometres (nm), they are one millionth of a millimetre. To appreciate this more easily, a human hair is about 80,000 nanometres in diameter, red blood cells are about 5,000 nm across, the AIDS virus is approximately 100 nm long, and DNA is less than 3 nm. At the nano-scale properties change, and the reason for this can be understood if one considers a cube of just about any substance, which would have one in ten million of the atoms on the surface. However, for a one nanometre cube, 80% of the atoms are on the surface. This increased surface area can be beneficial for many technologies and products. For several years now, car exhaust catalysts have relied on nanotechnology and the increased surface area it provides to breakdown exhaust fumes.

A project on emerging nanotechnologies at the Woodrow Wilson International Centre for Scholars to date has catalogued over 500 manufacturer-identified, nanotechnology-based products.[1] These include numerous sun-screen products in which the nanoparticles of titanium dioxide, TiO_2, let the *good* ultraviolet light through but reflect the *bad* UV. In addition, there are an increasing number of products—ranging from socks and towels to refrigerators and food storage containers—that incorporate silver nanoparticles, which provide antimicrobial properties. Many wound dressings now contain silver nanoparticles to aid faster recovery. The earliest applications for nanotechnology products have been in sporting goods,[2] where the margins are high, *e.g.* Federer's tennis racket, lighter weight materials in Formula One racing cars, and Floyd Landis's cycle frame. Once established in those markets, the technology moves down to commodity products, which is certainly what is happening with most automotive companies.

For consumer products that provide healthcare, we are seeing many new types of toothpaste on the market which are based on nanotechnology. Guaber in Italy make BlanX®, containing NANOREPAIR™, which is treated nanoparticulate hydroxyapatite for filling the minute cracks that occur in the enamel of teeth. A similar system is available for hair damage repair, which is called TANAGRA, and contains *nano-molecular* keratin to block cracked or rough hair. L'Oreal has the largest number of patents relating to nanotechnology in health and personal care,[3] many of which are for anti-ageing products. Their significant R&D expenditure in nano-capsule technology is directed at delivering agents such as vitamin A and retinol into the skin.

However, the most promise for nanotechnology will come from *nanomedicine*, which has been described as the application of nanotechnology to achieve breakthroughs in healthcare.[4] Nanomedicine promises to impact all stages of healthcare:

- preventative medicine
- diagnosis
- therapy
- follow-up care

Proponents of nanomedicine hold that it will lead to earlier detection of diseases and novel therapies that will minimize discomfort for the patient, and hence provide cost savings all around. The European Strategic Research Agenda for nanomedicine[4] has set priorities based on a number of parameters, namely: mortality rate, level of suffering, burden on society, prevalence of the disease, and the ability of nanotechnology to diagnose and overcome illnesses.

The strategy is to attack the diseases that are the greatest burden on society first:

- cardiovascular diseases
- cancer
- musculoskeletal disorders
- neurodegenerative diseases and psychiatric conditions
- diabetes
- bacterial and viral infections.

According to the World Health Organization,[5] cardiovascular diseases are the most frequent cause of death in the EU. However, efforts to reduce heart-related problems by encouraging more exercise and a reduction in cholesterol levels are paying off, and cancer instead may soon become the leading cause of death. In the US, nanotechnolo-

gy solutions for cancer therapy are receiving high priority. A *Cancer Nanotechnology Plan*[6] is being spearheaded by the National Cancer Institute, which acknowledges that *nanotechnology offers the unprecedented and paradigm-changing opportunity to study and interact with normal and cancer cells in real time, at the molecular and cellular scales, and during the early stages of the cancer process.*

It is worth listing part of the vision statement for the Cancer Nanotechnology Plan, which says that nanotechnology will be the enabling technology for:

- early imaging agents and diagnostics that will allow clinicians to detect cancer at its earliest, most easily treatable, pre-symptomatic stage
- systems that will provide real-time assessments of therapeutic and surgical efficacy for accelerating clinical translation
- multifunctional, targeted devices capable of bypassing biological barriers to deliver multiple therapeutic agents at high concentrations, with physiologically appropriate timing, directly to cancer cells and those tissues in the micro-environment that play a critical role in the growth and metastasis of cancer
- agents capable of monitoring predictive molecular changes and preventing precancerous cells from becoming malignant
- surveillance systems that will detect mutations that may trigger the cancer process and genetic markers that indicate a predisposition for cancer
- novel methods for managing the symptoms of cancer that adversely impact quality of life
- research tools that will enable investigators to quickly identify new targets for clinical development and predict drug resistance.

Nanomedicine can be divided into four main areas:

- drug delivery
- molecular diagnostics
- tissue engineering
- cell/gene therapy

Drug Delivery

In addition to all the proposed new work discussed above, the challenge for the traditional pharmaceutical companies is to deliver the right therapeutic to the right target with no (or minimal) side effects, and at reduced cost. Drug delivery using nanostructures offers considerable potential to accomplish this. Some early successes have come from the Elan Corporation, a neuroscience-based biotechnology company headquartered in Dublin, Ireland. They have developed proprietary NanoCrystal technology for active pharmaceuticals that have poor water solubility. This technology reduces the particle size and increases the surface area of drugs leading to an increased dissolution rate. The nanoparticles are processed into finished dosage forms for all methods of administration. There have been four commercial approvals for products that have incorporated this type of technology, *viz*.:

- *Rapamune*, Wyeth's immunosuppressant, now in tablet form; previously it was available as a refrigerated product in packets or in bottles.
- *Emend*, which was developed as a new chemical entity by Merck for cancer treatment.
- *TriCor*, a reformulated drug for lowering cholesterol from Abbott; previously it had to be taken with a meal.
- *Megace ES* is an appetite enhancer for AIDS sufferers from Par Pharmaceuticals who have licensed the Megace name from Bristol-Myers Squibb. NanoCrystal technology improves the rate of dissolution and bioavailability of the original unpalatable oral suspension.

In the US, the company Esprit Pharma markets *Estrasorb* (from Novavax). This is an emulsion containing nanoparticles that is rubbed onto the legs to reduce hot flushes. There are two other cancer therapy drugs that are now nanotechnology-based: *Abraxane* for fighting metastatic breast cancer (Abraxis BioScience and AstraZeneca) and *Doxil* for ovarian cancer (Ortho Biotech).

A NanoMarkets report[7] suggests that $US 65 billion p.a. in drug revenues are accounted for by active agents with low bio-availability, which can lead to inefficient treatment, higher cost and risk of toxic side effects - hence the drive to develop reformulations based on nanotechnology. The report estimates that nano-enabled drug delivery systems will reach $US 1.7 billion in 2009.

For cancer therapy, the goal is to target cancerous cells while leaving healthy cells intact. With nanotechnology, it is possible to specifically target the cancerous cells and then activate the nano-structures to kill just those cells. Naomi Halas and Jennifer West[8] are working on gold nanoshells that can be tailored to absorb near infrared light. This passes harmlessly through soft tissue but when nanoshells are activated under near infrared light, enough heat is generated to burst the walls of cancerous cells.[8] This breakthrough has led to the set up of spin-off company Nanospectra Biosciences, which is to begin trials with the nanospheres in humans.

Molecular Diagnostics

As indicated earlier, biological structures exist at the nano-scale, and nanomaterials are being used for both *in vivo* and *in vitro* biomedical research, especially for diagnostic devices. Some technologies are now mimicking the effectiveness of *sniffer dogs* at airports, which are able to rapidly detect minute amounts of drugs or explosives very quickly. Such sensitivity has recently been reported[9] from the University of Manchester where gas sensors that use graphene to detect single gas molecules are being developed. The promise for molecular diagnosis is that diseases will, in the future, be easily and quickly detectable before they gain a foothold on the body, resulting in less severe and expensive therapy.

Hand-held *lab-on-a-chip* devices are already being used in hospitals to detect whether someone is having a heart attack. A similar development is being used to distinguish among several different narcotics in the bloodstream.

An area that has seen huge growth in the last decade is medical imaging. Here, nanotechnology applications are beginning to appear for both imaging tools and marker and contrast agents. It is expected, in the foreseeable future, that nano-imaging will lead to the detection of single cells in very complicated environments.

In 2005, the molecular diagnostics market was about $US 5.5 billion, and the nano-diagnostics share was about $US 1 billion. It is estimated that by 2015, the nano-diagnostics market will be worth $US 9.5 billion and will predominate the molecular diagnostics market.[10]

Tissue Engineering

Tissue engineering uses developments from materials engineering and the life sciences to provide biological substitutes that will reproduce or repair damaged tissue. Tissue engineering stimulates cell proliferation using nanomaterial scaffolds, which are porous or solid. It is expected that in the future, tissue engineering will enable the replacement of artificial implants and organ transplants.

Nanotechnology is already contributing to commercially available products. *NanOss* and *Vitoss* are basically fillers for damaged bone. *NanOss* (from Angstrom Medica Inc. in the US) was the first nanotechnology medical device to receive approval from the US Food and Drug Administration. It is an innovative structural biomaterial, based on nanocrystalline calcium phosphate technology, that is highly osteoconductive and remodels over time into human bone. The material has applications in sports medicine and trauma, spine, and general orthopaedics markets. *Vitoss* (from Orthovita) also is based on calcium phosphate. It comes in blocks that can be shaped with a scalpel and gently tamped into place, or in granules that can be packed into irregularly shaped voids in the defect site. A third product, *TiMesh*, (from GP Surgical) is described as a soft-tissue reinforcement implant for hernia repair and is based on titanised polypropylene.

The market described as tissue engineering[10] was worth $US 6.9 billion in 2005 and is predicted to rise to $US 23.2 billion in 2015.

Cell/Gene Therapy

This type of therapy repairs or replaces damaged tissue by using cells from the patient or a donor that have been multiplied and sometimes altered outside the body. A good example of this is stem cells, which can be grown and transformed into specialized cells, such as nerves and muscles, through cell culture.

SiBiono Gene Tech (based in China) was the first company to commercialize gene therapy. Its product *Gendicine* makes use of the fact that over 50% of tumours have a dysfunctional gene that makes protein p53 a cellular anti-cancer agent. Effectively, *Gendicine* works by inserting the p53 gene into a virus that is then injected into patients. The gene is naturally present in healthy cells but is *turned off* or mutated in many cancer patients. When reinserted into tumour cells by the virus, it causes them to self destruct. *Gendicine* is finding success among sufferers of head and neck squamous cell carcinoma. The only other commercially available gene therapy is from Shanghai-based Sunway Biotech, which has a virus that kills tumours. The product, called *Oncorine*, is a genetically modified virus that selectively replicates inside tumour cells with dysfunctional p53 genes.

Nanomedicine is moving forward at a rapid pace, and we are only just seeing the tip of the iceberg. The best is yet to come.

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Article

The Origins of Organic Contaminants in Antarctica

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The continent of Antarctica is often described with words such as *pristine* and *untouched* due to its remote location and distance from the inhabited regions of earth. Environmental contaminants have, however, been detected[1,2] in various Antarctic media since the 1960s. These early findings provided impetus for further study, confirming the ubiquity of contaminants in Antarctica but raising questions about how these chemicals came to exist in such a secluded area.[3,4]

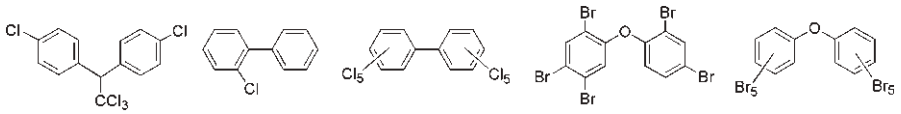
The isolation of the Antarctic continent has meant that it has endured very little contact with people throughout history, making it a rather unique case in the field of contaminant study. Traditionally, human activities in this area have been involved with either exploratory or scientific endeavours.[5] More recently, the tourism industry has also brought noteworthy numbers of visitors to the Antarctic continent, thus increasing the likelihood of contamination.[6] Collectively though, these activities still represent a relatively low level of human interaction and are generally confined to specific and restricted areas of the continent. As a result, the detection of contaminants throughout Antarctica conventionally has not been attributed to *in situ* human activity.[7] Nonetheless, this assertion requires further analysis through consideration of specific contaminants and their distributions in Antarctica. The term *contaminant* encompasses a wide variety of chemicals, including heavy metals, hydrocarbons, radionuclides and so forth.[8] A large proportion of the contaminant research in Antarctica focuses on the class of compounds known as Persistent Organic Pollutants (POPs). These are chemicals *that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment*.[9]

It is the potential detrimental impact on humans and wildlife that originally instigated interest in the global distribution of the POPs. The first organic contaminant detected in Antarctica was the pesticide 1,1-trichloro-2,2-di(*p*-chlorophenyl)ethane (DDT, **1**; Chart 1), as reported by Sladen *et al*.[1] in 1966. DDT was used extensively to control disease vectors throughout WWII and the subsequent two decades. Its discovery in Antarctica was regarded as highly significant because it signified that harmful contaminants had achieved a global distribution well beyond that previously anticipated. Moreover, the detection of DDT in Antarctica occurred not long after concerns about the biological effects of such pesticides were first raised in the book[10] *Silent Spring* in 1962. Research at that time elicited concern for high trophic-level bird species, in which high concentrations of DDT were being measured because of biomagnification. It was also implicated in egg-shell thinning, which led to the fracturing of egg shells during incubation and decreased hatching rates.[10] Thus, Sladen's publication[1] was ground-breaking in its time and it prompted an emerging environmental concern. The most prominent issue exposed was the question of how such chemicals came to be found in the Antarctic region without ever having been used there.

Based upon Sladen's initial measurements, the authors proposed various mechanisms by which DDT may have come to be found in Antarctica.[1] These were limited by a lack of information about the physical properties, degradation rates, and partitioning behaviour of DDT in the environment. Despite the limitations, the authors were able to estimate the effect of human activity in Antarctica and they concluded that this was unlikely to be responsible for the observed levels of DDT. Instead, they suggested that the pesticide may have reached Antarctica by ocean-, atmosphere- or biologically-mediated transport.[1]

Subsequent research has furthered not only a better understanding of the physical properties of DDT but also of its distribution in Antarctica.[11,12] The properties most relevant to the distribution of DDT, now accepted as entering the Antarctic by long-range atmospheric transport, are shown in Table 1;[7] it has been detected in biota and sediments at most sites across Antarctica thereby decreasing the possibility of local point sources being responsible.[13,14] Furthermore, the notion of atmospheric transport is supported by DDT's vapour pressure of 2 x 10^{-5} Pa (Table 1), which falls within the 1 x 10^{-5} to 100 Pa range for semi-volatile chemicals.[11]

Table 1. Some physicochemical properties of persistent halogenated contaminants **1-5**.[a]



1 **2**, PCB-1 **3**, PCB-209 **4**, PBDE-99 **5**, PBDE-209

Property	*1, DDT*	*2, PCB-1*	*3, PCB-209*	*4, PBDE-99*	*5, PBDE-209*
Vapour Pressure (Pa)	2 x 10^{-5}	1.84	1.4 x 10^{-6}	5 x 10-5	5 x 10^{-11}
Log K_{ow}	6.19	4.56	8.20	6.71	11.15
Half-Life in Air (h)	170	170	55,000	467	7,620
Half-Life in Water (h)	5500	5,500	55,000	3,600	3,600

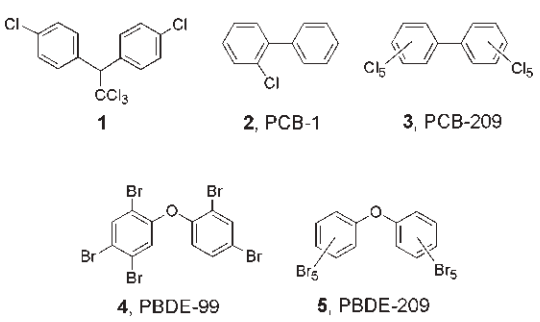
[a]Data for **1-3** are taken from ref 11; data for **4-5** are taken from ref 23.

Semi-volatile chemicals tend to enter the air in the warmer regions of earth where they also tend to be used. Provided that the chemical is sufficiently air-stable, which is true for DDT (Table 1), it will be transported in the direction of the prevailing wind. On a global scale, atmospheric circulation transports air from the equator towards the poles so that the chemicals eventually make their way to the polar ice caps. The temperatures in these regions are significantly lower than at the site of use and thus the molecules condense[15] thereby explaining the detection of semi-volatile chemicals in cold remote regions where they have never been used in notable quantities.

The fate of atmospherically deposited semi-volatile chemicals is dependent upon their partitioning characteristics. The medium of greatest interest for organic contaminants is biological tissue since this is where they exhibit their toxic effects. The parameter that best quantifies the tendency of a molecule to bioaccumulate in living systems is the octanol-water partition coefficient (K_{ow}). Log K_{ow} for DDT is 6.19 and it is therefore expected to bioaccumulate because chemicals with a log $K_{ow} \geq 4$ are considered lipophilic.[16] This lipophilicity is particularly significant for Antarctic organisms because many of them must store energy in the form of fatty adipose tissue in order to survive the winter and, therefore, have relatively large quantities of lipids. This hydrophobic tissue is ideal for accumulation of compounds such as DDT that already show considerable potential for bioaccumulation, and this explains the numerous reports of measurable levels of DDT found in Antarctic biota.[1,2,13]

The polychlorinated biphenyls (PCBs) were the next class of semi-volatile organic contaminants to be measured in Antarctica. PCBs consist of a biphenyl core with varying degrees of chlorination that can giving rise to as many as 209 possible structures, termed *congeners*, *e.g.* **2** and **3** (Chart 1). Following their discovery in the 1920s, PCBs had wide-ranging applications throughout industry, based largely upon their desirable properties of low flammability and high boiling points. However, as time progressed, various detrimental health effects, including chloracne, skin rashes, and liver damage were observed in people who had experienced high exposures to PCBs.[17] As a result of these observations, PCBs were banned from use in the developed countries in the 1970s.

Chart 1



1 **2**, PCB-1 **3**, PCB-209

4, PBDE-99 **5**, PBDE-209

PCBs have been detected in samples from Antarctica since 1976. However, their origin was not as easily discernable as that of DDT because of local sources,[4] one of the most obvious being the historical dumping ground at McMurdo Base in the Winter Quarters Bay. A variety of PCB-containing materials were disposed of directly into this bay giving rise to considerable contamination in the area.[18] In order to establish whether local sources such as these have a notable effect, or whether long-range atmospheric transport again is responsible, it is necessary to consider the properties of different PCBs.

The properties of the PCB molecules change as the degree of chlorination increases and two molecules were selected for comparison, namely congeners 1 (**2**) and 209 (**3**) (Chart 1). The vapour pressure of a PCB decreases as the degree of chlorination increases (Table 1) because of the increasing van der Waals attraction between the molecules. The progressive change is so significant that whilst the lower chlorinated congeners such as PCB-1 (**2**) have vapour pressures that allow them to be classified as semi-volatile, the heavier congeners such as PCB-209 (**3**) have vapour pressures that place them outside the accepted range. This is a key factor in the elucidation of the source of PCBs in the Antarctic as it means that the larger congeners would not be expected to undergo efficient long-range atmospheric transport. Therefore, larger congers are associated with local sources whilst lighter congeners are associated with long-range atmospheric transport.

Numerous studies were conducted into the levels of PCBs found in a variety of Antarctic biota collected from Terra Nova Bay during the 1990s. The congener profiles were compared to those in common commercial PCB mixtures, and those from biotic samples were found to contain more of the less-chlorinated congeners than was found in the commercial mixtures.[14,19] Based on their K_{ow} values, all PCB congeners are expected to bioaccumulate. Thus, the observed congener profiles indicated that long-range atmospheric transport was largely responsible for the presence of PCBs in Antarctica. This conclusion was strengthened when similar congener profiles were found in sediment samples, removing the potentially confounding variable of biological uptake.[20]

Banned, historic-use contaminants are no longer the only focus of contaminant research in Antarctica. Recent studies have revealed the potentially detrimental health effects of brominated fire retardants - polybrominated diphenyl ethers (PBDEs), *e.g.* **4** and **5** (Chart 1). These chemicals are used in a wide range of household items, particularly electronics, plastics and furniture. PBDEs have been identified as possible neurotoxins, causing their distribution in the natural environment increasingly to become a prominent human health and environmental issue.[21]

A recent publication has described the detection of PBDEs in Antarctic wildlife.[22] Although they make up a relatively small proportion of the detected organic contaminants, at *ca*. 1%, their increasing usage in the Antarctic environment is cause for concern.[22] Consideration of the structure of the PBDEs again is important in determining their origins. In analogy to the PCBs, PBDEs are brominated to varying degrees and there are again 209 possible congeners. The major commercial classes of these products are the penta-, octa- and deca-brominated compounds. One of the major constituents of the pentabromo formulation

is PBDE-99 (**4**) whereas PBDE-209 (**5**) is the principal constituent of the decabromo formulation.

The differing properties of the two compounds reported in (Table 1) illustrate the importance of the bromination level on the behaviour of a PBDE in the environment. The more heavily brominated **5** has a vapour pressure of 5×10^{-11} Pa that is well below the 1×10^{-5} Pa minimum for a semi-volatile compound.[23] In contrast, PBDE-99 at 5×10^{-5} Pa is semi-volatile and is expected to undergo long-range atmospheric transport.

In an early investigation on PBDE fate in the environment, computer modelling was used to determine that they are likely to undergo long-range atmospheric transport.[24] In a subsequent study, the low-brominated congeners were detected in three species of Antarctic penguin, leading the researchers to conclude that they had confirmed the proposed mechanism of long-range atmospheric transport.[22] However, a more recent publication[5] challenged this assertion by investigating the possibility that PBDEs may be originating predominantly from local sources, namely the Antarctic research bases, the major human activity centre in Antarctica.[5] The aim of the authors' investigation was to *determine whether the indoor dust and wastewater sludge from Antarctic research bases contain substantial burdens of PBDEs* and to *establish whether PBDEs have been released locally*.[5] In order to do this, they took dust and wastewater samples from two bases in McMurdo Sound, Scott Base (NZ) and McMurdo Station (US), and analysed the levels of PBDEs in these matrices.

The analysis revealed particularly high levels of PBDEs in the indoor dust samples - even higher than those commonly found in residential dwellings in developed countries, including NZ.[25] This validated the authors' hypothesis that Antarctic research bases may be local sources of PBDEs. However, a high PBDE concentration in indoor dust does not necessarily mean that the bases are polluting the environment. Thus, PBDEs were also measured in wastewater samples from the same two bases. The untreated wastewater would be expected to contain high levels of PBDEs since that used for cleaning would contain indoor dust, and it did. In contrast, the treated wastewaters had no detectable levels of PBDEs fully compatible with the bases under investigation not contributing PBDEs to McMurdo Sound.[5] Nonetheless, the findings are highly significant for two reasons. Firstly, compared to the high-grade treatment facilities at Scott and McMurdo Bases, many Antarctic research bases fall short and often release untreated waste directly into the environment. Thus, based upon the untreated wastewater measurements, these other bases currently may be releasing notable amounts of PBDEs to the surrounding environment.[5] Secondly, Scott Base and McMurdo Station had their treatment facilities upgraded some 18 months prior to sample collection and, given the long environmental half-life of the compounds, it is distinctly possible that PBDEs would still have been detectable in the area surrounding the bases due to the relatively recent release of PBDEs into the environment.

It was with this latter possibility in mind that the authors measured the levels of PBDEs found in sediments and biota at sites of increasing distance from the wastewater outfall of McMurdo Station. The sum for congeners 47, 85, 99, 100, 153, and 154 (ΣPBDE6) - the major constituents of the pentabromo commercial product - was quantified in biological samples. In the sediment samples, both ΣPBDE6, and the concentration of PBDE-209, the major constituent of the decabromo analogue, was measured.

PBDE concentrations were found to decrease with increasing distance from the McMurdo wastewater outfall, thus providing strong evidence that prior to installation of the treatment facility, the wastewater from McMurdo Base was, in fact, releasing PBDEs to McMurdo Sound. The importance of these results cannot be underestimated as they indicate that long-range atmospheric transport may not be the primary source of PBDEs in Antarctic marine ecosystems. This is particularly noteworthy because, although little can be done about the presence of banned, historic-use chemicals like DDT and PCBs in Antarctica, PBDE inputs are on-going and current environmental management decisions may affect their fate and future impacts on Antarctic ecosystems.

Prior to the implementation of any mitigation plans, it would be wise to repeat a similar experiment to that described above at an Antarctic research base that does not treat its wastewater. This would provide further information and give a more logical basis for the formulation of a plan to reduce the anthropogenic inputs of contaminants to Antarctica. Further initiatives could also include research into the level of other chemical contaminants currently overlooked. Until very recently, research on contaminants in remote ecosystems focussed on banned, historic-use contaminants for which analytical methods are well-established. However, as the PBDE case illustrates, chemicals in current-use may be accumulating in these ecosystems without our knowledge. Other compounds of concern, which may be worthy of investigation due to their toxic properties, include *pharmaceuticals, fragrances and cleaners*.[5] Without adequate knowledge of their distribution, these may be having untold effects on organisms within this unique ecosystem. It is clear that efforts directed at understanding the sources of contaminants in Antarctica are critically important to the future welfare of Antarctic ecosystems.

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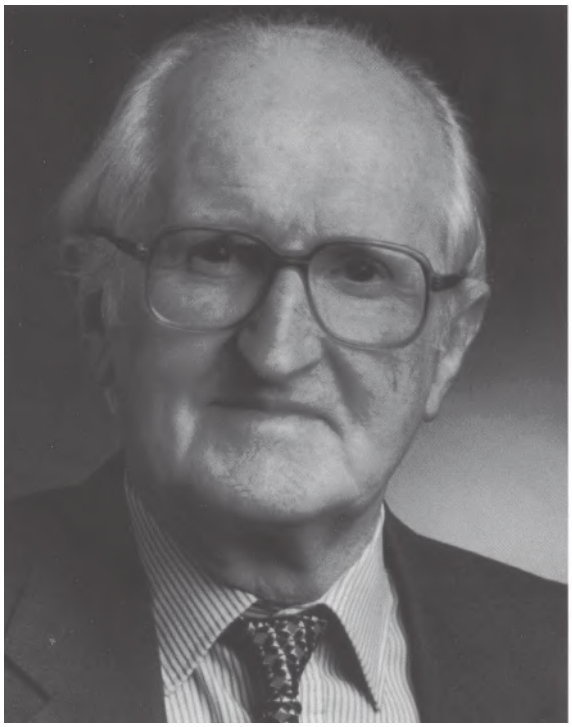
OBITUARY

Rowland Albert (Roy) Kennerley — 9 March 1923 – 7 January 2009



The death occurred recently at the age of 85 of Roy Kennerley, a scientist with the former Chemistry Division of DSIR. Roy was born in Carterton, gained secondary education at Levin District High School, and joined the public service as a cadet. Initially assigned to the Public Service Commission Office in Wellington, he moved to the Social Security Head Office as a clerk, and then signed up for military service in 1941. He served first with 2nd Field Regiment and then the RNZAF as a radar operator in the Solomon Islands campaign.

After the war, Roy graduated with his MSc in chemistry from Victoria University and then joined DSIR's Dominion Laboratory in 1948, where he led a research team working on the inorganic chemistry of cement and concrete. This was particularly important in the era of public works construction following WWII, when millions of tonnes of cement and concrete were used in bridges, hydro dams, airport runways, geothermal projects, and public buildings. Roy and his colleagues at DSIR's inorganic materials unit at Gracefield played a vital part in these developments, testing materials, advising cement works, and ensuring standards were drafted and complied with.

By the time Roy retired in 1983, the inorganic materials team had grown to twelve, five of whom held PhD degrees. Retirement saw Roy begin a second career - as a technical manager and consultant to the Milburn NZ Ltd. cement company from which he retired permanently in 1998.

In addition to his science, Roy Kennerley's other great interest was music. He was a church organist, teacher, accompanist, ensemble leader and conductor who set high standards. He first sang in 1949 with the Eastbourne Lyric Singers conducted by Maxwell Fernie, and later formed or led choirs at Tawa, Upper Hutt, and, more recently at Eastbourne. After studying the organ, he began a career as an itinerant church organist, accompanying church services wherever the need arose. He also performed with Schola Polyphonica and the NZSO, which he accompanied on the Wellington Town Hall organ.

Roy is survived by his wife Agnes, and a son from an earlier marriage.

Ken MacKenzie

Help getting the message across

The British Science Media Centre has produced some one page brochures on communicating in soundbites. They have one entitled "Communicating risk in a sound bite" which can be freely downloaded at the address; *www.sciencemediacentre.org/uploadDir/admincommunicating_risk.pdf*

A second is entitled "Communicating uncertainty in a soundbite", available at the address *www.sciencemediacentre.org/uploadDir/adminuncertainty_in_a_soundbite.pdf*

They also have a tips brochure for working with the media. Some of this is less relevant to New Zealand but still has helpful suggestions. It can be found at the address; *www.sciencemediacentre.org/uploadDir/admintop_tips.pdf*

Article

Environmental Aspects of the Disposal of Pharmaceuticals in New Zealand

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Introduction

The use of pharmaceuticals is on a global increase and NZ is no exception to this trend. The magnitude of this usage has led to an increasing international awareness of the disposal of pharmaceutical compounds, either to landfills or as sewage where they can have potential detrimental effects on the aquatic environment.[1] For example, trace levels of the contraceptive ethynylestradiol (**1**, Chart 1) found in waterways have been shown to impair sexual development and increase feminization of fish.[2] There is also evidence that the presence of antibiotics in waterways has impact on the bacteria present and may lead to antibiotic resistance.[3]

While researchers generally concur that the risk of acute toxicity of the pharmaceuticals at the levels found in water is negligible, the outcome of long term chronic exposure is less certain.[4] Many believe that as the detected levels are significantly below therapeutic levels there is no risk. However, this may not be true for all drugs and for all members of the public as the elderly, children, or those who have renal or hepatic impairment will be at increased risk.[2] As the production and use of pharmaceuticals continues to increase, the measurable levels in water systems will also increase. The question needs to be asked: *Is it acceptable to have active pharmaceuticals in drinking water even at subtherapeutic levels regardless of the predicted risks?*

It is not just the impact on human health that is relevant, but also that on other animals, marine life, and ecosystems. For example, environmental exposure of diclofenac (**2**) has been revealed as the cause of the declining vulture population in Pakistan.[5] Even our household pets may be at increased risk due to them having differing metabolic pathways. Thus, cats are deficient in the glucuronidation pathway and can accumulate and actually increase the half-life and toxicity of paracetamol (**3**) upon ingestion.[6]

The main sources of pharmaceutical contamination of water systems are humans (medications either ingested or improperly disposed of) and animals (veterinary treatments including medications),[7] but they can also include hospital wastewater and manufacturing facilities.[8]

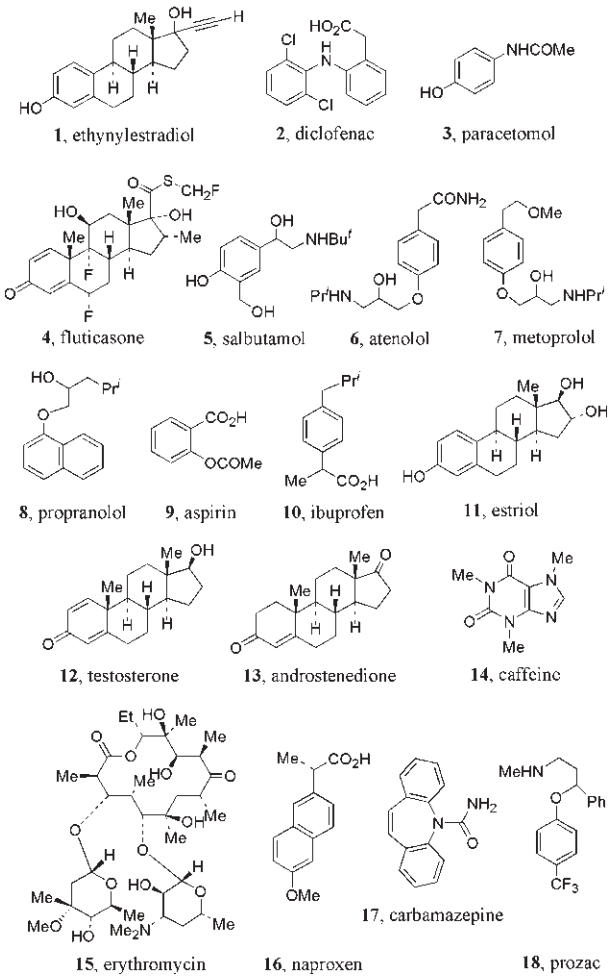
Disposal Pathways for Pharmaceuticals

The main pathways associated with the human disposal of pharmaceuticals that lead to their transport into the environment are summarised in Fig. 1.

Excretion

Pharmaceuticals can act either directly on various organs in the human body without any chemical modification or

Chart 1



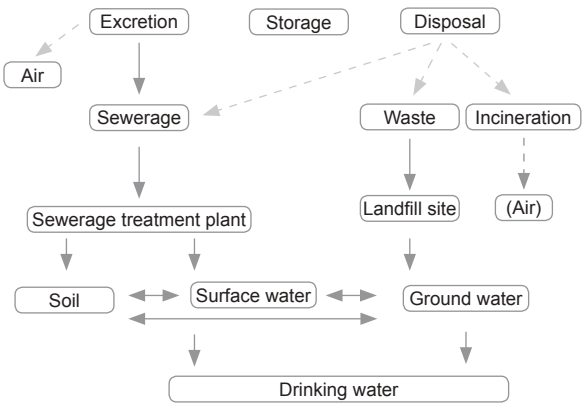


Fig.1. Potential pathways for entry of human pharmaceuticals into the environment (taken from European Medicines Agency Document EMEA/CHMP/SWP/444700, 2006).

they can be modified during their interaction. After their pharmacological action they are excreted in either an unchanged or modified form. Most pharmacologically active molecules are lipophilic and, following biotransformation, they are converted into water-soluble (and hence excretable) metabolites.[9] These metabolites are often less active than the parent compound although some may have enhanced activity or toxicity.[9] Additionally, many pharmaceuticals are excreted unmetabolised and in combination these products typically are discharged into sewage to be treated by a municipal authority.

A small fraction of human pharmaceuticals are used in a volatile liquid or gaseous form, *e.g.* inhalers used to treat asthma in NZ often contain fluticasone (**4**) or salbutamol (**5**) in an aerosol form. This can lead to the *excretion* into the atmosphere of the parent or metabolised forms, but rapid dilution with the air soon makes any subsequent environmental impact very unlikely.

Disposal of Unused Pharmaceuticals

Unused pharmaceuticals are either returned to the retail source or, more likely, disposed of as solid waste to a landfill or flushed down a toilet to become a component of liquid sewage. A study conducted in the US found that less than 2% of those surveyed returned unused medication to a pharmacy, 54% added them to household solid waste, and 35% disposed of them down the toilet or sink.[10] In the UK, 63% of those surveyed discarded unused medications in the household waste, 22% returned them to a pharmacy and 11% emptied them into the sink or toilet.[11]

Landfill and Sewage Treatment of Pharmaceutical Waste

Once a pharmaceutical compound is deposited as a solid in a landfill it is subject to all of the aerobic and anaerobic degradation processes that occur for any other type of organic solid waste. These lead to the formation of an organic-rich liquid leachate that can be contained or released in a controlled manner onto nearby soil, where it can make a contribution to the groundwater, or into a natural river course, where it is rapidly diluted. Studies conducted in the US have shown that landfill leachate can be a significant source of organic waste water contaminants and that the detected concentrations decreased along a gradient from a landfill.[12] To lessen this effect, many states are now recommending the disposal of pharmaceuticals in plastic containers before entry into the landfill to reduce the leaching of pharmaceuticals. While these plastic containers can remain undegraded for decades,[13] they can only delay the environmental exposure of the pharmaceuticals. If the organic material in a landfill is incinerated, then the pharmaceuticals present are combusted largely to form harmless gases that rapidly disperse in the atmosphere.

Conventional sewage treatment facilities involving primary and secondary treatment were never designed specifically to remove pharmaceuticals and, given their variable physical and chemical properties, their removal efficiency varies substantially.[1,8,14-17] The solid digested sludge can be spread on land or buried in a landfill, while the treated effluent is typically discharged into a natural waterway such as a river, lake, estuary or the open ocean. These aquatic systems can, in time, become the source of drinking water for humans, yet conventional drinking water treatment processes also have been found to be ineffective in removing many of these trace pharmaceuticals.[18,19] A specific example is the common *β*-blocker drugs atenolol (**6**), metoprolol (**7**) and propranolol (**8**) which have been widely detected in the aquatic environment.[14,20] Less than 10% of both **6** and **7** is removed by conventional sewage treatment using activated sludge.[21]

Environmental Impact (Ecotoxicology) arising from Pharmaceutical Usage

In assessing the likely impact of pharmaceuticals discharged into the environment, an important question is: *What is the ecotoxicological impact of the parent compounds or their metabolites and any degradation products arising from the treatment of the waste water containing these compounds?*[22] Some of the potential adverse effects that have been identified include lethal and sub-lethal toxicity on aquatic organisms, resistance development in pathogenic bacteria, genotoxicity, and endocrine disruption.[1,23-25] It is also important to consider the stability of these compounds in water,[26] and the potential for their bioaccumulation in marine life.[2]

One of the most common pharmaceuticals typically observed throughout the world in aquatic environments such as raw and treated sewage is the non-steroidal anti-inflammatory drug (NSAID) diclofenac (**2**, Chart 1). Median concentrations of **2** of 0.81 μg/L have been measured in municipal sewage treatment plant (STP) effluents and 0.15 μg/L in rivers and streams in Germany.[14] The sub-lethal toxic effects of this drug have been studied on rainbow trout (*Oncorhynchus mykiss*) by exposing them to concentrations of **2** in the range 1–500 μg/L over a 28 day period.[4] Histological changes were observed in kidneys and gills with the lowest observed effect occurring at a concentration of 5 μg/L. However, significant concentration-related bioaccumulation of **2** occurs in the liver, kidney gills and muscle of the trout. This suggests that prolonged exposure to environmentally relevant concentrations of **2** would lead to an impairment of the general health condition of the fish. Such an effect, however, is much less dramatic than the massive population decline reported for Indian vultures that fed on carrion of diclofenac-treated domestic livestock and cattle.[5]

Although the concentrations of individual pharmaceuticals can now be relatively easily measured, it is also important to remember that leachate and sewage waste streams contain a very large number of pharmaceutical components and such mixtures of pharmaceuticals may lead to additive or synergistic ecotoxicological effects. For example, NSAIDs including aspirin (**9**), diclofenac (**2**), and ibuprofen (**10**) have an estimated annual production of several kilotons worldwide. In ecotoxicity tests their toxicities in combination were considerable despite their individual toxicities being low.[27]

The current knowledge of the effects of trace amounts of pharmaceutical compounds in aquatic systems on the behaviours of a wide range of organisms has been summarised, but it is acknowledged that the information is

sparse and limited to a few substances and organisms.[22,28] The environmental concentrations of pharmaceuticals have been measured and generally fall within a range 10^3-10^7 times lower than the known LC_{50} or EC_{50} values for various organisms. Hence it is unlikely that lethal or acute toxicity effects will occur. However, the example of **2** in trout discussed above suggests that many presently unrecognised sub-lethal or chronic toxic effects may still occur.

A risk assessment database has recently been established to evaluate the potential risks of pharmaceutical contaminants in the environment with a focus on marine and estuarine environments.[29] The compounds are ranked using five different combinations of physicochemical and toxicological data that emphasise different risks. The results suggested that drugs used to treat infections pose the greatest risk to the environment. However, in setting up this database it became clear that there were still significant gaps in the knowledge available to accurately predict the impact of many other pharmaceuticals on the environment.

Chemical Aspects

Analytical Determination of Trace Levels of Pharmaceuticals

In order to assess the real or potential impact of the discharge of pharmaceuticals into the environment, it is essential to establish the concentrations of the parent and metabolised forms of these compounds in waste streams that discharge into the environment. This task is currently impossible for a landfill given the extreme heterogeneity of the solid contents. Atmospheric levels are so low as to be undetectable with current analytical instrumentation. Instead, much research[30-32] has been undertaken to develop methods for accurately and precisely measuring their typically very low levels in liquid streams associated with either landfill leachate or treated and untreated forms of sewage. Most of these methods, like that detailed below,[33] involve the extraction of the lipophilic pharmaceuticals using solid-phase extraction, separation by liquid chromatography, and detection using some form of mass spectrometry, *e.g.* LC-MS-MS. The limits of instrument detection in the separation and quantification of 27 such compounds in a diverse group of pharmaceuticals, steroids, pesticides, and personal care products were reported to be < 1.0 pg (<1.0 x 10^{-12} g) and recoveries of most compounds were >80%.

Observed Trace Levels of Pharmaceuticals in Waste Waters

The analysis of waste water to determine the trace levels of pharmaceuticals has developed only over the last 10-15 years following the pioneering study of German waste water (1998),[14] in Italy (2000),[34] and in a wide range of US streams (1999-2000) by the US Geological Survey.[35]

Other countries for which studies have been reported[36] of the concentrations of selected pharmaceuticals in various natural waters include Brazil,[15] the Netherlands, Switzerland, Spain, Finland, Canada[15,36] and Italy,[37] Mexico,[38] South Korea,[39] and Australia.[40,41] Typical levels reported for a range of different types of water in overseas countries for some of the common pharmaceuticals used in NZ (Table 1) are listed in Table 2.[26,35,38,40,42,43]

Strategies for the selection of those pharmaceuticals to monitor in natural waters have involved those based on calculation of sales volumes multiplied by percentage of metabolic excretion,[7,44] those that had previously been measured in water,[37] and those that were likely to be problematic due to high activity and potential activity even at low usage volumes.[7] A list of *priority pharmaceuticals* that have been selected for analysis in Italian waste waters has been described by Castiglionoi *et al*.[32]

Sewage Treatment Processes

As noted above, many conventional waste treatment processes such as those involved in primary or secondary waste water treatment, have minimal, if any, effect in degrading pharmaceuticals. Hence there is a significant current research effort to develop new processes that can degrade them to levels that are likely to have minimal environmental impact. Typically, more advanced (tertiary) treatment methods have been developed to degrade pharmaceuticals in the aquatic environment[45] that include membrane filtration, such as nanofiltration and reverse osmosis,[16,46,47] and activated carbon adsorption.[18,39] For example, a membrane filter system is in removing hormone compounds such as estriol (**11**), testosterone (**12**) and androstenedione (**13**), and certain pharmaceuticals such as paracetamol (**3**), ibuprofen (**10**) and caffeine (**14**) from sewage influent.[39] However, this membrane treatment did

Typical method for the extraction and analysis of pharmaceuticals from natural water sources; see ref. 33

1. Collect ≥ 1 L of natural water, *e.g.* sewage influent and effluent, groundwater, estuarine water, in a clean, darkened glass container.
2. Adjust the pH to < 2 using conc. H_2SO_4 to minimize degradation of the analyte(s) during storage at 4 °C.
3. Extract the analytes by loading 1 L of water sample on a pre-conditioned Waters HLB solid phase extraction cartridge, elute with methanol followed by a 1:9 mixture of methanol/*t*-butyl methyl ether (MTBE).
4. Add appropriate amounts of internal reference pharmaceutical compounds.
5. Concentrate the eluate to a volume of 1 mL using a stream of N_2.
6. Separate the analytes using a Synergi Max–RP C12 column and a binary gradient involving 0.1 % HCO_2H in H_2O and 100 % MeOH.
7. Analyze a 10 μL sample using an Applied Biosystems Model API 4000 triple quadrupole tandem mass spectrometer in one of the three modes: ESI positive, ESI negative or APCI positive.

Table 1. Top 15 NZ pharmaceuticals by number of prescriptions dispensed between July 2006 and June 2007.[a]

Rank	*Common Name*	*Treatment Condition*
1	Paracetamol	Analgesic/Antipyretic
2	Aspirin	Analgesic/Anti-platelet
3	Simvastatin	Cholesterol and cardiovascular control
4	Omeprazole	Dyspepsia, peptic ulcer disease
5	Amoxycillin	Broad spectrum antibiotic
6	Amoxycillin clauvulanate	Broad spectrum antibiotic
7	Metoprolol succinate	*β*- blocker (blood pressure control)
8	Salbutamol	Asthma (inhaled)
9	Diclofenac sodium	Analgesic/Anti-inflammatory
10	Cilazapril	ACE inhibitor
11	Frusemide	Diuretic
12	Bendrofluazide	Diuretic
13	Quinapril	ACE inhibitor
14	Fluticasone	Asthma (inhaled)
15	Prednisone	Steroid

[a]Data kindly provided by Pharmac NZ

Table 2. Concentrations in overseas natural waters of pharmaceuticals commonly prescribed in NZ (2006-07).

Pharmaceutical	*Country*	*Water Type*	*Typical Conc. (ng/L)*	*Ref.*
Paracetamol	Australia	STP[a] influent	8.1-23.3	40
"	USA	streams	110	35
Aspirin	Australia	STP influent	9.0-38.5	40
Simvastatin	USA	surface water	< 4 (LOD)[b]	42
Omeprazole	Spain	STP influent	2.17	43
Amoxycillin	Italy	STP influent	4.7	26
Metoprolol	Mexico	STP influent	210-250	38
Salbutamol	Italy	river	2.5	26
Diclofenac	Mexico	STP influent	250-340	38
Frusemide	Italy	river	255	26
Fluticasone	USA	surface water	< 13 (LOD)	42
Prednisone	USA	surface water	< 2.2 (LOD)	42

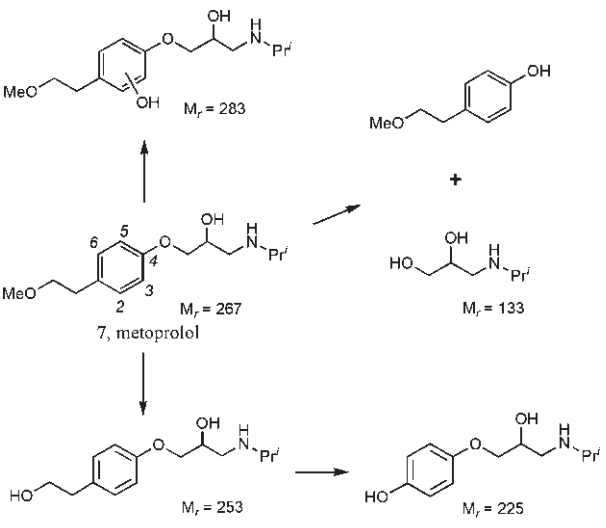
[a]STP: sewage treatment plant [b]LOD: limit of detection reported

not decrease the concentrations of other pharmaceuticals such as erythromycin (**15**), naproxen (**16**), diclofenac (**2**) and carbamazepine (**17**) as previously noted.[15,16] In comparison, reverse osmosis and nanofiltration processes show removal rates in excess of 99 % for all of these pharmaceutical compounds from sewage influent.

Nonetheless, there are issues that can complicate the successful implementation of these treatment processes, *e.g.* reverse osmosis and activated carbon adsorption require a high input of energy and material.[48] Similarly, it has been noted that many pharmaceutical compounds are polar and thus less likely to removed by the hydrophobic interactions involved in carbon absorption.[49] Recent studies conducted in Australia compared different wastewater recycling schemes to determine the impact of the treatment processes and found that the addition of reverse osmosis technology can concentrate many of the compounds of concern.[40.41]

Other successful tertiary treatments, including advanced oxidation processes (AOPs) involving the reaction of the very strong hydroxyl radical (•OH) oxidant and/or the solvated electron (e_{aq}^-) are also effective in removing many pharmaceutical compounds from waste water.[18,50] These reactive species can be generated by chemical reactions involving a range of chemical agents such as O_3, H_2O_2, transition metals, *e.g.* Fe^{2+} – Fenton reaction, and metal oxides, *e.g.* TiO_2, together with auxiliary energy sources such as UV-visible radiation, electron beam, γ-radiation and ultrasound. Thus, the *β*-blocker metoprolol (**7**) present in waste water is only degraded by 10% using conventional activated sludge treatment,[21] but it can be removed efficiently from aqueous solution by reaction with •OH and e_{aq}^- produced by an electron beam.[51] The degradation products were separated and detected with LC-MS techniques leading to the degradation pathway of Scheme 1. Of course nothing is known about the ecotoxicty of the degradation products and so the overall assessment of how eco-friendly this advanced oxidation process might be in reducing the levels of metoprolol in natural water remains unknown.

Photochemical AOP reaction can be affected by anything present in the water that absorbs or reflects the incident light. Variable levels of dissolved organic matter, carbonate, bicarbonate, and chloride ions are always present in all naturally occurring waters and these species can also react with •OH and e_{aq}^- to reduce significantly the overall efficiency of the removal of trace levels of pharmaceuticals.[52]



Scheme 1. Degradation products and proposed reaction pathways for •OH oxidation of metoprolol - see ref. 64

Modelling Pharmaceutical Concentrations in Raw and Treated Sewage

An alternative to actually measuring the trace levels of pharmaceutical compounds in raw sewage after various levels of treatment is to calculate these levels. Such an approach has been reported[53] using fugacity modelling and data on i) pharmaceuticals usage (prescription records), ii) human metabolism and excretion of pharmaceutical residues, iii) the chemical and physical properties of the compounds, and iv) information on the design and operating characteristics of the sewage treatment process. This model was successfully tested using Australian data from which 29 of the *top-50* most commonly dispensed pharmaceuticals were predicted to be present in raw sewage influent at concentrations of ≥ 1 μg/L while 20 of the compounds were predicted to remain at effluent concentrations of ≥ 1 μg/L after secondary treatment. While it was conceded that the model *possesses a high degree of uncertainty*, it was a useful screening tool for providing a) a basis for estimating any correspondence between the quantities of prescribed pharmaceuticals and their observed concentrations, b) an estimate of concentration and likely distribution of compounds that have not been measured, and c) an indication of likely future effluent concentrations of any new drugs.

Green Chemistry

In the current era of environmental sustainability, the rapidly developing area of chemistry described as *the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances* (green chemistry) clearly is very relevant to any chemical process that can degrade and detoxify pharmaceutical compounds discharged into the environment.[28] Current *green processes* that might be used for the treatment of waste- or drinking-water include all of the ozone-based advanced oxidation processes, *viz*.(O_3 and O_3/H_2O_2 with and without UV light). The Fe-TAML activation of peroxide[54] has also been found to degrade recalcitrant pharmaceuticals such as fluoxetine (**18**)[55] and a range of estradiol-based hormone compounds.[56]

Of course, the ultimate Green Chemistry challenge as identified by Khetan and Collins[30] would be to design pharmaceuticals that contain a structural component that is unreactive in the human body while treating an ailment, but *turned on* to become reactive when the compound or its metabolites are excreted and released into the environment. Alternatively, this special structural component might enhance removal of the compound once it is present in the waste water undergoing treatment in a sewage plant using a selective process such as surface adsorption.

Conclusions

The profession needs to be proactive now rather than reactive when a health problem emerges. We need to determine the key contributors and/or potentially harmful compounds, and put processes in place that minimise their entry into the waterways.

There appears to be almost nothing known about the levels of pharmaceuticals disposed in the NZ environment or of the impact they have. Although annual data on the quantities of various pharmaceuticals sold in NZ are available from Pharmac (see Table 1), there appears to be no published measurements on the levels of these compounds in NZ waste streams either in parent form or in degraded form(s). Moreover, the recent Ministry for the Environment publication *Environment New Zealand 2007* that updates the state of NZ's environment,[57] has no mention of the levels of possible organic contaminants arising from the use of pharmaceuticals. Seemingly there are no limits currently specified in the resource consent monitoring conditions for maximum allowed levels of *specific* pharmaceutical compounds in the effluent discharged from sewage treatment plants in this country. However, current overseas research clearly indicates that even trace levels of some of the compounds likely to be present in NZ waste water (based on Pharmac records) can have a potential detrimental effect on the environment by affecting aspects of biological activity. They could also affect the quality of NZ drinking water obtained from groundwater and other natural sources. If NZ is to maintain its international *clean green image*, then it is essential that all aspects of pharmaceutical use and disposal in NZ are investigated.

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Darwin Celebrated

Charles Darwin's legacy was celebrated in Auckland on the 200th anniversary of his birth.

Darwin was born on February 12 1809 and on February 12 2009, a free public symposium; "(R)evolution – a Celebration of Darwin", was held at Auckland University.

The day included speakers discussing the impacts of Darwin on various areas of science as well as the humanities. Professor Brian Boyd (pictured) from the Auckland University English Department gave a talk on imagination and story telling in the evolution of humans.

A poster competition was held for graduate students involved in evolution research. This was won by Katie Hartnup from Massey University with her poster; "Historical DNA analysis of kiwi feather cloaks: Kahu Kiwi".

The event was well attended with the 600-seat Fisher & Paykel Appliance Auditorium full and overflowing into another venue for some of the talks. Three hundred secondary school students were part of those present. Organiser, Professor Allen Rodrigo (pictured) from the University's Biological Sciences Department, was delighted at the response.

Other events to celebrate Darwin included a series of lectures in March by Dr Frans B. M. de Waal that started with; "Our Inner ape – Morality: A Darwinian view of the moral emotions in man and animals" and also covered empathy and culture. In May a two day course entitled "Resolving the Creation versus Evolution Controversy" is also being held.

Allen Rodrigo and Brian Boyd at the symposium (Picture credit: University of Auckland photographer, Godfrey Boehnke)

Article

A Possible Candidate for the Fight against Alzheimer's Disease

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Introduction

Although there are examples of individuals living for a century and more with little decline in brain function, many others are not so fortunate and are afflicted with debilitating disorders like Alzheimer's disease (AD) even by the age of 65 years. It is projected that by 2050 there will be a 106 million people affected worldwide with this disease with 10% of the population affected in NZ.[1] Alzheimer's disease is a progressive neurodegenerative disorder resulting in cognitive, memory and behavioural impairments, and is the leading cause of dementia in humans. There is no currently known cure or treatment to reverse its pathology. The cognitive decline associated with AD has been proposed to result from overproduction of a *sticky peptide* amyloid-β (Aβ) in the brain. Excessive production of this peptide arises from an imbalance in the processing of the large parent amyloid precursor protein (APP) by proteases.

Although Alois Alzheimer made the connection between the combined presence of senile plaques and tangles in the brains of the deceased who had dementia in 1906, it took another 80 years before a clearer understanding of the genetic and biochemical complexities underlying the formation of these plaques and tangles began to emerge. Since the 1980s studies have shown that the plaques are primarily composed of Aβ,[2] and Aβ is neurotoxic.[3] Soluble aggregates of Aβ induce synaptic dysfunction leading to learning and memory deficits,[4,5] and eventually form insoluble plaques in the brain (Fig 1).[6]

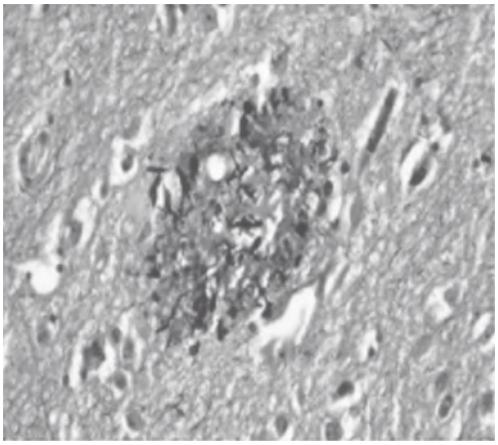


Fig 1. An insoluble amyloid-β (Aβ) plaque in a human brain histology section from an Alzheimer's patient (adapted from Selkoe (1999) in ref. 14 with permission from Macmillan Publishers Ltd.).

Risk Factors

While AD is age-related, environmental factors such as behaviour, consumption of a high calorie and fat diet, history of head trauma, and a sedentary lifestyle are also likely to increase the risk.[7] A recent study in an AD mouse model induced insulin resistance (as seen in humans with obesity and type 2 diabetes mellitus) by feeding the mice with 10% sucrose-sweetened water and also exacerbated memory deficits and increased insoluble Aβ deposition in the brain.[8] Low dietary folate intake and high intakes of lipids and metals such as copper and iron may also influence disease risk.[9] Controlling the consumption of sugar-sweetened beverages,[8] regular physical exercise, a strict dietary regimen, and maintaining a cognitively stimulating environment could be protective against the development of AD – and, as we are constantly reminded, for most other diseases![10]

Genetic factors that affect the precursor protein APP in particular, also increase the risk of AD, and causative mutations in APP have been discovered.[11] Subsequent studies also identified mutations within the genes presenilin-1 and -2 (PS-1, PS-2) to cause inherited AD.[12] It also seems likely that other genes will influence one's susceptibility to AD. One such example is apoliprotein E (ApoE), with individuals who produce the ApoE4 isoform being more likely to develop the disease.[13] The mutations within these genes are believed to be either directly or indirectly responsible for the increased production of Aβ by promoting an imbalance in APP processing.

Cleaving the Precursor Protein APP

A molecule of APP is cleaved at *one of two competing sites* by two distinct proteases, α- or β- secretase, releasing either a 612 amino acid (612aa) or a 596aa (Fig. 2) extracellular *N*-terminal product, respectively.[14] Following this first cleavage, another intramembrane cleavage follows downstream and is caused by another enzyme, the γ-secretase. When APP is cleaved by the α- and γ- secretases, an *N*-terminal fragment sAPPα is generated but, importantly, the amyloidogenic Aβ peptide cannot be produced. In healthy individuals there exists a fine balance between this pathway and the β-secretase pathway where Aβ can be produced. However, in AD patients, APP enters the β-secretase pathway more frequently and increases the production and concentration of Aβ whilst decreasing the production and concentration of sAPPα.[4,15]

Is the C-terminal 16aa of sAPPα Neuroprotective?

sAPPα from the α-secretase pathway is identical in sequence to the *N*-terminal protein, sAPPβ (Fig 2) derived from the β-secretase pathway, with the exception of the C-terminus. sAPPβ is shorter by 16 amino acids. However, sAPPα is neuroprotective, is 10- to 100-fold more potent than sAPPβ in protecting neurons against Aβ mediated toxicity,[16-18] and it protects cells against oxidative stress resulting from glucose deprivation.[17,19] It has been demonstrated experimentally that the neuroprotection offered by sAPPα could be a result of its ability to signal an up regulation of the expression of neuroprotective genes.[20,21] While Aβ interferes with synaptic plasticity, neurite outgrowth, and elongation, *i.e.* axon/dendrite projections from a neuron, these pathogenic effects are prevented in

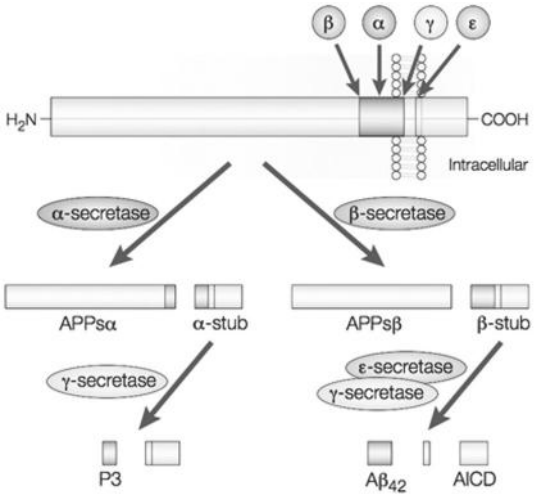


Fig 2. The two competing pathways driven by *α*- and *β*-secretases (adapted from LaFerla (2002) in ref. 14 with permission from Macmillan Publishers Ltd.).

the presence of sAPP*α*.[22] Even more exciting is *the observation made by our group that sAPPα alone plays an important role in the retention and restoration of spatial memory in rats*.[18]

A Specific Role for the C-terminal 16aa of sAPPα?

sAPP*α* is neuroprotective and plays important roles in preventing neurodegeneration and promoting memory. We believe that sAPP*α* may be as important as A*β* in the dynamics of the pathogenesis of AD. However, it carries 612 amino acids (aa – 612aa), and getting it across the blood brain barrier (BBB) poses a significant problem to any use as a therapeutic reagent. Could a region within sAPP*α* be a key to its neuroprotective function? A good place to start such a search is with the 16aa region present in sAPP*α* but absent in sAPP*β*, as this marks a very large change in effective function. Our study aims to test whether this region of sAPP*α* plays a critical role in its signalling function.

The C-terminal 16aa region of sAPP*α* is strongly hydrophilic in keeping with our proposal that it could be involved in ligand-receptor interactions, perhaps even the key to initiating sAPP*α* functions. Within this 16aa region there is also domain that could possibly bind heparin,[23] which could then play a crucial role in cell-substratum adhesion and neuroprotectivity. Indeed, preventing heparin binding causes a loss of both functions.[16] This region is a good candidate for association with the cell surface, preventing neurite retraction and conferring neuroprotection against A*β* toxicity. Additionally (or alternatively), the 16aa C-terminus could confer critical structural parameters on sAPP*α* crucial for its functions and thus have an indirect influence. Studies to test this hypothesis are to be carried out.

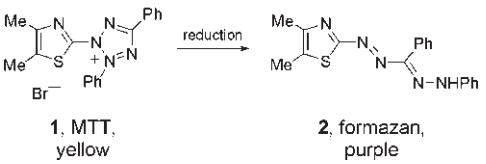
Experimental Strategy

A: Can the Neuroprotective Function of sAPPα be Validated?

We have produced recombinant sAPP*α* to circumvent the processing that occurs *in vivo*. Functional validation of native recombinant sAPP*α* produced in the laboratory was necessary before developing the sAPP*α* variants for testing the function of the C-terminal region. Two cell based assays namely, *cell viability* and *activation of a responsive promoter*, were chosen for this purpose.

Cell viability assay — The cell viability assay (often referred to as the MTT [**1**, 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in the literature) is a colorimetric assay used to compare the viability of cells subjected to different treatments, from their oxidant/antioxidant status. MTT is a yellow coloured compound that is reduced by cellular mitochondrial dehydrogenases to purple coloured crystals of formazan (**2**; Scheme 1). The assay depends on measuring the health of the cells by their ability to produce **2**.

Scheme 1



Our experimental strategy involved pre-incubating neuroblastoma cells (SHSY5Y - a model-neural cell line) and African green monkey kidney fibroblast-like cells (COS-7 - a commonly used mammalian cell line) with sAPP*α* and determining in what way they reacted to the stress of glucose deprivation. The aim was to confirm that our laboratory-produced recombinant sAPP*α* could protect cells from the adverse effects of hypoglycaemic damage (oxidative stress).

NFκB promoter activation assay — The second assay used luciferase as a reporter gene and was based on the activation of the NFκB promoter (nuclear factor kappa-light-chain-enhancer of activated B cells). This promoter encompasses a major family of transcriptional factors involved in the regulation of the transcription and expression of neuroprotective genes. Previous studies[17,24] had shown that sAPP*α* can somehow activate this family of transcription factors. The COS-7 cells were used because they have a functioning NFκB promoter that can be further stimulated by sAPP*α*. They were transiently transfected with the NFκB-dependent luciferase reporter plasmid (pNFκB-Luc) and, after cell lysis, luciferase activity was assayed *in vitro*. The light emitted was then measured in a luminometer.

B: Generation of sAPPα Variants

Substitution and deletion variants of sAPP*α* within its C-terminal region were designed to test our hypothesis that the 16aa C-terminal region, present in sAPP*α* but absent in sAPP*β*, plays an important role in its function. These variants have been prepared and now are available for testing either to mimic or interfere with the function observed in recombinant sAPP*α* with the natural sequence. In addition, chemically synthesized peptides (by Professor Margaret Brimble, Auckland University) corresponding to the 16aa region are to be tested at the same time.

As changes had to be introduced at the C-terminal end of the sAPP*α* gene, site-directed mutagenesis was per-

formed using reverse mutagenic primers to introduce variations within sAPP*α*. Following amplification by the polymerase chain reaction (PCR) to incorporate the mutations, the gene products were then cloned into a special vector (the mutated sAPP*α* gene was inserted into a small DNA molecule, termed the vector, which then replicates within bacteria). Following confirmation that the introduced sequences were correct, the different vectors were then integrated into the genomes of cells of a mammalian cell line—the process of transfection for the creation of stable cell lines. The human embryonic kidney cell line (HEK293) has been used for this purpose as it can be easily transfected with the specialized vector to give stable cell lines. After transfection, the cells were grown in media containing the antibiotic geneticin. The transfecting vector has a gene resistant to geneticin thereby ensuring that only stably transfected cells survive. Individual colonies that survived 2-3 weeks of treatment with geneticin were selected for the establishment of permanent stable cell lines. These stable cell lines produced the variant sAPP*α* proteins.

We designed and generated five variants of sAPP*α* within the unique C-terminal 16aa of sAPP*α* (see Fig. 6a). One targeted two consecutive histidines by substituting them with neutral alanines. These consecutive histidine moieties could be crucial to sAPP*α*'s ability to behave like a receptor and provide a hydrophilic face for interaction with other proteins. Two deletion variants removed i) five amino acids (VHHQK) by inserting a stop codon after a glutamic acid codon in the gene (see also Fig. 6a) - a region implicated in heparin binding[23] - and, ii) ten amino acids by inserting a stop codon after an arginine codon in the gene. The remaining two variants of sAPP*α* are based upon the claim that the terminal lysine (the 612th aa of sAPP*α*), plays a role in the trafficking of full length APP for cleavage.[25] Tests will be made to determine if sAPP*α* function is impaired by substituting the lysine with either an alanine (charge and size difference) or a valine (charge difference but similar size).

Experimental Results

A: Functional Validation of sAPPα

The development and validation of assays that could reliably demonstrate the protective functions of sAPP*α* produced as a recombinant protein in the laboratory were necessary before the development of the variants themselves. Both the cell viability assay and the promoter activation assay have demonstrated that recombinant sAPP*α* could behave as a protective compound and gave us confidence to develop the sAPP*α* variants.

Cell viability assay — Using the cell viability assay, we aimed to demonstrate that recombinant sAPP*α* applied in the culture media could protect cells against the adverse effects of hypoglycaemic damage. As noted above, this assay measures the anti-oxidant status of the cell as an indicator of viability. The time required for glucose deprivation to affect significantly the viability of cells is between 48-72 h (Fig. 3). We demonstrated for the first time that exposure of cells to sAPP*α* for just 2 h was sufficient to protect cells against the effects of glucose deprivation (Fig 4a). This suggests that sAPP*α* behaves like a switch, which can induce changes to the cell physiology when cells are subjected to stress, perhaps by the expression of protective genes and proteins. We carried out this assay with sAPP*β* that lacks the C-terminal amino acids as well. Interestingly here, the sAPP*β* had a *toxic* rather than a protective effect on cells. Intriguingly, in the different COS-7 non-neural cell line, sAPP*α* was biphasic in titrations of over a range of concentrations and actually toxic at higher concentrations (10 nM) (Fig. 4b).

NFκB promoter activation assay — COS-7 cells have endogenous NFκB promoter activity as shown by the expression of the luciferase from the NFκB promoter (see Fig 5), but they have potential for further enhancement by treating the cells with sAPP*α*.[17] An increased emission of light over that from the untreated sample will occur if the

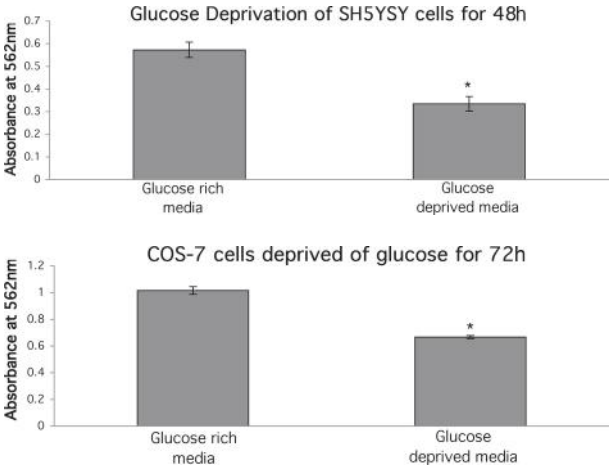


Fig 3. Cell viability in response to deprivation of glucose in SHSY5Y (upper) and in COS-7 (lower); * indicates significance at p<0.05 (student's two-tailed T-test) between cells with and without glucose; Y-axis = absorbance values.

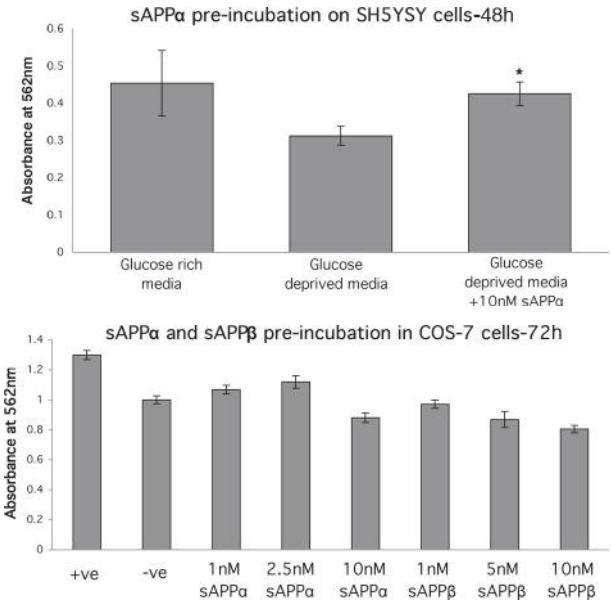


Fig 4. Upper: sAPP*α* protection of glucose deprived cells; * indicates significance at p<0.05 (student's two-tailed T-test) between samples treated with 0.1 x PBS and 10 nM sAPP*α*. Lower: sAPP*α* protection and sAPP*β* sensitization to glucose deprived cells;. +ve, glucose rich media; -ve, zero glucose media treated with 0.1 x PBS; data are normalized to the -ve control.

NFκB promoter is more activated as more of the luciferase will then be expressed. Treating transfected COS-7 cells with sAPP*α* demonstrated sAPP*α*'s ability and sAPPα's inability to signal the downstream activation of NFκB-dependent transcription (Fig. 5); the positive control used was lipopolysaccharide (LPS).

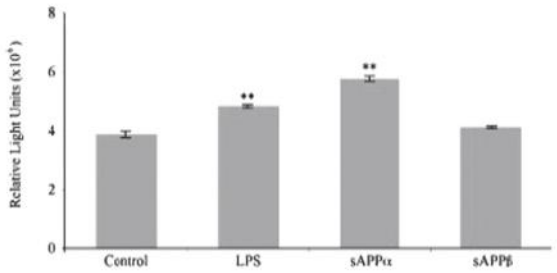


Fig 5. Activation of NFκB promoter by sAPP*α* but not by sAPP*β*; PBS (control), LPS (+ve control), sAPP*α* (2.5 nM) or sAPP*β* (2.5 nM); results are the average of four separate experiments following normalization; error bars show standard deviation.

B: Generation of sAPPα Variants

By designing appropriate primers and employing PCR, the desired sAPP*α* mutant genes were generated (Fig. 6) and then cloned into the specific integration vector for the production of stable cell lines. Positive colonies were confirmed diagnostically by a restriction enzyme digest using the *Bam*H1 enzyme - a 1.3 kb band along with a 6.3 kb band indicates a positive clone (Fig. 6 lower) and the correct modifications were confirmed by sequencing.

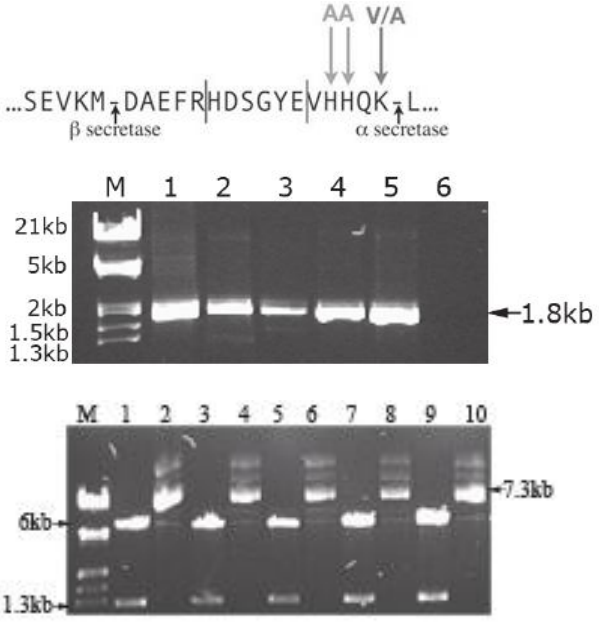


Fig 6. (Upper) Deletion and substitution variants of sAPP*α*.; vertical lines after arginine and glutamic acid represents insertion of stop codon. ***(Centre)*** synthesis of mutant sAPP*α* genes by mutagenic primers and PCR; **M**-Marker – **Lanes 1-5**: H609&610A, 1-607, 1-601, K612V, K612A, – **Lane 6**: -ve control. ***(Lower)*** Diagnostic restriction digest for successful clones –.the 1.3 kb band confirms the presence of insert; **M**-Marker – **odd lanes**:-digested H609 & 610A, 1-607, 1-601, K612V & K612A; .– even lanes: undigested H609 & 610A, 1-607, 1-601, K612V & K612A.

C: Expression of sAPPα Variants by Stably Transfected HEK293 Cells

HEK293 cells were transfected with the recombinant DNA to create stable cell lines that produced the recombinant sAPP*α* protein variants. The experimental design allowed the protein to be secreted directly into the culture media.[17] The presence of heparin binding domains within the sAPP*α* and sAPP*β* has allowed the development of a one-step protein purification strategy.[17]

Cells are usually maintained in serum enriched media. However, serum free media can be substituted during protein harvest to ensure that only the required protein and a small number of other proteins are present, ensuring a simple purification process. To confirm that the required protein was being produced and secreted into the media by the HEK293 cells, an immunoblot for each of the variants was carried out using an antibody that recognized the *N*-terminal of the protein variants. The immunoblot demonstrated that the HEK293 cells had been successfully transfected and were producing and secreting the required sAPP*α* variants into their media (Fig 7). Each of these is to be tested for neuroprotective functions and compared with the native sAPP*α* and sAPP*β*.

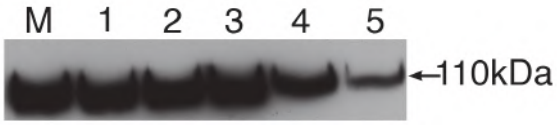


Fig 7. Immunoblot to detect sAPP*α* variants in culture; **lane 1**: H609&610A; **lane 2**: 1-607 sAPP*α*; **lane 3:** 1-601 sAPP*α*; **lane 4:** 1-601 sAPP*α*; **lane 5:** K612V: **lane 6**: K612A.

Discussion

AD is beginning to impact on health budgets both here and abroad and this will only intensify over time. There is no effective therapeutic strategy currently to reverse the pathology of AD, and in NZ even the best available drugs, acetylcholinesterase inhibitors, remain to be funded by Pharmac.

In common with other neurodegenerative disorders, AD has abnormal accumulation of aggregated proteins (amyloidosis). Hence, a therapeutic strategy that alleviates the pathology of AD might also be applicable to other neurodegenerative disorders such as Parkinson's and Huntington's diseases.

Our assays show that laboratory produced sAPP*α* is protective and/or beneficial to cells of both neural and non-neural cell lines. This implies that the effect is likely to be general. Furthermore, we have demonstrated sAPP*α*'s ability to influence and promote the transcription of protective genes indirectly, by showing that it can activate the NFκB family of transcription factors.

A quite brief pre-exposure of cells to sAPP*α* is sufficient to protect them from the adverse effects of glucose deprivation. However, while low concentrations of sAPP*α* are protective, higher concentrations become toxic as previously demonstrated by us;[18] low concentrations of sAPP*α* restored memory mechanisms in rats, but higher concentrations (10 nM) were counterproductive and even

toxic. This implies that there exists a delicate balance in the proteolytic processing of APP and that any imbalance results in detrimentally higher concentrations of one product over the other.

Since sAPPα can be applied in the culture media, the studies strongly suggest both neuroblastoma and COS-7 cells have a sAPPα-specific surface receptor(s) that have yet to be identified and characterized. We are currently directing our work toward this.

The only difference between sAPPα and sAPPβ is the 16aa at the C-terminus. Yet, sAPPα is almost 10- to 100-fold more potent than sAPPβ, as we have indicated and reported also by others.[16,19] We have suggested that this region of the protein could be responsible for the activity of sAPPα. To test this hypothesis deletion and substitution variants of sAPPα have been generated with the changes within the C-terminal end of sAPPα. We are now poised to assess whether the variants mimic or interfere with the function of wild type sAPPα. In addition, we have prepared a synthetic 16aa peptide that corresponds to the C-terminal end of sAPPα and its activity will be tested independently to see if it acts as a mimic or a competitor. A previous study[20] suggested a protective function for a 10aa peptide spanning the β-secretase junction and we aim to see if this finding can be replicated. Should the 16aa peptide itself prove be a potent neuroprotector, then it has the potential to be utilized for a viable therapeutic strategy.

Acknowledgements

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Science Scene continued

Astronomy's Year

2009 is the International Year of Astronomy. It was chosen because it is the 400th anniversary of the first use of an astronomical telescope by Galileo Galilei.

The aim of the organising groups, the International Astronomical Union and the United Nations, Educational, Scientific and Cultural Organisation, is a global celebration of astronomy and its contributions to society and culture.

John Hearnshaw from the University of Canterbury is participating in the cosmic diary blogs as part of the celebrations. You can follow his blog at http://cosmicdiary.org/blogs/john_hearnshaw/

Part of the celebrations is to encourage worldwide interest in astronomy using the theme; "the universe, yours to discover." Many events have been planned and educational resources are available through the website; *www.astronomy2009.org*

To find out about events happening in New Zealand visit the website; *www.astronomy2009.org.nz*

Getting the measure of things

New Zealand's Virtual Institute of Metrology in Chemistry (VIMC) is finding demand increasing for information and help on chemical and biological measurements.

The website is *http://msl.irl.cri.nz/si-units/chemical/index.html* Here you can find information on reference materials, proficiency tests and chemical standards programmes, as well as other useful references.

Head of the VIMC is Dr Laly Samuel at Industrial Research Limited. She was part of a group that set up the National Metrology Institute in Japan in 2001. She says, "the institute is helping laboratories to ensure New Zealand's routine analysis is up to international level."

Many of the requests to the VIMC involve uncertainty around test results that require traceability back to a referenced measurement.

"The VIMC is an intermediate and cost effective solution but there is a clear need to establish a fully accredited facility in New Zealand," says Dr Samuel.

Bringing Inquiry-Based Learning to the First-Year Laboratory: Experimenting with Conducting Polymers

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Introduction

As might have been predicted, the introduction of NCEA has had a significant influence on students taking 1st-year university chemistry courses, although the feared *lack of chemistry knowledge* does not appear to be one of them. One unanticipated outcome noticed at Otago University is the students' noticeable focus on assessment, seemingly a direct result of the numerous assessments that they have to cope with during Years 11-13 (and in some cases Year 10). This results both in tutorials run by the Chemistry Department being poorly attended and in few students completing the pre-laboratory exercises. On the positive side, students seem better able to prioritize, work independently, and are equipped with a better range of practical skills than previously. The average student has had a greater exposure to laboratory work, in part as a result of the internal assessments such as AS90306 *Carry out an acid-base volumetric analysis*.[1] This has led to a noticeable increase in overall laboratory skills, especially in titrations. When practice assessments are taken into account, the average student who comes through Levels-2 and -3 NCEA Chemistry will have performed several different titrations over the two years, and have great familiarity with the technique as well as general confidence in performing experiments. Those students who have carried out the Level-3 extended practical investigation (AS90694)[1] actually need to develop and demonstrate analysis, evaluation and planning skills to achieve *excellence* (23% of students taking this standard received *excellence* in 2008, *i.e.* they need to exhibit what are frequently referred to as *higher order cognitive skills*.[2-4] The AS90694 achievement standard[1] indicates that students must carry out *high quality practical investigations*, and goes on to define such an investigation as including *a description of the final procedure that shows an* ***understanding*** *of the technique used, and reasons for any modifications to the procedure, a comprehensive* ***evaluation*** *of the whole investigation that considers: the* ***significance of the results*** *in relation to the background information and the* ***validity of the conclusion*** *the reliability of the data by* ***evaluating the procedure*** *used and sources of error*. One system used by educationalists to classify levels of cognitive skills is Bloom's taxonomy,[4] first developed in the 1960s and subsequently revised (Table 1). When the higher order skills described in this table are examined, the terms used are very similar to those in the achievement standards mentioned above. The implication of this is that students are bringing into 1st-year Chemistry courses a range of abilities and skills that were not previously taught at school. Thus, the question needs to be asked: *are current 1st-year chemistry courses meeting the needs of the students?* Carnduff and Reid have pointed out that *it is possible to plan university chemistry laboratories so that they can avoid repeating school laboratory experiences*

Table 1. Bloom's Revised Taxonomy[a]

	Actions	***Examples***
Creating (Putting together ideas or elements to develop an original idea or engage in creative thinking).	Designing Constructing Planning Producing Inventing Devising Making	Film Story Project Plan
Evaluating (Judging the value of ideas, materials and methods by developing and applying standards and criteria).	Checking Hypothesising Critiquing Experimenting Judging Testing Detecting	Debate Panel Report Evaluation Investigation Verdict Conclusion Persuasive speech
Analyzing (Breaking information down into its component elements).	Comparing Organising Deconstructing Attributing Outlining Structuring Integrating	Database Abstract Report Graph Spreadsheet Checklist Chart Outline
Applying (Using strategies, concepts, principles and theories in new situations).	Implementing Carrying out Using Executing	Illustration Demonstration Presentation Interview Diary Journal
Understanding (Understanding of given information).	Interpreting Exemplifying Summarising Inferring Paraphrasing Classifying Comparing Explaining	Recitation Summary Collection Explanation Show and tell Example List Label Outline
Remembering (Recall or recognition of specific information).	Recognising Listing Describing Identifying Retrieving Naming Locating	Definition Fact Worksheet Test Label List Reproduction

[a]Adapted from refs 2, 3, and 4.

but also ***build*** *on the kinds of thinking skills which school courses seek to inculcate.*[5] It is the opinion of the authors that this should be an aim of any University course and is one of the motivations for the work reported here.

Over the last two years several local high schools have used the Otago teaching laboratories to carry out Level-3 investigations, leading us to a sobering awareness that many of our first-year laboratories did not build on the skills that students brought with them, which in some cases actually were far simpler than the work the students were capable of. This realisation led us to the conclusion that by incorporating the current student skill set, our laboratory course could be less prescriptive, more stimulating and contain some modern and *relevant* chemistry.

Traditional laboratory courses are designed to help students gain new laboratory skills and experience, and support lecture materials; they also tend to have definite *right* and *wrong* outcomes.[3,4] This places constraints on the type of material that can be used in such courses, resulting in student experiences that are frequently based on well worn *safe* material, but for which the cost effectiveness has been questioned.[5,6] On the other hand, first-year lectures are frequently illustrated with examples of chemistry that are designed to show students the relevance of theory or models, and modern textbooks contain examples of chemistry based on new research or applications to illustrate the study material.[7]

The Otago's Chemistry Department offers two first-year chemistry papers. CHEM191, which is part of the First Year Health Science course, is in the first semester and attracts some 1600 students, while in the second semester CHEM111 has ~130 students. Given the numbers involved, CHEM111 was the logical choice to redevelop. Until 2008, the laboratory component of CHEM111 was traditional in its approach. Several laboratory sessions (see Table 2) had closely defined outcomes, so that 95% of the students obtained very similar answers and the work neither stretched the more able students nor demanded any of the higher order skills described above. Each laboratory was assessed by twelve-minute exit tests that assessed students' immediate recall and understanding of the laboratory, but not their development of practical skills.

As described below, new laboratory experiments were designed and a significant change was made to the assessment of the course. There was a move from short end-of-laboratory tests to a structured progression through the laboratory report writing process, each step focussing on one aspect of a report prior to the need for a full report on the three-week *Schiff* base laboratory. Positive feedback is an important source of motivation in students, as it builds confidence and encourages improvement.[6,8] Also, it is important for students to have time to reflect on new ideas and concepts so they can use them to establish new patterns of work and thinking.[2,3,6] Thus, an important part of the laboratory report process was that students had time to work one section at a time, time to reflect on supervisors comments, and then time to prepare the next stage of the report. The progression through the report process started with a *practice* abstract for which no mark was awarded; for the next laboratory the abstract was marked and students wrote a *practice* ***method***; the subsequent report had a marked abstract and method with a *practice* ***results***, and so the process evolved into the full marked report for the three-week Schiff base laboratory described below.

In designing the laboratory course, it was recognised that a more *open-ended* approach to laboratories might allow the incorporation of new teaching material to illustrate the application of recent developments in chemistry. Students are more focussed and self- motivated if they can see the importance or relevance of the subject they are studying, so this change was seen as vital to the success of

Table 2. Comparison of the existing CHEM111 course with the 2008 course.

Old course	***Type of lab and assessment method***	***New course***	***Type of lab and assessment method[a]***
Systematic nomenclature	Computer based lab, exit test		
Boyles law	Practical lab, exit test	Boyles law	Practical lab, no mark, *PRACTICE abstract*
Spectroscopic analysis of aspirin	Practical lab, exit test	Spectroscopic analysis of aspirin	Practical lab, abstract *PRACTICE Results*
Intro spectroscopy	Practical lab, exit test	Intro spectroscopy	Practical lab, exit test
Synthesis and resolution of a cobalt salt	2 week practical lab, 'report'	Conducting polymers	Practical lab, Abstract and experimental *PRACTICE Results*
Coloured coordination complexes	Practical lab, exit test	Synthesis and resolution of a cobalt salt	2 week practical lab, Abstract, Experimental, Results. *PRACTICE Discussion*
IR spectroscopy	Computer based lab, exit test	Synthesis of a Schiff base and its copper salt	3 week practical lab, full lab report
Spectroscopic identification of organic compounds	Computer based lab, exit test	Spectroscopic identification of organic compounds	Computer based lab, exit test
Chemistry of colloids	Practical lab, exit test	Chemistry of colloids	Practical lab, exit test

[a]In addition to the assessment item, students were also given the opportunity to write a *practice* for the assessment item due in the next laboratory session; this process builds towards a full lab report.

the new course. One of the sessions in the existing course was the preparation and resolution of a cobalt salt; whilst this taught some new skills to students, from a synthetic chemist's point of view the final step in the process would be the analysis of the product. To build on the synthesis skills acquired in synthesizing the cobalt salt a new three week (9 h) investigation was developed that followed from it. This new component was based around the preparation and analysis of a Schiff base and its copper complex. After the preparation, students either were provided with, or obtained, data so that they had mass, UV-vis, IR, and NMR spectra of their products thereby allowing for an elucidation of both the final structure and that of the starting materials. It was presented to the students as a *complete* synthesis-analysis problem akin to that experienced by chemists in research situations.

Modelling Innovation: The Polypyrrole Experiment

In an age where science *heroes* are noticeably lacking, Alan McDiarmid's Nobel Prize-winning work with conducting polymers,[9] combined with the establishment of the Polymer Electronics Research group at Auckland University[10] and the ready availability of web sites containing examples of their use, suggested that a laboratory exercise based around these materials would be fitting and appropriate. It was seen as important that this was not just a case of making the materials (as in the previous example) but that some of their unusual properties were illustrated whilst keeping the chemistry relevant to areas of study.

After reviewing literature,[11] it appeared that polypyrrole offered the greatest potential, and so it was decided that students would make, and then measure the temperature-dependant resistance of polypyrrole films in a simple experiment designed to show an inherent property of the material. There would also be a demonstration of an actuator based on two layers of polypyrrole.

The polypyrrole component of the laboratory course (in 2008) is described below. The students prepared films of polypyrrole by electrochemical oxidation of a pyrrole solution. The solution contained negative counter-ions to stabilise the positive charged centres generated in the polymer film during synthesis. This charge stabilization process results in the conducting properties of the polymer film, there are a range of dopants that can be used, but for this exercise students used 4-dodecylbenzenesulfonate, 4-methylbenzene sulfonate and lauryl sulfate. We have included more experimental detail than is usual in the hope that others may wish to use a similar exercise in their course. The authors would be happy to provide further details.

Experimental

Methods and Materials

The polypyrrole films were prepared in aqueous 0.10 mol/L solutions, ideally degassed with N_2 prior to use. Note, however, that the solutions appear to show no significant change upon standing for a few days before use. The dopants used for Part A were sodium 4-methylbenzenesulfonate (MBS), sodium 4-dodecylbenzenesulfonate (DBS) and sodium lauryl sulfate (LS), again at 0.10 mol/L concentrations. Students had the option of diluting these two-fold with water. Three dopants at two alternative concentrations meant that a total of six dopant solutions could be chosen from by the students. The dopants were relatively inexpensive and, in the case of the LS especially, interesting from their *real world* applications. It should be noted that the DBS, in particular, can take a considerable time to dissolve in water. For Part C, lithium perchlorate was used. However, a number of other salts, such as the tetrafluoroborate, hexafluorophosphate and trifluoromethylsulfonate can be used.

The electronic device used for the synthesis of the polymer film was prepared in-house and was designed to work in concert with a standard multimeter. A knob for adjusting the applied current and a switch to change between applying current (Part A) and measuring current (Part B) are the only parts that the students need to interface with. A circuit diagram is given in Fig. 1. The electrode holder (Fig. 2) was fabricated in-house and the electrodes constructed from spatulas.

The compounds used (prices ex-Aldrich 2007-08 catalogue) are: pyrrole [(CAS 109-97-7] A$34.00 per 25 mL], sodium 4-methylbenzenesulfonate [(CAS 657-84-1) A$44.00 per 100 g], sodium 4-dodecylbenzenesulfonate [(CAS 25155-30-0) A$49.00 per 25 g], sodium lauryl sulfate [(CAS 151-21-30) A$41.00 per 25 g], lithium perchlorate trihydrate [(CAS 13453-78-6) A$53.00 per 50 g).

The instructions given to students and modified from the CHEM111 laboratory manual are:

Preparation of the Conducting Polymer Film (45 min –1 h) – Part A

Into a clean, dry 50 mL beaker place 10 mL of 0.1 mol/L aqueous pyrrole solution. To this add 40 mL of the dopant solution, using measuring cylinders. The positive electrode, upon which the polymer film is to be deposited, is placed in the middle slot of the electrode holder and positioned so that about 3 cm of it is submerged in the solution. The negative electrode is put into the outer slots in the holder and the entire assembly is placed onto the beaker (Fig. 2). The red alligator clip is connected to the central positive electrode and the black alligator clip to the outer negative electrode. Turn on the device and adjust the applied current to about 9.2 mA. Leave for 30 minutes. During this time some bubbling should be observed from the central electrode and a black film should slowly form on it.

After 30 min turn off the device, remove the crocodile clips and remove the electrode holder from the solution. Carefully remove the central electrode, which will now have a black film of the polypyrrole polymer coating it (Fig. 3). Rinse the polymer film with distilled water and dab dry with tissue paper.

Using a sharp knife, shave off the polymer on the edges of the electrode. Place the electrode on the bench and then carefully place a piece of sticky tape over the film. Using

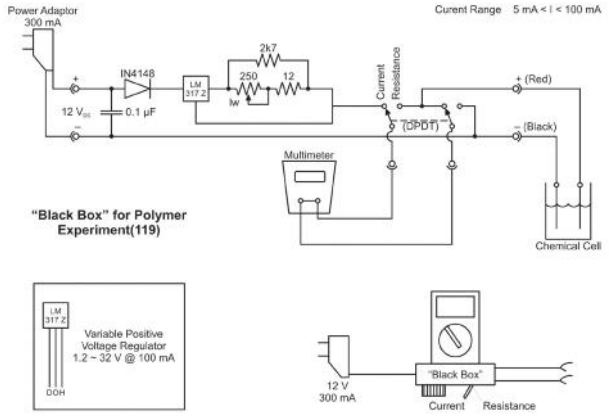

Fig. 1. Circuit diagram of the electronic box required for the laboratory.

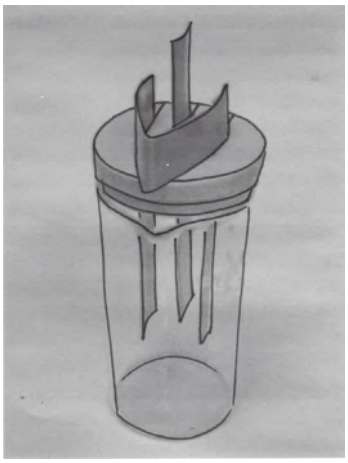

Fig. 2. A schematic representation assembled electrodes.

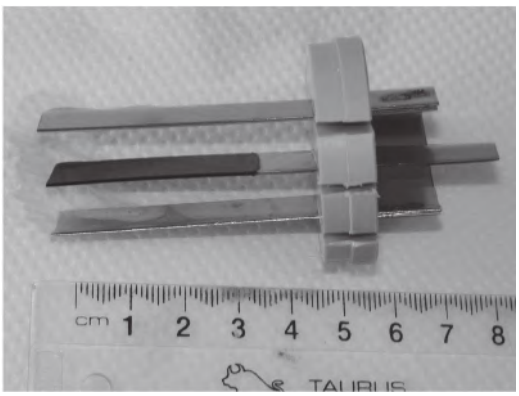

Fig. 3. Photograph showing the polypyrrole film on the central anode.

a sharp knife, remove the film from the electrode so that it is now attached to the sticky tape (note that the other side of the electrode also has a polymer film formed on it, for emergencies). This film can be stored in water for several weeks if required.

Relationship between Conductivity and Temperature - Part B

Place some warm water (about 50 °C from the water bath provided) in a 50 mL beaker and clamp a digital thermometer in the water. Using the crocodile clips, carefully connect your sticky tape/polymer film to a multimeter set to read resistance. The actual value of the resistance will vary between films but is generally about 500 Ω at room temperature. Place the polymer film in the water, ensuring that the clips are not in the water.

Record the temperature and the resistance of the polymer film at two minute intervals as the temperature of the water slowly decreases. If the cooling becomes slow, place the beaker into a larger beaker containing first cold water, then ice water. Record the resistance of the polymer film at temperatures down to about 5 °C and plot the data graphically. Ideally the plot should look like that in Fig. 4.

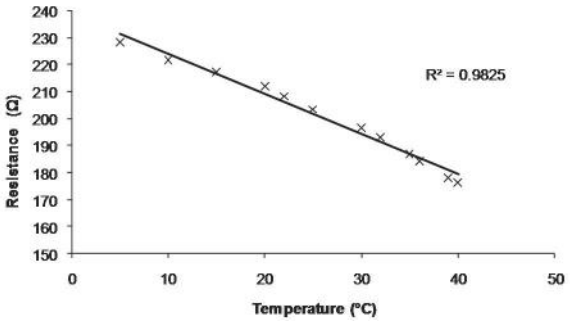

Fig. 4. Typical results from the experiment using a polypyrrole film doped with DBS.

Preparation of an Artificial Muscle - Part C

The preparation of the polymer film proceeds in the same way as in Part A except that the dopant used is $LiClO_4$ (40 mL of 0.1 mol/L aqueous solution) When the polymer synthesis is complete, the central electrode is carefully removed, washed with distilled water and patted dry with tissue. A sharp knife is run around the edges of the electrode to remove the polymer, and then, in a similar manner to Part A, each of the two pieces of polymer (from each side of the electrode) are attached to the two sides of a piece of double-sided tape, giving a trilayer assembly.

To observe the redox-driven bending of the assembly, it is suspended in a solution of aqueous $LiClO_4$ (0.1 mol/L) in such a way that one side of the assembly is attached to the positive electrode and the other to the negative electrode. When a current of 30-60 mA is applied, the film assembly will bend upwards. Reversing the electrodes will reverse the bending. As before, the film assembly can be stored in aqueous $LiClO_4$ solution for some weeks.

Discussion

The Polypyrrole Experiment

The first part of the experiment went very well and students prepared relatively good quality films (~50 µm) thick that were easy to remove from the electrode assembly and stood-up well to the typical robust student handling when being stuck to the tape. It was found that the type of dopant affected the surface appearance of the film when examined by scanning electron microscopy (SEM - Fig. 5).

The second part of the experiment presented more significant problems. The expected increase in resistance as the film temperature decreased was observed by almost all students, but obtaining linear data proved problematic

for many. Typical results for the DBS-doped film can be seen in Fig. 6 (note: temperature decreases from right to left). The steps that appear in the plot seem to correspond to students moving the beaker to put it into ice-cold water to increase the rate of cooling. Worse still was that several groups found little pattern present in their plotted data. We infer that both problems are caused by movement (either from the transfer to water bath or the stirring action of the magnetic flea) and result from unreliable contact between the crocodile clip and the film. To overcome these problems we plan to make the following changes. Instead of attaching the polymer film to a piece of sellotape only, we will use double sided tape to allow attachment to a stiff plastic support and avoid crocodile clips with their potential to puncture the film. Also, rather than cooling the film, we plan to warm it. To this end, we have purchased a class set of thermostatted stirrer hotplates. These changes will make it easier for students to compare the effects of different dopants, and identify the best one.

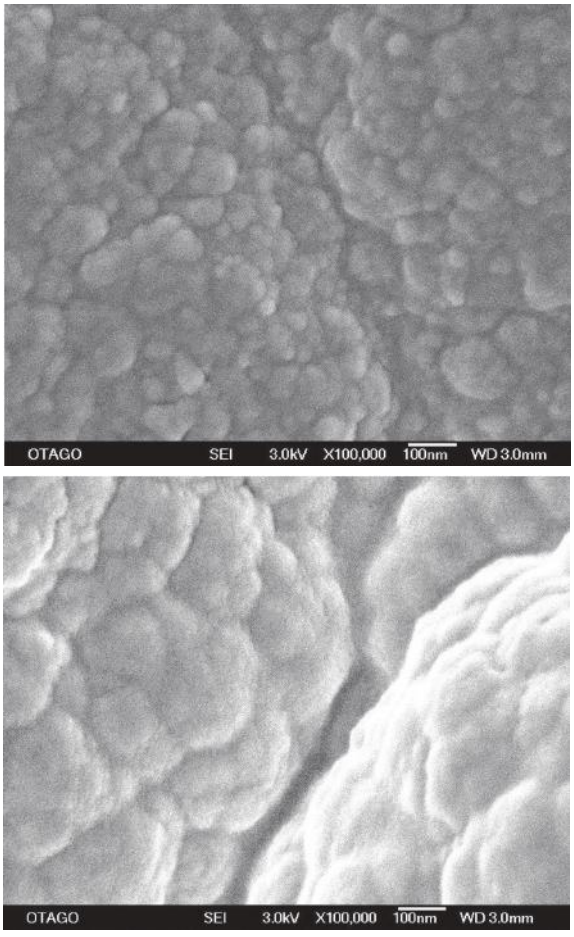

Fig. 5. SEM images of polypyrrole grown using DBS (upper) and with ClO4– as dopant (lower).

The demonstration of the artificial *muscle* was well received but it was a little temperamental at times. To their credit, the students were remarkably patient, appreciating that we were trying to demonstrate some *cutting edge* chemistry using basic apparatus. This was preferred to illustrating the effect taking an *here's one we prepared earlier* approach using material prepared in the research laboratory under optimum conditions. The description of the actuation mechanism was kept simple. During film preparation the polymers are oxidised slightly and this

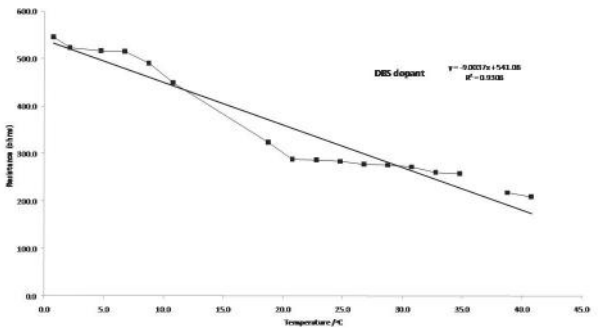

Fig. 6. Typical results from student using DBS dopant; the steps result from poor electrode/film contact.

results in negative counter ions (dopants) being present within the film to neutralize the positive charge. Reduction of one film decreased the positive charge on the polymer chain so dopant ions moved out of the polymer layer, whilst at the same time oxidation to the other film in the trilayer resulted in dopant ions moving into the film, *i.e.* the chemistry was kept to a level they were familiar with and used to illustrate how redox chemistry they knew about could be seen in action.

Student Perceptions of the Polypyrrole Experiment

In a survey of the course that was conducted in the final week (see below), the student's impressions for this experiment overall were good. There were many positive comments about *cool chemistry* and several students used the World Wide Web to look at the robotic arms and peristaltic pumps based on conducting polymers. There were only two negative comments where the student complained about the time needed to prepare the polymer (the dislike of waiting was common to other laboratory sessions and is further highlighted below) and from one further student that the laboratory did not appear to work consistently – we presume the fault with the contact as outlined above.

Students were asked to name their favourite and least favourite laboratory sessions and comments on each. Finally, they were asked to compare the end-of-laboratory tests with laboratory reporting as the assessment, which one of the two they preferred and why. The overall results are shown in Fig. 7 for 89 students of the 122 students that sat the final examination. The total number of responses to some questions exceeded 89 because students gave more than one response to some of the questions. As can be seen, the *polypyrrole* received far more favourable than unfavourable responses. Surprisingly, the Schiff base session received many unfavourable votes. When the comments are examined (Table 3) it appears that most of the negative ones come because of time spent waiting, *e.g.* filtering and washing crystals or for access to an instrument, and this is being addressed for the 2009 course.

Surprisingly, feedback for the assessment process was equal between exit tests and laboratory reports. Many students liked the short sharp end-of-laboratory tests as they were over quickly and involved no homework. However, a large number of students who preferred the end-of-laboratory test also acknowledged that they learned

Table 3. Summary of student comments about specific laboratory experiments.

Expt	***Comments***	
	Pro	***Con***
Boyles Law	enjoyed it, like physics	maths is too hard, is this chemistry? tedious, boring-physics like
Aspirin	was able to test common product and see if claims of aspirin content were true, Lots of hands on, Something to do the whole lab no waiting	
Intro spectroscopy	nice colours, Fun to do	little chemistry, very repetitive, physics not chemistry, too simple,
Conducting polymers	cool new chemistry, product was cool, different and interesting with cool applications like robotic arms, Because we tested the implementation of our experiment	too much waiting, Didn't seem to work well and had varying results
Cobalt enantiomers	large pretty crystals	lot of waiting around
Schiff base	working at own pace allowed better time management, synthesis is fun, 3 weeks of fun! Good to work through entire chemical process get results and analyse	waiting around for instruments, long lab, should be done after spectral id, too long, lab report too long and confusing,
Colloids	no equations! Basic instructions easy to follow, Very interesting, Short and easy,	needs more theory to explain the chemistry, Mayonnaise didn't seem very scientific
Spectral i.d.	working on computer was refreshing, very helpful, should have been done before lab 6	labs supposed to be hands on, boring (though helpful!), slow stupid computers, Sitting in a small room with a computer is not my idea of a lab Computers are boring, that stuff was so easy

much more in preparing a laboratory report and that it gave them a much better chance to reflect on the work and understand it at their own pace. A few of the more astute students observed that the two methods tested different aspects of learning and liked the mixture.

The comments offered by the students for each type of laboratory exercise, both pro and con, are collated in Table 3 and, in general, these are *as written* save for where there were several very similar ones about a laboratory, when they have been paraphrased. It is interesting and pleasantly surprising to find that students want to be kept busy during laboratory periods; they appreciate challenges but do not like laboratory sessions that are easy or repetitive. A little worrying is the dislike of mathematics and physics. This is an area of concern that was noted at the recent NZIC conference in Dunedin. The maths skills that many of first-year students come with are not always adequate and we aim to provide guidance to local Otago/Southland schools to make sure students thinking of taking chemistry courses are aware of maths skills required.

One particularly interesting outcome of the survey relates to the experiment set to determine the amount of aspirin in a tablet. It is typical of the type of 1st-year experiments that are less demanding than the equivalent high school analogue, *e.g. how does temperature affect the amount of Vitamin C in fruit juice*, which typifies the investigations we see when schools use the laboratory facilities for Level-3 investigations. The aspirin exercise is one that is unacceptable as a Level-3 chemistry investigation under the current curriculum as it is a simply an *how much* type of experiment – and it made almost no impression on the students in either a positive or negative manner. Contrast this with the Schiff base sessions where all the positive comments are about the actual experiment, whilst the negative ones reflect a laboratory management issue rather than a dislike of the experiment itself; this is easily fixed. Whilst the Schiff's base is demanding in terms of effort, the students responded well to the chemistry and the processes involved. They also demonstrated excellent time management skills to pace their workload over the three weeks duration of it - another skill that comes from managing NCEA assessments?

The general comments from the 2008 CHEM111 cohort, which are not specific to any experiment, *cf*. Table 3, are (verbatim):

awesome 2nd semester doing chem111 good experience.

set up of laboratory report assessment was good, being able to practice each session before it was marked.

really well put together laboratories that were quite relevant to course content, really enjoyed my time.

best series of laboratories I've done. Better than 191 and far better than physics.

chem laboratories are still easily my favourite. Thanks.

I love chem.

It was fun, better than 191

good how we didn't get asked to do a full laboratory report

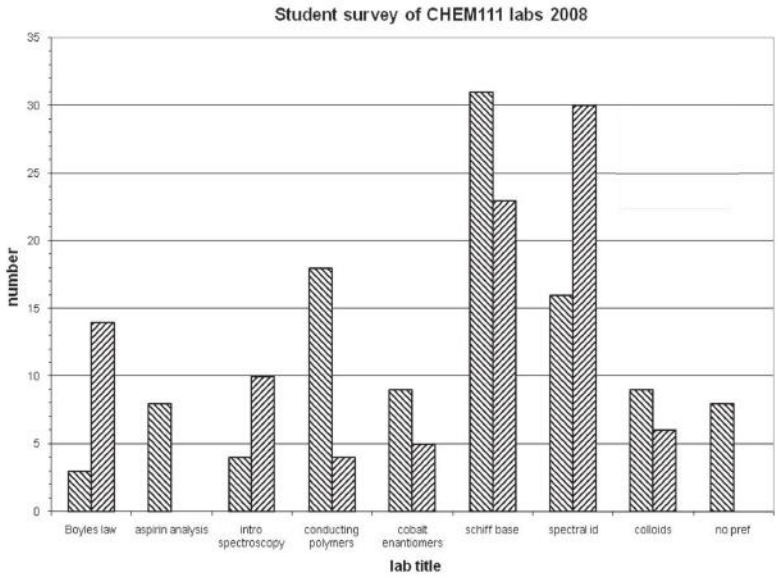

Fig. 7. Results of the student survey indicating favourite [\\\] and least favourite [///] experiments arranged in the order performed; survey conducted after the end-of-laboratory course.

right from the beginning

better than 191, smaller groups and more interesting expts.

shorter laboratories more productive and efficient would be appreciated

should have less laboratories, fortnightly would be better

supervisor gave no feedback on reports

The last comment reflects an important aspect of this type of laboratory course, namely, that it is vital to provide feedback expressed in a positive manner to students, not by a whole class address but to individual students about their particular work, working around the class during the particular session.

Summary and Conclusions

We have described a laboratory course that is a *work in progress* but we feel, from the comments made by the students, that we are on the right track and have met our original aims of designing a course more appropriate for students' knowledge and skills; we feel that we have given them a taste of some modern chemistry techniques and exposed them to some new materials. There are still things to improve as we would like to further enhance the programme with, *e.g.* a session based around nanoparticles. A long term goal is to establish a suite of experiments suitable for the course that can be rotated in and out of the course. Doing the same thing for ten years taxes the most enthusiastic of supervisors and, after all, education should also be about the instructor and teacher!

Finally, in offering this *work in progress* we hope to encourage other NZ course coordinators to assess their own courses and ask themselves if they are making the most of the skills their students bring into their courses and building upon them.

Acknowledgements

The authors thank Paul Reynolds for the construction of the electronics, Ian Billinghurst and Katie Wilson for working out many of the wrinkles in the method, and the CHEM111 class of 2008. We also thank the Chemistry Department at Otago for a Summer Scholarship (to RS).

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Article

Inhibitors of Phosphatidylinositol 3-kinases: The Next Wave of Anti-Cancer Drugs?

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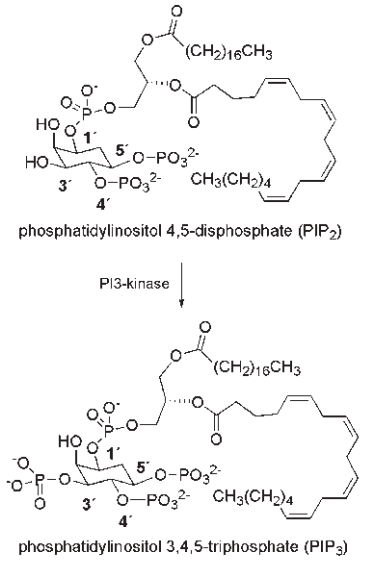
*This article, originally printed in the January issue of this Journal (2009, 73, 9-11), was subject to a production error with the loss of structural displays critical to its understanding. In fairness to the authors, and to you the reader, the article is reproduced here in full; the editors of Chemistry in New Zealand apologise unreservedly.

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinase enzymes, which catalyse the phosphorylation of the 3′-hydroxyl position of the inositol ring of phosphatidylinositol 4,5-diphosphate (PIP_2) to give the messenger molecule phosphatidylinositol 3,4,5-triphosphate (PIP_3) (Scheme 1). This then participates in a variety of physiological processes, including cell growth and differentiation.[1] The PI3Ks are divided into three classes (I-III) based on their structure, mode of regulation, and substrate specificity. Class 1A PI3Ks are comprised of three isoforms (p110*α*, p110*β* and p110*δ*) that share a common regulatory subunit (p85) activated by signals from receptor protein tyrosine kinases, while the Class IB PI3K (p110γ) is structurally similar but lacks a regulatory subunit, and is activated by G protein-coupled receptors.[2] The pathway through p110α is the most frequently activated signalling pathway in human cancer, and its corresponding gene (*PIK3CA*) undergoes amplification in tumours, with activating *PIK3CA* mutations being relatively common in late-stage colon, brain, breast, and gastric cancers.[3,4]

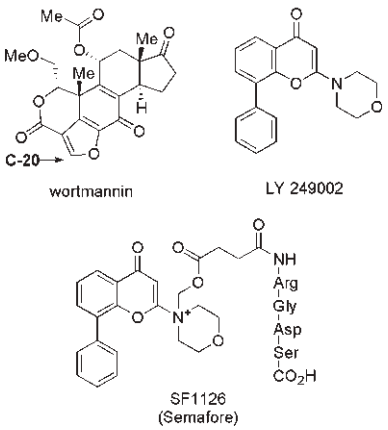


Scheme 1. PI3K phosphorylation of phosphatidylinositol 4,5-disphosphate (PIP_2).

Early investigations into the mechanism of PI3K inhibition were aided by two compounds, the fungal natural product wortmannin, first isolated[5] from *Penicillium wortmanni* in 1957, and the synthetic inhibitor LY294002 (Chart 1), which was first synthesized by Eli Lilly in the early nineties.[6]

Wortmannin is a potent and irreversible inhibitor in which the furan ring adds to the amino group of a lysine residue in the ATP binding pocket of PI3K giving an enamine at C20. X-ray studies with the p110γ isoform confirmed that this is with the amino group of Lys-833 and they also showed an H-bond between the C17 carbonyl oxygen and the backbone NH of Val-882.[2] However, since similar amino acid residues are found in all of the PI3K isoforms, wortmannin shows very poor isoform selectivity, and displays considerable liver toxicity at low doses in animal studies. Several wortmannin analogues have been prepared in an attempt to reduce this toxicity[7] but, since they all function as prodrugs of wortmannin itself, they show no advantage in terms of PI3K selectivity.

Chart 1



LY294002 binds reversibly with moderate potency and has proved useful as a tool due to its stability. It was the first synthetic PI3K inhibitor to have its complex with PI3Kγ structurally elucidated.[2] The morpholine oxygen makes an H-bond with the backbone amide NH of Val-882, the same residue that forms an H-bond with wortmannin and, in fact, this is a much conserved interaction that is now known to be shared by all current PI3K inhibitors and ATP itself. LY294002 is too insoluble for investigation as a drug, although a prodrug derivative, SF 1126 (Chart 1) has now entered human clinical trials as a pan-PI3K inhibitor, targeting cell growth, proliferation and angiogenesis.[8]

The issue of isoform selectivity is potentially important since each of the isoforms has a suite of significant biological effects; the p110*β* isoform is important in thrombus formation, while the p110*δ* and p110γ isoforms are important in aspects of inflammation. However, despite high-quality crystal structure data on both the α- and γ-isoforms, obtaining compounds with high selectivity for p110α has proved difficult. This is illustrated (Table 1) by the IC_{50} values (concentration of drug for 50% inhibition of PIP2 phosphorylation) of LY294002, wortmannin, and the first of the other new PI3K inhibitors that have

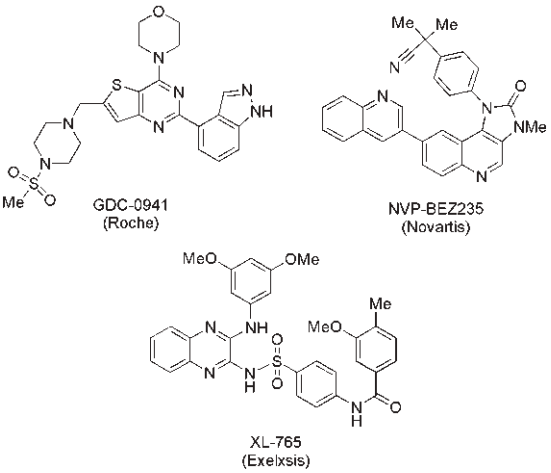
begun clinical trial; GDC-0941, NVP-Bez235, XL-765 (Chart 2).

Table 1. Isoform selectivity of PI3K inhibitors.

Compound	IC_{50} (nM) p110			ratio
	-α	-β	-δ	β/α
Wortmannin	~4	~4	~4	~1
LY294002	800	1000	700	1.2
SF-1126	NA*	NA*	NA*	NA*
GDC-0941	3	33	11	11
NVP-Bez235	20	160	12	8.0
XL-765	13	113	43	8.7

*NA - not applicable; prodrug

Chart 2



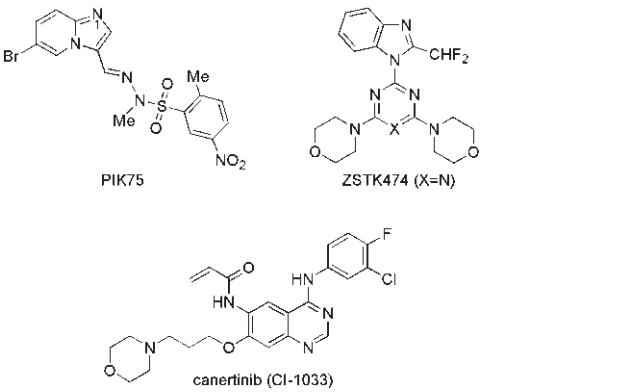
GDC-0941 (Genentech/Roche) is the result of much study with other morpholine-containing analogues of LY294002, and is currently undergoing Phase I human cancer clinical trials.[9] Heteroaromatic nitrogen atoms can also participate in hydrogen bonding to the NH of Val-882 and examples of this class include the Phase I clinical agent NVP-Bez235 (Novartis),[10] where it is believed that the primary H-bond is via the quinoline nitrogen of the imidazo[4,5-*c*]quinoline core of the molecule. Another azaheterocycle that is reported[11] to have entered clinical studies for the treatment of solid tumours is the quinoxaline derivative XL-765 (Chart 2), although few details are available. There is, therefore, high interest in the development of PI3K inhibitors as anticancer agents,[4,11,12] although most of the current compounds are pan-inhibitors, rather than specific inhibitors of p110*α*, the PI3K isoform most often mutated in human cancers.

From its outset in 2005, our programme has thus been focused on the development of more selective inhibitors of p110*α* as anticancer drugs, and began by studying the literature to see where we could make positive improvements.[13] We started with the imidazo[1,2-*a*]pyridine derivative PIK75 (Chart 3), which is a moderately selective inhibitor of p110*α* compared to the other Class I PI3K isoforms (p110*β*, p110δ and p110γ) (Table 2),[14] and is also active in human cancer xenograft models.[15] Our systematic study of the changes to the imidazopyridine chromophore indicated tight structure-activity relationships (SAR),[16] but did lead us to a new chromophore that had both high potency and much higher selectivity for p110*α*. A patent application has been filed on this new class of inhibitor,[17] and we are continuing to optimise the structure. To date, we have been able to retain high potency and improve p110α selectivity.

Table 2. Inhibitory effects of PIK75 on PI3K isoforms.

Compound	IC_{50} (nM) p110			ratio
	-α	-β	-δ	β/α
PIK75	20	300	1000	15

Chart 3



In order to try and rationalize the high p110*α* specificity of the imidazo[1,2-*a*]pyridines and the new chromophore, we studied the binding mode of the known inhibitor PIK75 to p110*α* using a molecular modelling approach.[16] For this work it was necessary to develop a p110*α* homology model,[16,18] since prior to December 2007 structural data were available only for the p110γ isoform.[2] We used the high level of sequence identity shared across the PI3K isoforms around the ATP binding cleft to develop this model.[16,18] As expected, the primary interaction involves an H-bond between the *N*-1 of PIK75 and the backbone NH of Val-851 (equivalent to Val-882 in p110γ) but, in addition, a possible hydrogen bonding interaction between one of the oxygen atoms of the sulfonyl group and the NH of a histidine residue (His-855) was identified.[16] Since this histidine is unique to the p110*α* isoform, it was proposed that the additional interaction could account for the high selectivity of PIK75 against p110*α*. However, in December 2007 the structure for the full length human p110*α* catalytic subunit in conjunction with a portion of its p85*α* regulatory subunit was published,[19] and demonstrated that while most of the ATP binding site residues had a similar 3D structure, there were some notable differences at certain positions. Significantly, the most notable difference from our homology model related to His-855 which was *tied-back* due to an H-bond with Asp-925 and therefore not accessible to the sulfonyl oxygen atoms of PIK75. Whether this is a crystallization artefact or a real phenomenon remains to be determined, but in the interim we are developing a refined model based on this new data.[20]

The second literature lead that we investigated in detail, was the dimorpholino-1,3,5-triazine derivative ZSTK474 (X=N; Chart 3) that is reported to be a reversible and non-selective PI3K inhibitor, but with excellent oral activity against human xenografts in mice.[21,22] This is a very competitive field, with a Japanese patent application filed by

Zenyaku Kogyo Kabushiki Kaisha[23] in 2006 that covers both the triazine and its 2-pyrimidine derivatives (X=CH), where the second morpholine has been replaced by a piperazine group, and a suite of 12 patent applications from AstraZeneca covering a variety of different morpholine replacements, and all three possible pyrimidine isomers.[24] We modelled the binding of ZSTK474 (X=N) in the ATP-binding site of the p110γ crystal structure,[25] and identified a binding mode in which the key H-bond with the NH of Val-882 was with the oxygen atom of one of the morpholine groups, rather than with the benzimidazole nitrogen as proposed,[22] with the latter nitrogen actually H-bonding to the NH_2 group of Lys-833 (the amino group responsible for the irreversible interaction with wortmannin). Our binding model allowed us to design new analogues that are not predicted by the published model, and enabled us to identify several potent new lead structures.

With the exception of wortmannin and its analogues, all of the approaches discussed so far have involved reversible PI3K inhibitors that must compete with ATP for binding in the catalytic site of the enzyme. Irreversible inhibitors have advantages in that they allow for longer-term inhibition of the enzyme, promising greater therapeutic effect, while allowing for longer times between treatments, as shown by the erbB irreversible inhibitor canertinib (CI-1033; Chart 3) that we developed earlier to Phase II clinical trial.[26] Thus, our aim was to develop compounds able to bind irreversibly to the p110*α* site, but only reversibly to the other isoforms. Such specific p110*α* irreversible inhibitors should have better therapeutic potential than pan-PI3K irreversible inhibitors based on wortmannin. Preliminary results suggest this approach is feasible.[27]

Our work in the PI 3-kinase area began in 2005 with in-house funding and support from the government-funded Maurice Wilkins Centre for Molecular Biodiscovery. A successful 2006 HRC grant application, coupled with 2007 support from Auckland's Faculty Research Development Fund, enabled sufficient results to be obtained for the commercialization arm of the University (Auckland UniServices Ltd.) to set up the spinout company *Pathway Therapeutics Ltd*., which has recently successfully raised $A10 million from two Australian-based venture capital companies, CM Capital Investments (Brisbane) and GBS Venture Partners (Melbourne), and the new Trans-Tasman Commercialisation Fund.

Our initial PI3K research team consisted of the authors with cell biologists Bruce Baguley and Elaine Marshall, and biochemist Peter Shepherd. More recent additions to the team include chemistry PhD student Andrew Marshall, biologist Claire Chaussade, molecular modellers Raphael Frederick and Jack Flanagan, pharmacologist Phil Kestell, and technicians Claire Mawson and Mindy Chao. New additions to the team resulting from the Pathway funding are chemists Swarna Gamage, Anna Giddens and Sophia Tsang, and five technical positions are to be filled.

The pharmaceutical development of PI3K inhibitors has taken great strides during the last five years. Several compounds are now in clinical trial, and large amounts of structural and biological data are becoming available. We are hopeful that the future will see even better therapeutic results being achieved with more selective inhibitors.

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The Oxidation of Red and White Wines and its Impact on Wine Aroma

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*This article, originally printed in the January issue of this Journal (2009, 73, 18-22), was subject to a production error with the loss of structural displays critical to its understanding. In fairness to the author, and to you the reader, the article is reproduced here in full; the editors of Chemistry in New Zealand apologise unreservedly.

Introduction

The oxidation of wines has quite different consequences for red and white varieties, although the underlying chemistry is similar.[1,2] Oxygen additions are usually required in the maturation of red wines prior to bottling, to enhance wine quality (through the removal of unwanted aromas), to stabilize colour and to improve mouth feel, but it is difficult to predict the optimum level of oxygen exposure. On the other hand, oxygen additions seldom improve white wines where preservation of fruity aromas is sought, and where oxidative browning can detract from the appearance of the wine. This article summarizes the chemistry behind wine oxidation with a focus upon polyphenol-mediated processes and how these impact upon aromas in red and white wines.

Oxygen in Wine

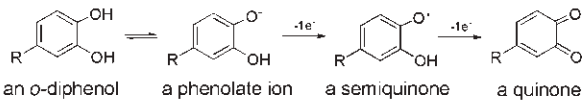
It is inevitable that wines are exposed to O_2 at various stages of production. Air-saturated wine can take up to 6 mL/L (8.6 mg/L) of O_2 at room temperature, with greater solubility at a lower temperature. Larger doses are supplied to red wines during deliberate pump-overs, while slower rates of O_2 ingress occur for wines in barrels. For example, while mixing wines from different casks was found to raise the O_2 concentration to around 1.8 mg/L, racking of a wine at 15-20 °C produced an O_2 concentration of 0.4 mg/L, but this value increased three-fold when the temperature of racking was lowered to 10 °C.[3] An alternative to barrel aging is the new technology of micro-oxygenation now commonly used with red wines. This involves continuous, slow bubbling of oxygen into the wine for several weeks at a rate of a few mL of O_2/L of wine per month. Under these conditions the dissolved O_2 has been measured at 0.2 to 0.25 mg/L.[4]

Once a wine is bottled it might be expected that oxygen is largely excluded, but wine closures vary considerably in how much O_2 they allow into the wine. Synthetic plastic corks allow the entry of larger amounts of O_2 to enter the wine than natural corks and screw caps and are thus best suited for wines that are to be consumed soon after bottling. The effects of closure type upon the colour and aroma in trials on red and white wines are referred to below. The conditions used for bottling are also very important, as the small headspace above the bottled wine can contain a few mg of O_2,[5] equivalent to several months of the oxygen entry through the closure, unless a special vacuum or inert gas system is used on the bottling line.

The Oxidation of Wine Polyphenols

There are many organic compounds in wine that are potential targets for oxidation processes. These range from ethanol itself through to various acids [tartaric acid (**1**) being the major wine acid (see Chart 1) and aroma compounds, but these are not, in fact, the main initial substrates of oxidation. An important finding in the research undertaken by Vernon Singleton (UC Davis) in the 1970s, was that ethanol oxidizes to acetaldehyde at a significant rate only through the coupled oxidation of readily oxidizable polyphenols such as caffeic acid (**2**, typical of white wine hydroxycinnamic acids) and catechin (**3**, a flavanol at high levels in red wine – see Chart 1).[6] Without these polyphenols ethanol and tartaric acid are remarkably stable to oxidation. The oxidation of polyphenols generates a strong oxidant, presumed to be H_2O_2, that can oxidize other substances in wine such as ethanol.

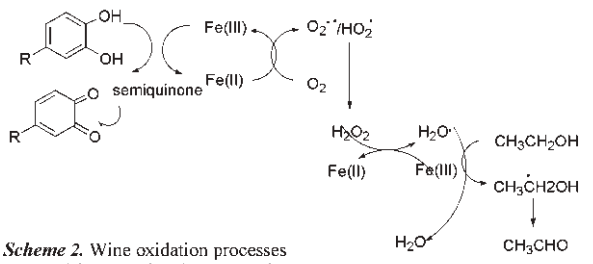
Wine polyphenols containing a 1,2-diphenol (an *o*-catechol group), such as **2** and **3**, can be oxidized through to quinone forms easily as shown in Scheme 1. Model studies have shown that in solution this process is more rapid at a higher pH, due to a higher percentage of the phenolate that reacts with oxygen.[7] Only a small proportion of phenolate ions are expected at wine pH (pK_a polyphenols *ca*. 9-10), but many more will be present in a pH 4 wine than a pH 3 wine, consistent with higher pH wines being more susceptible to oxidation problems. It has also been shown that one of the subsequent reactions of the quinones formed is with remaining polyphenols and leads to brown products, but the process regenerates the catechol group making it available for further oxidation. Overall, more oxygen is taken up than would be expected given the original number of polyphenol molecules present.



Scheme 1. Oxidation of polyphenols

Oxygen itself is a triplet, and requires activation of some form before it can be reduced progressively to hydroperoxyl radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\bullet}$), and eventually H_2O. In wines, the activation of oxygen is thought to involve catalysts, particularly iron and copper as these complex O_2 and facilitate the oxidation process with polyphenols (Scheme 2).[8] In the coupled oxidation process, Fe(II) converts H_2O_2 to the very reactive $OH^{\bullet}$ (the Fenton reaction) that oxidizes most organic compounds, including ethanol to acetaldehyde and glycerol to glyceraldehyde, *etc*.[9]

Polyphenols containing a 1,2-diphenol (an *o*-catechol moiety) or a 1,2,3-triphenol (a galloyl group) are the most easily oxidized, and show the lowest oxidation-reduction potentials in a model wine solution measured at a glassy carbon electrode.[10] The current peak in cyclic voltam-



Scheme 2. Wine oxidation processes (adapted from Danilewicz - see ref. 1)

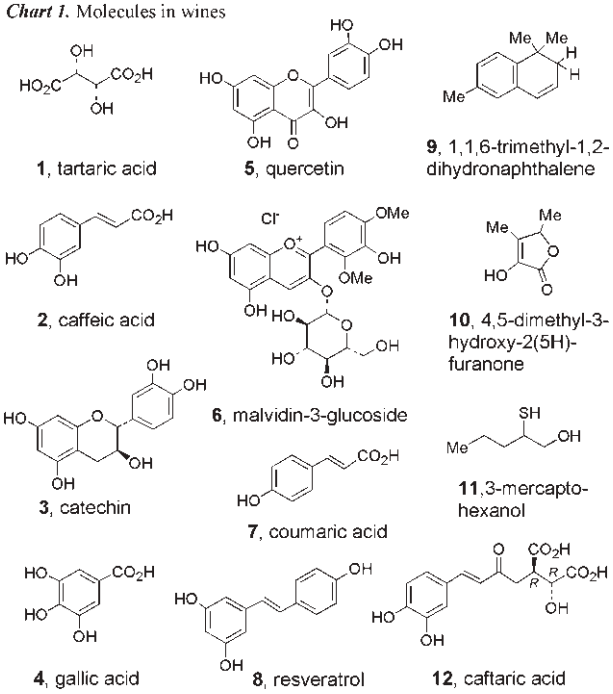
mograms for common wine polyphenols such as **2**, **3**, or gallic acid (**4**), and quercetin (**5**; Chart 1) is seen at a similar potential, *ca*. 0.4 V (*vs*. Ag/AgCl), as is the main current peak for diluted red and white wines. This further confirms that such polyphenols are the main initial substrates in wine oxidation.[11] Integration of the current peak can quantify the level of catechol- and galloyl-containing polyphenols in wine.[10-12] Further compounds, such as the malvidin anthocyanins (see **6**), the major coloured species in red wines, and compounds with more isolated phenolic groups, such as *p*-coumaric acid (**7**) and resveratrol (**8**; Chart 1), are oxidized at higher potentials. However, despite their lower ease-of-oxidation, anthocyanins such as malvidin-3-glucoside (**6**) degrade faster in wine than, *e.g.* **2** or **7**, the catechol-containing hydroxycinnamic acids,[13,14] as other reactions involving the anthocyanins come into play, including the formation of bridges between the polyphenol moieties.

The aldehydes produced by coupled polyphenol oxidation, and through yeast activity, have important roles in wine aging. They provide links between various flavonoid polyphenols (including anthocyanins) to produce new polymeric pigments that explain the change in red wine hue with age.[15] These components are often more stable than the anthocyanins that they are formed from and are resistant to bleaching by the bisulfite added as a wine preservative. There is considerable current interest in the way in which anthocyanins combine with wine tannins (larger oligomeric and polymeric polyphenols made up of catechin-type units) and lower the astringent effect of the tannins. Such studies help explain the *softening* of red wine astringency with age, an important area of sensory science where the underlying chemistry is still poorly understood.

Oxidation and Effects on Wine Aroma

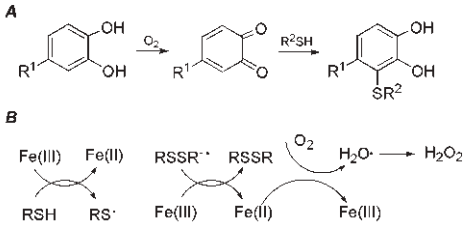
A range of off-odours can be formed from wine oxidation.[16-18] At low concentrations these may add to the complexity of a wine, but as these increase they begin to detract from wine quality. Some examples of the compounds associated with sensory terms for aged wines such as *farm-feed* and *woody-like* include phenylacetaldehyde ($PhCH_2CHO$), 3-(methylthio)propionaldehyde ($MeSCH_2CH_2CHO$), 1,1,6-trimethyl-1,2-dihydronaphthalene (**9**; responsible for the *kerosene* odour in aged Riesling) and 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (**10**).[17] At the same time, the concentration of acetaldehyde itself does not always increase markedly during wine oxidation experiments, and it is recognised that many important wine oxidation aromas remain to be identified.[16]

Chart 1. Molecules in wines



Alongside the production of new odours, wine oxidation can lead to the removal of existing aroma compounds, particularly those containing sulfur. This can be a positive development, as many sulfur-containing compounds produce unwanted aromas reminiscent of rubber or cooked cabbage.[19] Winemaking processes involving the introduction of O_2 to wine (as in racking) provide the first means for their removal, while fining with copper salts is also used. At the same time, there are sulfur-containing compounds present that add to the varietal character of the wine, but these may be lost through oxidation processes. These include 3-mercaptohexanol (3MH, **11**) which provides important grapefruit and passion fruit-type aromas in Sauvignon Blanc and other wines.[20]

One mechanism proposed for the removal of sulfur-containing compounds is by reaction with the quinones formed during polyphenol oxidation (Scheme 3A). Experiments exposing catechol-containing polyphenols to oxygen show losses of **11** consistent with a polyphenol-mediated oxidation mechanism.[21,22] The oxidation of thiols to disulfides (Scheme 3B) has also been suggested as a possible pathway.[19,23,24] In one recent survey of wines of different ages, the tendency towards higher levels of dimethyl disulfide (MeSSMe) and diethyl disulfide (EtSSEt) in the older wines was seen as implicating disulfide formation during aging.[25] The rapid reaction of the thiol-containing amino acid cysteine in the presence of O_2, Fe(II) and Cu(II) has also been ascribed to the metal-catalysed oxidation of thiols as shown in Scheme 3B.[22] However, while the addition of O_2 was seen to lower the concentrations of methane and ethane thiols in a micro-oxygenation study, no disulfides were seen.[26] Thiols with low sensory thresholds potentially can be released from disulfide forms by reduction with bisulfites in wine,[27] or through the hydrolysis of thioacetates.[24] However, there is a lack of experimental data on the effects of oxidation upon sulfur-containing compounds, and research is being undertaken in this area at the University of Auckland.



Scheme 3. Oxidation of S-containing compounds in wine; ***A:*** polyphenol-mediated; ***B:*** metal-catalyzed thiol oxidation (adapted from Danilewicz - see ref. 24)

Influence of Wine Antioxidants

In addition to controlling the rate of O_2 entry into a wine, winemakers can make use of antioxidants to control oxidation, using those already present in the grape juice, such as glutathione, or through added SO_2 (bisulfite in solution) and ascorbic acid. SO_2 is almost universally used in modern winemaking at levels of 20 mg/L or more of free SO_2 (and to 100 mg/L or more of total SO_2 once forms bound to acetaldehyde and other compounds are included). Sulfites are added to grape juice to inhibit the rapid oxidation caused by polyphenol oxidase activity.[28] Here it can act as a scavenger of H_2O_2 formed from further oxidation processes, but it does not react rapidly with O_2 itself.[1] On the other hand, SO_2 has a further role in the rapid reduction of oxidized polyphenols,[29] thus removing polyphenol quinones from further browning and aroma degradation processes.

Related protection is provided in grape juice and young wines by the presence of free glutathione at 30 to 100 mg/L with the actual concentration being dependent upon the pressing conditions used.[30] An important role for glutathione in white grape juice is to react with the quinone formed from the main hydroxycinnamic acid, caftaric acid (**12**), to produce an S glutathionyl caftaric acid, which is more stable to enzymatic oxidation and limits the browning of the juice.[28] Glutathione also appears to have a protective role in wines by reacting with oxidized polyphenols in preference to varietal aroma compounds such as thiol **11**, or other polyphenols.[31]

There has been some interest in finding replacements for SO_2 additions in winemaking owing to potential health-problems in sensitive individuals and ascorbic acid has been considered. As the dienol moiety is readily oxidized[1] by O_2, it can be used for its direct removal, a role that is not ascribed to SO_2 or glutathione. However, ascorbic acid additions to wine have a controversial history in that certain pro-oxidative effects have been observed and ascribed to the formation of H_2O_2 or other reactive oxygen species following the initial antioxidant activity. This is analogous to the polyphenol oxidation of Scheme 2. In model studies, ascorbic acid was shown to rapidly form acetaldehyde in ethanolic solutions, a process that could be slowed but not completely eliminated through SO_2 additions,[6] and a change from anti-oxidative to pro-oxidative activity has been seen after a certain time in accelerated aging trials.[32] On the other hand, wine storage trials have shown mixed results regarding added ascorbic acid, with some trials showing little benefit to wine browning from the addition.[33] In other trials, such as a three year trial on Chardonnay and Riesling at the Australian Wine Research Institute (AWRI) in Adelaide, wines without ascorbic acid additions were browner, and the additions either led to no difference in aroma or to less oxidized and more fruity aromas, with little change in SO_2 levels.

Red Wine Oxidation

Red wines contain polyphenols at a higher concentration (1 to 5 g/L) than white wines, particularly much higher levels of the anthocyanin flavonoids responsible for colour and astringency (flavanol oligomers and polymers). Some of the established effects of O_2 additions to red wine include a decrease in certain smaller polyphenols and an increase in red polymeric pigments, alongside a loss of sulfites.[34] Several recent reports on the effects of micro-oxygenation in red wines have confirmed the loss of monomeric anthocyanins and other polyphenols, along with the enhanced formation of polymeric pigments (resistant to SO_2 bleaching), often with an increase in wine colour density.[13,14,35,36] Further changes in red wine pigments have included the formation of ethyl-bridged compounds associated with the acetaldehyde released during wine oxidation processes,[35,37] while a build up of acetaldehyde has been recorded in the later stages of regular micro-oxygenation,[38] and during an electrochemical micro-oxygenation approach.[39] Overall, micro-oxygenation has been shown to increase the rate of a range of red wine aging processes, allowing wines to be prepared for bottling in a shorter period.[40] A further influence on the rate of oxidative changes during micro-oxygenation is the level of SO_2 in the wine. We have tracked the development of polymeric pigments from monomeric anthocyanins during a sixteen week treatment of a Merlot wine at an O_2 exposure of 10 mL/L/month, and observed that these processes are severely restricted as more SO_2 is added to the wines (Fig. 1).[14]

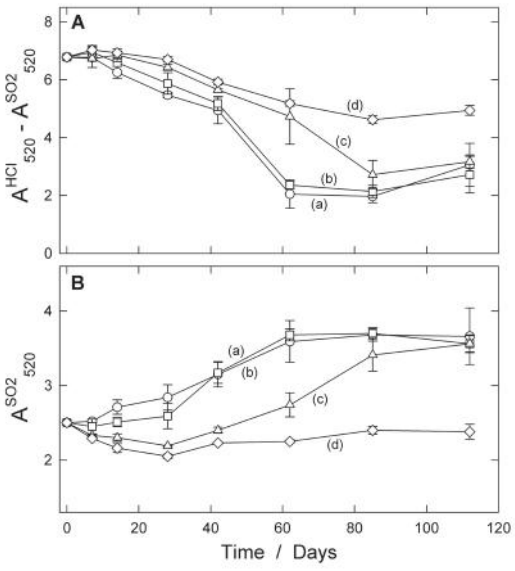


Fig. 1. Loss of monomeric anthocyanins given by the spectrophotometric measure ($A_{520}^{HCl} - A_{520}^{SO_2}$), and increase in non-bleachable (mainly polymeric) pigments ($A_{520}^{SO_2}$) during the micro-oxygenation of a red wine with different SO_2 additions: (a) 0, (b) 50, (c) 100, (d) 200 mg/L (n = 3).

The influence of red wine oxygenation upon aroma compounds and wine sensory properties has been more difficult to confirm compared to effects on wine colour. Micro-oxygenation is promoted as a technique that lowers unwanted vegetative characters in wines and elevates varietal, fruity aromas,[41] but the limited reports in this area show little change in levels of fruity esters, short chain fatty acids, or floral terpenes[42] while, in a separate report, the intensity of the berry/plum character and overall wine quality both fell in the micro-oxygenated wines.[13] Trends in aroma profiles have also been observed in wine closure trials with both white and red wines undertaken at the AWRI. In a three year closure trial on a Cabernet Sauvignon wine, that with the greatest air headspace showed significant losses of SO_2 soon after bottling and developed a higher *oxidized* aroma score.[43] Conversely, the wine under screw cap with the smallest air headspace showed the smallest loss of SO_2 and recorded higher, but not dominating, *struck flint/rubber* aromas. This shows how different wines can develop in the bottle according to the choice of wine closure and bottling procedures.

White Wine Oxidation

White wines contain lower levels of polyphenols (0.2-0.5 g/L), mainly hydroxycinnamic acids, *e.g.* **2** and **7**, but these remain very important for oxidation issues centred around wine browning and losses in varietal aroma. The low concentrations of flavonoids such as catechin (**3**) and quercetin (**5**) glycoside remain important particularly for wine browning and are more prevalent in musts exposed to longer skin contact times and harder pressings.[7,30] Tests on browning rates with different wines have shown varying results with respect to the importance of phenolic content, SO_2 level, pH, and metal content.[44]

Wine closure trials at the AWRI have again shown interesting trends in aroma development in the bottle. In the trial on the Chardonnay and Riesling wines referred to above, a higher rate of O_2 ingress through a synthetic closure led to lower levels of SO_2, higher browning and more advanced oxidized aromas.[45] By contrast, the limited O_2 ingress for wines under screw cap and cork, or for storage in glass ampoules, led to lower rates of browning and lower SO_2 levels, low oxidized characters, but again a discernable *struck flint/rubber* aroma for the screw cap and ampoule wines. This relates to the low oxygen ingress combined with the presence of certain sulfur-containing precursors at bottling.

For NZ Sauvignon Blanc, we have examined the effect of storage conditions on the decline in compounds responsible for the passion fruit and citrus aromas, particularly 3MH (**11**) and its acetate 3MHA.[20,46] Across sixteen Sauvignon Blanc wines bottled at the wine research hall in Auckland, under both cork and screw cap closures, a steady increase in absorbance at 420 nm (a widely used measure of wine browning) was seen (Fig. 2).[47] The rate of browning was greater under the cork closure, but this can be related more to the method of bottling at the University (which allows more O_2 into the wine than does a commercial operation) rather than to properties of the closure. The development of the two aroma compounds was very different, with 3MHA declining to very low levels over the first year in the bottle (Fig. 3), regardless of the closure type. This confirms the need to drink this wine young while such fruity aromas are at their most intense. A different aging pattern is shown by 3MH (**11**) and, in many cases, its concentration increased over the first three months in the bottle, likely due to hydrolysis of its acetate. A decline in level then follows with longer storage (Fig. 4). The 32% average decrease in **11** under cork versus a 21% average decrease under screw cap across the sixteen wines, matched the higher level of (oxidative) browning under the cork closure, related to conditions at bottling for this particular trial.

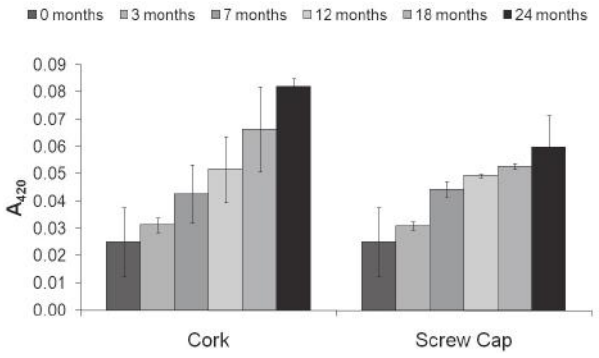


Fig. 2. Typical increase in 420 nm absorbance (browning) for a Marlborough Sauvignon Blanc in the bottle (n = 3).

Fig. 3. Typical loss in 3-mercaptohexanol acetate (3MHA) for a Marlborough Sauvignon Blanc in the bottle (n = 3) (same wine as for Figs. 2 and 4).

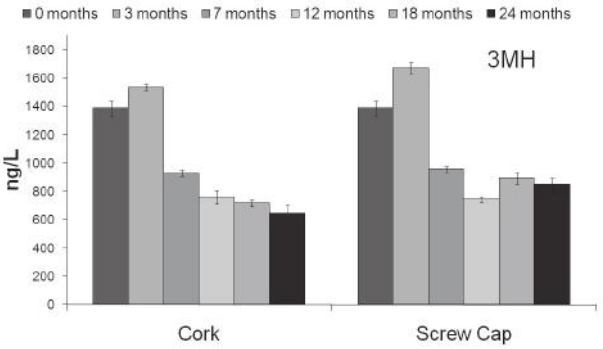


Fig. 4. Typical evolution of 3MH (**11**) for a Marlborough Sauvignon Blanc in the bottle (n = 3).

Final Remarks

The chemistry underlying wine oxidation processes has developed considerably over the past 10-20 years, and the role of polyphenol-mediated oxidation processes is a feature of this chemistry. The implications for red and white winemaking continue to grow and reveal both positive and negative contributions of O_2 for wine quality. Integrating chemical analyses with sensory studies remains an important area in the study of wine oxidation processes and it needs to progress. At the same time, a more detailed study of the chemical interactions between aroma compounds and oxidized polyphenols is needed to better appreciate the complexity, which makes wine such an interesting, and enjoyable, chemical matrix.

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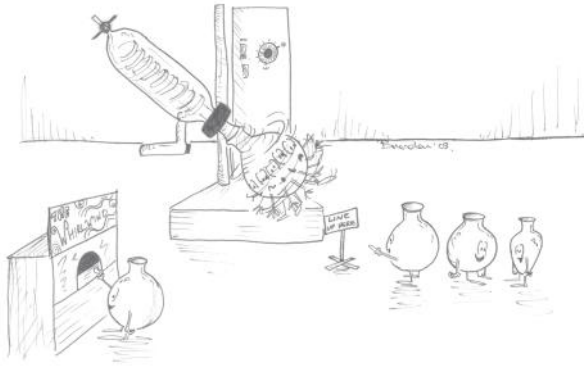


Brendan Burkett

Article

NZIC Conference – Dunedin

Chemistry in the Biosphere

The University of Otago hosted the 2008 NZIC Conference held jointly with the NZ Societies for Biochemistry and Molecular Biology (NZSBMB) and Plant Biologists (NZSPB) at the end of last November. The conference was opened by the Chairman, Prof Keith Hunter, and an historical recount of activities of the NZIC by Arthur Campbell followed. The meeting spanned five days and saw 130 presentations in four concurrent sessions covering areas of marine biogeochemistry, macromolecular structure and function, plant biochemistry and molecular biology, physical and theoretical chemistry, gene structure and function, plant physiology and ecophysiology, environmental and analytical chemistry, organic chemistry, human health, medicinal chemistry, plant development, and inorganic chemistry; the 329 registrant included 115 students.

Address to the Haggis

The Gala Dinner

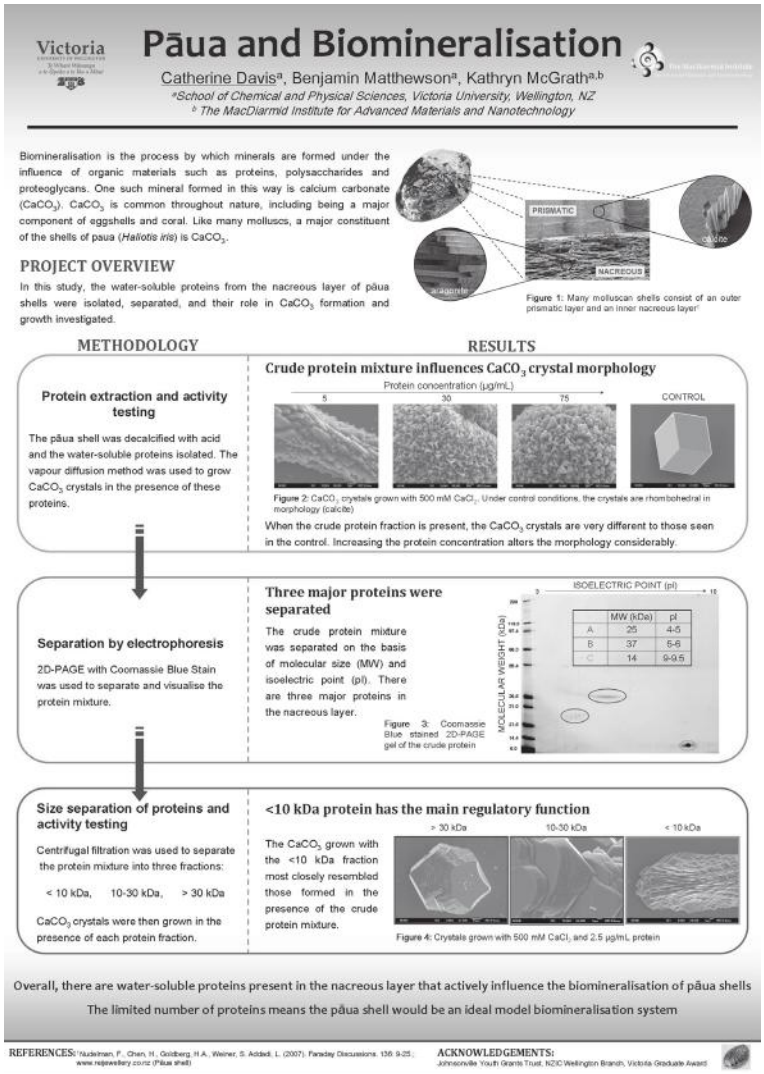
Gala Dinner participants where inspired by the reading of Robert Burns' *Address to the Haggis* and the dutiful Haggis Guards fully dressed in traditional Scottish attire including Mary Fowler (great grand-daughter of Ernest Rutherford), Max Coleman, and Keith Hunter (Conference Chairman). During the dinner ceremonies, Henrik Kjaergaard (Otago) was presented with the NZIC Maurice Wilkins Center Prize for his significant contribution to developing and using theoretical chemistry in studying atmospheric processes.

Prof *Henrik* ***Kjaergaard***, his group and the Maurice Wilkins certificate

NZIC student awards went to Jonathan Kitchen (Otago) for the best student talk and Catherine Davis (VUW) for the *CiNZ* Communicator of the Year award. The NZSBMB student awards went to Robert Fagerlund, and Tim Nalder and Steve Ballantine (all Otago) for best student oral and posters presentation, respectively. The NZSPB student oral went to Nick Albert (Massey), while Phuon Dinh (Massey) and Connie Wong (Lincoln) received the best posters awards.

Catherine ***Davis*** receiving the Communicator of the Year Award from the NZIC President

The physical and theoretical chemistry section of the conference enjoyed a truly international program with speakers from Aarhus to Auckland. A wide range of topics were covered from pure theory development to state-of-the-art computational simulations to some of the latest developments in laser based spectroscopy. The breadth and depth



The 2008 Communicator of the Year poster (daviscath1@myvuw.ac.nz)

of local delegate presentations at the conference was a testament to the exciting research currently taking place within physical and theoretical chemistry in NZ.

The marine biogeochemistry session was opened by Kim Currie (NIWA), who presented her eight-year time series of pCO_2 in sub-Antarctic waters. These waters and the Southern Ocean were continuing themes throughout the day with presentations on phytoplankton, sponges, cadmium, and dust storms in these unique ocean environments.

The use of analytical technologies in a range of biological applications ranging from human saliva to atmospheric pollution provided another well attended symposium. An application to monitoring stress by cortisol levels might well be applied to public speakers but those in this session from academia, CRIs and industry were all calm and concise! Dr Sami Damak (Nestlé, Switzerland) showed how the major food companies are investing in fundamental research on taste receptors. These are not just in the mouth but also in the gastrointestinal tract – so we do have gut feelings!

The excellence of organic chemistry in NZ was demonstrated in the various sessions that started with a superb plenary from a former colleague, Chris Hunter (Sheffield). He engaged us on developing models that explain the effect of solvent in weak non-covalent interactions and even managed to include a movie clip from *The Matrix* to illustrate modes of modeling molecular interactions. This was followed by many excellent presentations with subjects including new natural products, carbohydrate chemistry, aza sugars, glycolipids, and chitosan wound-healing gels.

Despite the joint meeting of the RACI and NZIC Inorganic groups at IC08 the week after *Chemistry in the Biosphere* there was a strong representation from the inorganic community. A Thursday session was devoted entirely to inorganic chemistry with recent Otago appointees James Crowley and Nigel Lucas, and NZIC President Bill Henderson and other Waikato participants describing their work. The broad ranging programme (from *Life on Mars* to *Molecular Recognition*) provided much to interest and inspire the inorganic chemist.

A feature of the conference was the NZIC Easterfield Address *Plastic Electronics: from Biosensing to Robotics* delivered by A/Prof Jadranka Travas-Sejdic (Auckland) on the chemistry and applications of electrically-conducting polymers. This followed the presentation of the NZIC/RSC Medal by NZIC President Bill Henderson. The medal is awarded biennially for research carried out in the 10-year period following the gaining of last qualification.

Dr *Jadranka* ***Travas-Sejdic*** receiving the Easterfield Medal

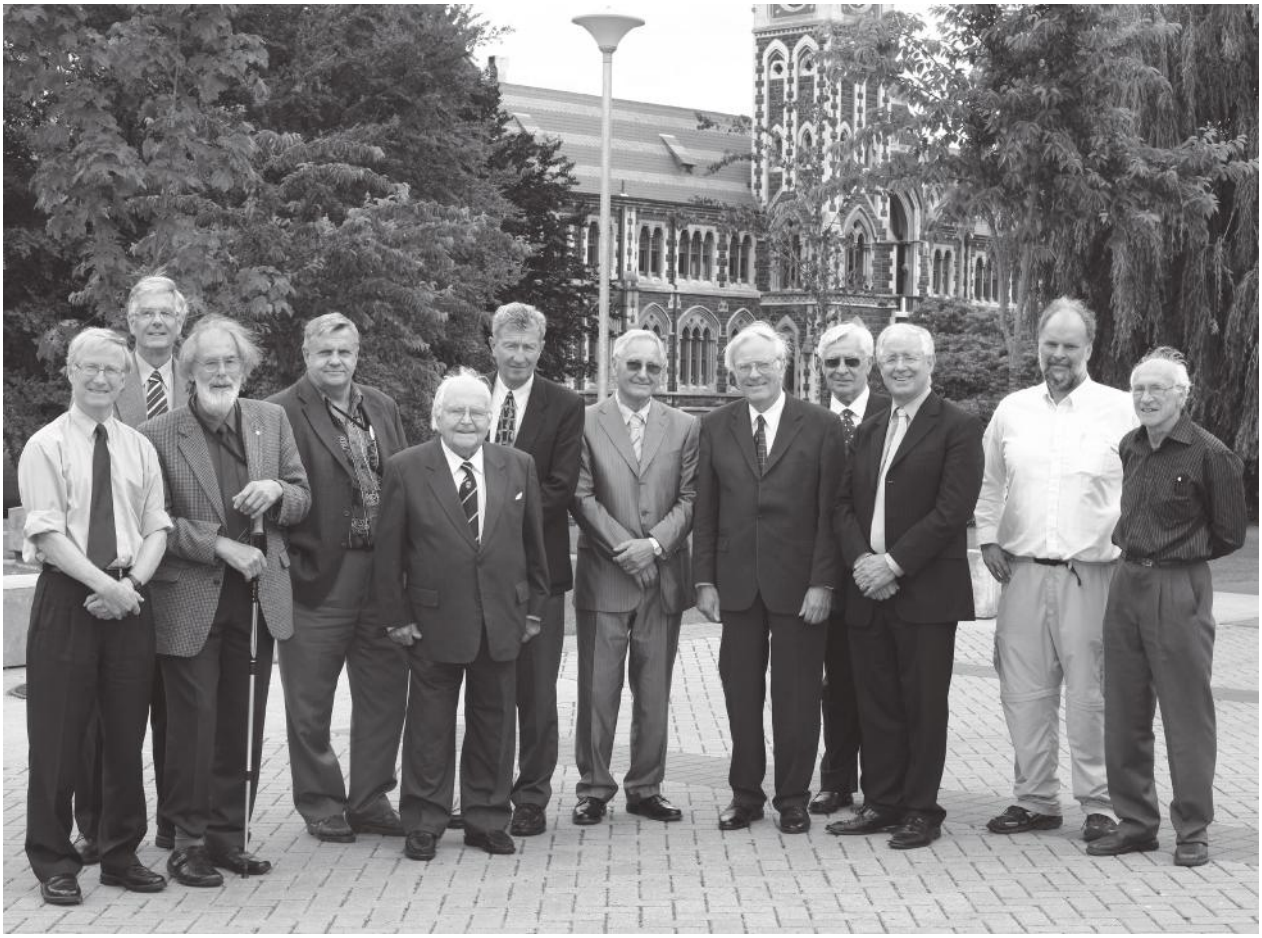
The NZSPB held six sessions with some twenty talks. Tina Summerfield (Otago) – the 2008 Roger Slack Awardee – gave a plenary lecture *on Global Gene Expression in the Cyanobacterium Synechocystis sp. PCC 6803* and Peter Nixon (IC-London) gave one entitled *Repairing the Engine of Life: How Plants Solved Their Energy Crisis* sponsored by *Annals of Botany*. In addition, there were over 20 posters from society members. The NZSBMB ran

four sessions and a session of student talks. Emily Parker (Canterbury) received the Applied Biosystems Award and spoke on *Evolving enzymes: Deciphering the evolutionary relationships in a family of crucial biosynthetic aldolases.*

A feature of the conference was a special joint one day education symposium *Education at the Interface* with John Hill (Curtin) as plenary lecturer opening the sessions. He described *an alternative curriculum framework for the tertiary chemistry course based on sustainability* and was followed by speakers from six universities and four schools who shared experiences of key aspects of Year-13 and University Chemistry and Biology. School teachers and science technicians were sponsored by the NZIC Otago Branch and NZSBMB. (Abstracts, PowerPoint presentations and videos are available from: http://nzsbmb.rsnz.org/Education.html).

The Rutherford Symposium

Celebrations to mark the centenary of Rutherford's 1908 Nobel Prize included a symposium at the conference to which the surviving winners of the Rutherford Medal, the highest scientific recognition within NZ, were invited. This provided the first (and likely the last) occasion when all of the Rutherford medallists could have gathered together in one room; it was a truly unique occasion. Few of the speakers were chemists, thereby providing the audience with talks ranging from hardcore mathematics to NZ soils. All but three of the awardees were able to attend and each gave a twenty minute presentation on their work. Also in attendance was Prof Mary Fowler (University of London), Ernest Rutherford's great grand-daughter. The symposium was sponsored generously by RSNZ and the Otago University Division of Sciences.



L-R: Profs Ted Baker, William Denny, Dr William Robinson, Profs David Penny, Thomas (John) Walker, Jeff Tallon, David Penny, George Petersen, Sir Ian Axford,, Profs Richard Faull, Vaughan Jones, and David Vere-Jones.

The Importance of a Seemingly Insignificant Poster Presentation…

Katherine Hebditch and Jarrod Ward

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A recent decision from the High Court of Justice Chancery Division[1] in the United Kingdom has accentuated the need for tight control on disclosure of any data pertaining to a potential invention. The case involved two companies, Laboratorios Almirall SA (Almirall) and Boehringer Ingelheim International GmbH (Boehringer), which both started out having patents with surprisingly similar subject matter. Obviously, this situation could not last for long.

Background

Both patents were essentially for a combination of two drugs for the treatment of respiratory disorders – aclidinium and a $ß_2$-agonist. The two patents in question were filed within a year of each other and the similarity in the subject matter was not discovered until both were published (patent applications are not made public until more than a year after filing).

Aclidinium is not a new compound, having been disclosed in an earlier patent application by Almirall.[2] In early May of 2003, Almirall presented two different poster presentations at a conference on two separate days.[3] The posters disclosed LAS 34273 (aclidinium) without disclosing the particular preferred enantiomer, and, more importantly, clinical data of the bronchodilatory and bronchoprotective properties on healthy males and those with Chronic Obstructive Pulmonary Disease (COPD). It is important to note that the posters did not disclose the use of aclidinium in combination with any other drugs.

The Judge summarised the facts of the matter as:

> *The first poster caught the eye of a passing Boehringer scientist […] who took four photographs of the poster. The photographs were then sent to Boehringer's International Project Management team in Germany for what was referred to as a "Competitive Assessment Update" on anticholinergics.*

Three months after Almirall's poster presentation, in July 2003, Boehringer filed three patent applications for the combination of aclidinium with various other drugs. The third of these applications was directed to the use of aclidinium in combination with a $ß_2$-agonist for the treatment of COPD. In the applications the preferred form of aclidinium was stated to be the *S enantiomer*.

In May 2004, with no knowledge of Boehringer's earlier patent application, Almirall filed its own patent directed to a combination of the *R enantiomer* of aclidinium with a $ß_2$-agonist for the treatment of respiratory disorders, particularly asthma and COPD.

On discovery of the Boehringer patents, Almirall applied for the revocation of the third Boehringer patent. Almirall used the grounds that Boehringer's invention was anticipated or was obvious and was insufficiently described. Boehringer counter-claimed that, if its patent was invalid, so too was Almirall's patent for the same reasons.

What to put in a patent specification

It appeared the patent application filed by Boehringer had been filed with little supporting information, such as experimental or clinical data. The presiding Judge stated:

> *No disclosure of any experimental work was given. Boehringer confirmed that they indeed had no disclosable documentation or laboratory records relating to experiments and/or tests with the anticholinergic compounds [aclidinium] in combination with $ß_2$-agonists.*

Boehringer later filed supporting information during the hearing to show the efficacy of its claimed combination. However, the further supporting information was directed to the racemate and the S enantiomer only, rather than the active R enantiomer.

Almirall's patent specification included detailed reasoning regarding its invention and why it was not obvious. It also contained detailed experimental and clinical data demonstrating the results of combining R-aclidinium with a $ß_2$-agonist.

Almirall argued that Boehringer's patent was obvious in light of the two poster presentations and its earlier patent application for aclidinium.

The Court found Boehringer's patent was indeed invalid and the invention was obvious. The Court also found the patent specification insufficient in that it did not contain enough information to enable a person skilled in the art to make use of the invention.

However, justice is a double edged sword. The Court then proceeded to determine the validity of Almirall's patent and found that Almirall's patent was also obvious over its disclosure in its earlier patent and the two posters. Both Boehringers and Almerill's patents were revoked.

Why is this important?

In a previous Patent Proze article we discussed patenting *vs*. publishing[4] and we mentioned that a poster presentation could be sufficient to destroy novelty for a patent application. In this case the poster presentations did not disclose the invention per se, but they did disclose enough information to render the invention obvious and therefore unpatentable.

This decision also highlights the need for sufficient description of an invention to be included in a patent specification. Although Almirall's disclosure in its posters and earlier patent were the nails in the coffins of both applications, if this

[1] *Laboratorios Almirall SA v Boehringer Ingelheim International GmbH* [2009] EWHC 102 (Pat)

[2] WO 01/04188

[3] The American Thoracic Society conference in Seattle

had not occurred, Boehringer's application may still have been found invalid on the grounds of insufficient description.

[4] Vol. 72, No. 2, April 2008

A reminder: if you have any queries regarding patents or patent ownership, or indeed any form of intellectual property, please direct them to:

Patent Proze
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Katherine Hebditch and Jarrod Ward of Baldwins Intellectual Property specialise in chemistry and biotechnology patents. Katherine obtained her PhD in organic chemistry from the University of Manchester in the UK in 2004. She is currently studying towards registration as a patent attorney. Jarrod obtained his PhD in chemistry from the University of Auckland in early 2008. He is currently studying towards a law degree and registration as a patent attorney.

Dates of Note continued

Sir *Arthur Conan* ***Doyle*** was born on May 22, 150 years ago, and the day marks 20 years since the first successful transfer of cells containing foreign genes into a human being (at the US NIH). *Waldo* ***Semon***, inventor of plasticized PVC, died on May 26 1999.

The highest temperature produced in a laboratory was attained on May 27, ten years ago. A plasma temperature of 510 million °C was reached in the Tokamak Fusion Test Reactor (TFTR) operated at the Princeton Plasma Physics Laboratory. Sir *Humphrey* ***Davy*** died on May 29, 180 years ago, while May 30 marks the 50th anniversary of the first hovercraft flight at Cowes on the Isle of Wight; its inventor Sir *Christopher* ***Cockerell*** died on 1 Jun 1999. On the last day in May 150 years ago a patent for flaked cereal was applied for by Dr *John Harvey* ***Kellogg***.

Irish chemist *Richard* ***Kirwan***, whose book *Elements of Mineralogy* (published 125 years ago in 1784) was the first English systematic treatment of the subject, died on 1 Jun 1812. On the same day in 1909, Swedish chemist *Theodor* ***Svedberg*** filed to patent his method of producing colloidal sols or gels, simultaneously in the UK, Germany, Denmark and Switzerland. By 1926, he had received the Nobel Prize in Chemistry for his work with disperse systems. On Mar 4, 1929, *George* ***Eastman*** demonstrated the first Technicolor movie.

Johan ***Gadolin***, who discovered the first of the 15 rare earth elements, yttrium in 1794 and after whom gadolinium is named, was born on 5 Jun 1760. June 9 marks the 50th anniversary of the death of *Adolf* ***Windaus***, the German organic chemist who showed the connection between sterols and vitamins and who received the 1928 Nobel Prize for chemistry, the first for work in human nutrition.

Anders ***Angstrom*** died 21 Jun 1874. Jun 21 also marks the day in 1808 when the isolation of boron was announced by *Joseph Louis* ***Gay-Lussac***, nine days ahead of Englishman *Humphrey* ***Davy*** who independently separated the element and made his announcement on 30 June.

Walther Hermann ***Nernst***, one of the founders of modern physical chemistry, was born 135 year ago on Jun 25. On this day 60 years ago, scientists in New York announced that the anti-tuberculosis drug *Neomycin* had been fully tested on animals. Moreover, Jun 25 1903 saw *Marie* ***Curie*** attend the examination committee for her PhD - she was awarded a Nobel Prize for her research later the same year! *Lord* ***Kelvin*** was born 185 years ago on Jun 26.

James ***Smithson***, who died 180 years ago on Jun 27, was the English scientist who provided funds in his will for the founding of the Smithsonian Institution in Washington *DC for the increase and diffusion of knowledge*. He was a chemist and mineralogist, and *smithsonite* (zinc carbonate) was named for him. *F. Sherwood* ***Rowland***, who has his 80th birthday on Jun 28, is one of the 1995 Nobel Chemistry Laureates (with Molina and Crutzen) for research on the depletion of the Earth's ozone layer.

July 1 marks the 75th anniversary of the first whole of body X-ray photograph. It was taken in Rochester (NY) with a one-second exposure using the ordinary clinical conditions common at an average hospital. On July 3, 1929, foam rubber was whipped up for the first time by *E.A.* ***Murphy*** at the Dunlop Latex Development Laboratories in Birmingham (UK).

Marie ***Curie*** died 75 years ago on July 4 and *Georg* ***Ohm*** 155 years ago on the 6th. *Robert* ***Woodward***, the most noted organic chemist of the mid-20th century, died on 8 Jul 1979. On the same day in 1895 *Joseph* ***Loschmidt*** died. He was an Austrian chemist and physicist and first to propose (1861) some kind of cyclic structure for benzene and many aromatic hydrocarbons; this was four years before Kekulé devised the correct ring structure. It is also the 225th anniversary of the death of *Torbern Olof* ***Bergman***, the Swedish chemist who experimented with carbon dioxide, which he named *aerial acid* and Priestley called *fixed air*. His investigation led him to successfully prepare artificial mineral water.

Erno ***Rubik***, inventor of the cube named after him, has his 65th birthday on July 13, a day that also marked the birth of *Stanislao* ***Cannizzaro*** in 1826 and the death of *August* ***Kekulé*** in 1896.

Emil ***Fischer***, who laid down the foundations for enzyme chemistry, died 90 years ago on July 15, the day 140 years ago that margarine was patented.