



**THE 30-YEAR ANNIVERSARY SYMPOSIUM OF THE
FINNISH SOCIETY OF TOXICOLOGY**

Challenges of Toxicology Today

May 26-27, 2009

Tampere University (Main Building, Lecture Hall 1A), Kalevantie 4, Tampere



**Edited by Tarja Toimela and Jenita Pärssinen
2009**

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Dear Colleagues,

The Finnish Society of Toxicology, FST, was established 29.05.1979 in Turku at a meeting of 78 Finnish toxicologists. During its 30-year existence, the society has grown markedly both in the number of members and in its activities. The 30-year anniversary meeting will provide an interesting scientific program, which highlights the development of toxicology in Finland and globally but most importantly it will discuss the challenges and critical goals of modern toxicology.

When reflecting challenges and goals of today's toxicological science to that of ten years ago, much are similar but there is one essential difference; the strategy for risk assessment has been moved from animal testing basis more to alternative-method basis. The driving forces for this movement are the new legislations for industrial chemicals (REACH) and the 7th amendment to the cosmetics legislation, the possibilities to use human stem cells and tissues in test models and large data banks. The growing resistance among the general public against the use of laboratory animals has also had a significant impact in the development of alternative approaches, but without biotechnology developments and impact of scientists 'the ship' would not have been turned. The change in practices takes time; as much as 50 years have elapsed since the publication of the 3R concept of Replacement, Reduction and Refinement of animal experiments.

The 30-year anniversary symposium starts with ToxGs-presentations of young scientists followed by an Industrial symposium, where key service and instrument providers present their products. After that we step into the past before the discussions on new developments starts where the focuses are on evidence-based toxicology, biomarkers and developments of alternative models. Intelligent testing strategy-symposium offers information on strategies and new developments for assessing human safety and environmental risks for pharmaceutical products and industrial chemicals. At the end of the second day the recently established Finnish Centre for Alternative Methods, FICAM, at the University of Tampere, will present its activities. The Research Tissue Bank Finland (FinTiB) in Tampere will also describe its services of providing human primary tissues for scientists.

With these words, on behalf of the organizers, I wish all the participants a scientifically valuable and socially enjoyable 30-year anniversary symposium. Especially I want to thank our guest speakers and my colleagues in the organizing committee for their contributions to the success of this symposium.

You are very welcome!

Tuula Heinonen

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Scientific Program

Time		Chair/Speaker
Tuesday 26.5.2009		
9-12	Registration ToxGs meeting	
11.30-12.00	<i>Coffee/Tea with sandwich, exhibition/posters</i>	
12.00-12.05	Opening of the 30-year Anniversary Symposium of the Finnish Society of Toxicology	Tuula Heinonen (FICAM, University of Tampere)
	Industrial Symposium	Chair: Tuula Heinonen (FICAM, University of Tampere)
12.05-12.20	Dynamic delivery solution for pharmaceutical and biotechnology research – what toxicology means to us?	Juha Saharinen (SBW Corporate, Turku)
12.20-12.35	FinnREACH - Finnish CRO cluster for REACH related services	Markus Soimasuo (FinnREACH)
12.35 -12.50	Machine vision system, Cell-IQ®, for addressing kinetic applications in high content screening (HCS)	Juha Korpinen (Chip-Man Technologies Ltd, Tampere)
	STY Symposium: Significant Past Milestones in Toxicology and Future Challenges	Chair: Hanna Tähti (FICAM, University of Tampere)
13.00-14.00	Finnish Society of Toxicology and International Union of Toxicology: Challenges for the Future - the IUTOX Perspective	Kai Savolainen (Institute of Occupational Health, Helsinki)
14.00-14.40	Evidence-based toxicology	Thomas Hartung (Johns Hopkins University, Baltimore)
14.40-15.10	<i>Coffee/Tea with sandwich, exhibition/posters</i>	
	Future Challenges	Chair: Arja Rautio (Thule Institute, University of Oulu)
15.10-15.50	Challenges of 3Rs for reproductive toxicology	Horst Spielmann (Zebet, Berlin)
15.50-16.30	Gene expression as a biomarker	Kirsi Vähäkangas (University of Kuopio)
16.30-17.10	Stem cells as a source for tissue models	Susanna Miettinen (REGEA, University of Tampere)
17.20-	Annual Meeting of the FST (Note: Lecture hall A2a)	
20.00	Dinner at Restaurant Ziberia (Siperia, Finlayson area, Itäinenkatu 9)	

Wednesday 27.5.2009		
	<i>STY and Fincopa Symposium: Intelligent Testing Strategy</i>	Chair: Pauli Ylitalo (University of Tampere)
9.00-9.15	Intelligent testing strategy (ITS)	Tuula Heinonen (FICAM, University of Tampere)
9.15-9.45	New trends in non-clinical safety assessment in pharmaceutical industry	Marja-Leena Toivonen (Orion Pharma, Espoo)
9.45-10.15	Integrated Testing Strategies as the challenge for industrial chemicals	Kimmo Louekari (ECHA, Helsinki)
10.15-10.45	QSAR(S) models for different end points?	Antti Poso (University of Kuopio)
10.45-11.15	<i>Coffee/Tea, exhibition/posters</i>	
	<i>Evaluation Environmental Risks</i>	Chair: Aimo Oikari (University of Jyväskylä)
11.15-11.45	International development and harmonization of methods for testing chemicals for regulatory purposes - roles of OECD, ISO, EU and Nordic co-operation	Jukka Ahtiainen (Finnish Environment Institute, Helsinki)
11.45-12.15	Risks of nanoparticles to the environment	Anne Kahru (Institute of Chemical Physics and Biophysics, Tallinn)
12.15-12.30	Discussion	
12.30-13.30	<i>Lunch</i>	
	<i>Tissue Models for Safety and Efficacy Testing</i>	Chair: Kirsi Vähäkangas (University of Kuopio)
13.30-14.00	Tissue models as tools for efficacy and toxicity testing	Timo Ylikomi (FICAM, University of Tampere)
14.00-14.30	Tissue banking – national and international aspects	Immo Rantala (Research Tissue Bank Finland, Tampere)
14.30-14.50	The Finnish Centre for Alternative Methods, FICAM	Tuula Heinonen (FICAM, University of Tampere)
14.50-15.00	Closing of the Symposium	Tarja Kohila Chairperson of the Finnish Society of Toxicology
15.00-16.15	Fincopa Business Meeting	
16.15 – 17.30	FST Toxicology Register Committee Meeting	

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Dynamic delivery solution for pharmaceutical and biotechnology research – what toxicology means to us?

Juha Saharinen

SBW – Systems Biology Worldwide and its subsidiaries Toxis and Infodix

The growing unwillingness to accept risks and hazards in everyone's daily life is putting a demand for the safety evaluation of substances, whether those are drugs, commodity chemicals, food additives or other molecules or mixtures. Toxicology and safety evaluations have a long history, dominated by various animal models as well as in vitro methods, associated with different international standards, like OECD assays and GLP requirements. While this battery of methods is considered to be the "golden standard" for safety evaluation and acknowledged by the regulatory authorities, there is a clear demand to compensate these tests with alternative in vivo models as well as in vitro, in silico and 'omics developments – in general referred as Toxicology for the 21st century.

Currently in Europe, the need for these compensatory methods is arising due the REACH legislation in addition to the trend of more strict safety evaluations for the growing number of novel drugs and food additives being developed. While these new methods are gaining support by some authorities, like US EPA, NTP and NCGC, their role currently is towards early-safety warning systems and prioritizing testing in the "golden standard" models.

SBW – Systems Biology Worldwide is a consolidated CRO, headquartered in Helsinki and serving pharma, chemical safety (REACH) and functional food industries. Our research service platform covers many areas in e.g. discovery and clinical development phases in drug research, including aspects of in silico, in vitro and in vivo methods. For safety and toxicology we offer multiple standardized in vivo models, both under non-GLP as well as OECD GLP standards (towards end of 2009). In addition, by the end of 2009, we will be offering OECD GLP in vitro toxicology testing.

In addition to these standardized assays, which under OECD GLP are required for regulatory preclinical and chemical safety testing, we also have extensive knowledge in in silico predictive toxicology and adverse effect models, including toxicogenomics, (Q)SAR models and well as OntoMine (US pat pending) expert system. OntoMine contains a knowledgebase, collected from literature and databases, hand curated and constantly expanded, containing thousands of endpoints of biological activity, mechanism/targets, adverse effects and various toxicities. The fingerprints of molecules in these endpoints allows fast prediction for toxicities, adverse effects, biological activities etc., for flagging toxic effects, HTS library selection, identification of promiscuous hits, lead chemistry scaffold hopping, drug repositioning and drug target identification.

With the various mentioned activities toxicology and safety assessments, SBW offers You a platform and experts for drug discovery, chemical safety and functional food research.

www.sbw.fi

FinnREACH - Finnish CRO cluster for REACH related services

Markus Soimasuo, CSO

Histola Research Ltd, Tampere, Finland

REACH is the European Community Regulation on chemicals and their safe use. The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. At the same time, innovative capability and competitiveness of the EU chemicals industry should be enhanced. The benefits of the REACH will come gradually, as more and more substances are phased into REACH.

The EU and the global picture for the chemical sector is largely changing due to REACH. Larger responsibilities and costs are laid on the industry in order to comply with the regulations. Especially, the obligations to test and to determine the properties and ways of safe handling of chemicals brought to the market, will force the manufacturers to undertake large and often time consuming and expensive research programs. Given the limited resources and complex requirements of this legislation, all companies manufacturing and/or importing chemicals are looking for external support that must be clear, consistent and reliable.

FinnREACH (www.finnreach.com), focusing on the REACH issues, is a large cooperation network of seven Finnish contract research organizations (CRO): AX Consulting, CRST, GenoSyst Ltd, Histola Research Ltd, Micoltech Ltd, Orthotopix Ltd and Zeus Tech Ltd. The FinnREACH cluster companies having extensive experience serving a wide variety of different manufacturers, distributors and r&d-organizations in the industrial, institutional, life science and personal care sectors are well prepared also to deal with the different tasks and issues raised on the implementation of the REACH directive.

Machine vision system, Cell-IQ[®], for addressing kinetic applications in high content screening (HCS)

Korpinen Juha¹, Ylikomi Timo²

¹ *Chip-Man Technologies Ltd, Tampere, Finland*

² *Medical School, University of Tampere, Finland*

The use of multiparameter measurements (high content analysis) instead of single endpoints increases the predictivity of in vitro toxicity testing. This is why high content screening (HCS) is becoming an important practice e.g. in drug discovery to study the toxicity and efficacy of the new drug molecules. HCS is currently usually carried out with microscopy by using different fluorescent labels. These tests are usually end point tests. Current fixed HCS endpoints do not usually allow kinetic analysis. Fixed endpoint tests are usually single snapshots after a certain time period for analyzing cellular functions. Extending HCS to kinetic live cell studies enables more comprehensive temporal evaluation of phenotypic response of dynamic cellular events e.g. cell migration, cell division, endocytosis and subcellular translocation.

Chip-Man Technologies has developed an automatic cell culturing and analysis system CELL-IQ[®]. Cell-IQ permits long-term culturing of cells. Cell-IQ can follow selected cell populations (collect time-lapse images) for days/weeks in different wells of well plates. Cell-IQ continuously collects visual information from cell cultures as a function of time. It is possible to do long-term analysis of cells with Cell-IQ to automatically identify and quantify changes in cell phenotype or phase. Once the user identifies the cellular features of interest, Cell-IQ can be taught to identify these features automatically. This enables multiple features to be monitored simultaneously. Once an experiment is completed, new features can be added to the analysis using the stored images, rather than repeating the same experiments. Practically any morphological parameter that can be differentiated by naked eye can be analyzed and quantified by Cell-IQ e.g.: i) Cell number, cell viability, cell division, cell death; ii) Morphological parameters; iii) Cell movement iv) Analysis of structures composed of groups of cells (either in 2D or 3D) in cell culture. This presentation will include user data on applications such as angiogenesis, neuronal cell growth and stem cell differentiation.

One limitation of many kinetic imaging approaches is the instability of the fluorescent probes used. Photobleaching often renders fluorescent intensity readouts meaningless during repeated imaging this is exacerbated by the risk of phototoxicity. Label free approaches such as phase contrast imaging (Cell-IQ) is a viable alternative.

Cell-IQ allows

- label free, kinetic assays without the risk of photobleaching or phototoxicity
- analysis of fluorescent labels is also possible
- combining phase contrast and fluorescent imaging

A powerful tool for HCS in vitro toxicity studies.

Finnish Society of Toxicology and International Union of Toxicology: Challenges for the Future - the IUTOX Perspective

Kai Savolainen

Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, 00250 Helsinki, Finland

Finnish Society of Toxicology (FST) was established in 1979, one year before the establishing of the International Union of Toxicology of Toxicology (IUTOX). Both actions reflect the increased impact of chemicals in the modern society. The timing the inaugural meeting of the Finnish Society of Toxicology indicates that Finnish toxicologists clearly understood the changes that had taken place in the society, or were about to happen in Europe and beyond regarding the need to promote chemical safety nationally and internationally. Over the years, FST has actively contributed to the IUTOX activities at all levels. An important milestone was the organization of the 10th International Congress of Toxicology in 2004 in Tampere by the FST jointly with the IUTOX. It thus seems that the close collaboration between the IUTOX and FST has assured also mutual understanding of the important local, regional and global issues in toxicology.

IUTOX has had a long standing goal, in addition to organizing international congresses, to contribute to human capital development in developing countries. This goal has become even more important over the years as the importance of safe management of chemicals in developing countries has increased. IUTOX has also recently emphasized the increased importance of having more impact on human health at a practical level. This has lead IUTOX to update its mission, vision and strategic goals which now more than before emphasize the importance of collaboration also with national societies and also governments to assure impact also at a country level. IUTOX wants to turn scientific knowledge into understanding and promote the use of this understanding to promote human health at a global scale. Some of the emerging issues and megatrends topic today include chemicals in products, nanotechnologies, global climate change, and life-cycle of products. These challenges require collaboration between IUTOX and its members as well as other parties around the world.

Evidence-based toxicology

Thomas Hartung

Johns Hopkins University, School of Public Health, Center for Alternatives to Animal Testing, Doerenkamp-Zbinden Chair for Evidence-based Toxicology, Baltimore, USA

The 3R concept to replace, reduce and refine animal experiments celebrates these days its 50th anniversary. In the meantime, a mechanistic toxicology has evolved which is effectively relying to large extent on methodologies which substitute or complement traditional animal tests. The biotechnology and informatics revolution of the last decades has made such technologies broadly available and useful.

Regulatory toxicology has only slowly begun to embrace these new approaches. Major validation efforts, however, have delivered the evidence that new approaches do not lower safety standards and can be integrated into regulatory safety assessments.

Political pressures especially in the EU, such as the REACH legislation and the 7th amendment to the cosmetic legislation, further prompt the need of new approaches. In the US, especially the NAS vision report for a toxicology in the 21st century and its most recent adaptation by EPA for their toxicity testing strategy have initiated a debate how to create a novel approach based on human cell cultures, lower species, high-throughput testing and modeling.

The lecture summarizes the lessons learned from the development, validation and acceptance of alternative methods for the creation of a new approach for regulatory toxicology. Beside the technical development of new approaches, a case is made that we need both conceptual steering and an objective assessment of current practices by evidence-based toxicology (www.ebtox.org). It is suggested to apply an approach modeled on Evidence-based Medicine (EBM), which over the last two decades has demonstrated that rigorous systematic reviews of current practices and meta-analyses of studies provide powerful tools to provide health care professionals and patients with the current best scientific evidence for diagnostic and treatment options. Similarly, a portal for high-quality reviews of toxicological approaches and tools for the quantitative meta-analyses of data promise to serve as door opener for a new regulatory toxicology.

Challenges of 3Rs for reproductive toxicology

Horst Spielmann

Freie Universität Berlin and ZEBET at the BfR Berlin, Germany

Reproductive toxicity refers to the adverse effects of substances on any aspect of the reproductive cycle, including the impairment of reproductive function, the induction of adverse effects in the embryo, such as growth retardation, malformations, and death. Due to the complexity of the mammalian reproductive cycle, it is impossible to model the whole cycle in a single *in vivo* or *in vitro* system in order to detect chemical effects on mammalian reproduction. However, the cycle can be broken down in its biological components which may be studied individually or in combination. This approach has the advantage that the target tissue/organ of a developmental toxicant can be identified. In specific areas of developmental toxicity, a number of useful and promising *in vitro* models are already available.

Today however, only testing in animals is accepted for hazard and risk assessment of chemicals affecting the reproductive cycle. The number of animals used for reproductive toxicity testing is expected to further increase due to the European legislation REACH and the 7th amendment to the Cosmetics Directive. A reduction in animal numbers can only be achieved, if testing strategies will be developed which will also integrate *in vitro* alternatives. Individual tests may be used as building blocks of a tiered testing strategy. This concept is currently being evaluated in the EU FP6 ReProTect project, which is coordinated by Michael Schwarz (U of Tuebingen, Germany) and was started in 2004 to develop / optimize *in vitro* assays that are able to detect adverse effects of chemicals on the reproductive cycle. The project consortium is composed of 33 partners from 12 European countries representing academia, industry, small medium enterprises and governmental institutions. The budget of the project is 13,2 m€ ReProTect is covering of four major areas: fertility, implantation, prenatal development, and cross-cutting innovative technologies, which may be applied in reproductive toxicity testing. In ReProTect more than 20 alternative tests have been developed or optimized and their reproducibility and transferability between labs have been studied.

Among the *in vitro* tests evaluated in the ReProTect project the embryonic stem cell test (EST) is particularly promising and due to its good overall performance, it is already established in laboratories of international drug companies. Thus EST may be used in commercial settings to aid in generating compound-development decisions. Possible improvements or refinements that might improve identification of developmental toxicity include the use of transcriptional expression profiling.

*To be presented at the symposium "Challenges of Toxicology Today" on the occasion of the 30-YEAR ANNIVERSARY THE FINNISH SOCIETY OF TOXICOLOGY
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Gene expression as a biomarker

Kirsi Vähäkangas

Department of Pharmacology and Toxicology, University of Kuopio

High throughput analysis of gene expression using microarrays, also referred to as toxicogenomics (Baken et al. 2007), has raised high hopes for many purposes including mechanistic and predictive toxicology (Gatzidou et al. 2007). Biomarkers are badly needed in various aspects of toxicology, for instance in exposure analysis and toxicological risk assessment, and for safety issues in drug discovery and development (Blomme et al. 2009). In biomarker development, gene expression patterns as biomarkers are being pursued for instance in immunotoxicology (Baken et al. 2007) and hepatotoxicity (Blomme et al. 2009). In both of these areas also the great challenges of toxicogenomics have become clear and the potential usefulness of the new discoveries remains so far open (Mendrick et al. 2008). Toxicogenomics reveals quantitation of mRNA and microRNA which are associated with gene regulation and mRNA stability (Gatzidou et al. 2007, Reamon-Buettner et al. 2008). However, because the expression pattern of gene products do not necessarily correlate with protein expression or function, studies on pathological and functional endpoints should accompany the obtained gene expression profiles for validation of toxicogenomic biomarkers and for conclusions on mechanistic aspects (Baken et al. 2007). Consorted efforts exist to validate toxicogenomic biomarkers by studying the reproducibility on different platforms and developing databases for comparison (Mendrick et al. 2008). For instance, the HESI Committee on the application of Genomics to Mechanism Based Risk Assessment has been on-going since 1999 (Pennie et al. 2004). In addition to proteomics and metabolomics, toxicogenomics will be complemented with epigenomics in future providing a new level for biomarker development (Reamon-Buettner et al. 2008).

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Stem Cells as a Source for Tissue Models

Susanna Miettinen

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Stem cells can differentiate into numerous types of cells with specific functions. It is well documented that stem cells might be of therapeutic value in regenerative medicine in the treatment of diseases including diabetes, heart disease, spinal cord injury, and multiple sclerosis, as well as in organ/tissue transplantation.

Stem cells are characterized by the ability to renew themselves as stem cells and to differentiate into a wide variety of specialized cell types. The three main types of mammalian stem cells are: 1) embryonic stem cells that are isolated from the inner cell mass of blastocysts, 2) adult stem cells that are found in adult tissues and 3) induced pluripotent stem cells (iPSCs) that are pluripotent stem cells derived from a non-pluripotent cell, typically an adult somatic cell, such as skin fibroblast, by inducing expression of certain genes. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells act as a cell reservoir and they maintain the normal turnover of organs and tissues. The iPSCs resemble natural pluripotent stem cells, such as embryonic stem cells in many respects, such as the expression of certain stem cell genes and proteins, embryoid body formation, teratoma formation, and potency to differentiate.

Stem cells and 3D models that capture both the organization and multicellular complexity of the target tissue provide powerful tools for screening the effects and safety of wide variety of substances including therapeutic drug candidates and toxins. Reprogramming of adult cells to obtain iPSCs may pose significant risks that could limit the use of iPSCs in cell therapies and regenerative medicine. Yet, the possibilities to create patient specific disease models and cell models for drug screening make this stem cells type a promising research tool for cell based testing systems.

Intelligent Testing Strategy (ITS)

Tuula Heinonen

FICAM, Medical School, 33014 University of Tampere, Finland

Every new chemical, whether pharmaceutical or industrial, has to be assessed for safety to human beings and for risks to environment before it enters into market. Safety evaluation is also a crucial element for cosmetics, disinfectants and GMO food. A pharmaceutical substance will not get marketing approval if it shows unacceptable health risks. The results from safety evaluation of an industrial chemical determine the classification and labeling of the chemical, its authorization and in more serious cases its restriction from use. All this means that a lot of safety studies have to be performed. Toxicity assessment today relies heavily on experimental animal models, where the priority endpoints are clinical signs and (histo)pathological findings.

In Europe over 12 million experimental animals are used annually. To comply REACH legislations, additional several millions of animals will be needed within the next nine years if the present (animal)testing models are used. Animal experiments are also expensive and time-consuming. It has been estimated that the testing costs to fill the safety data gaps of the 30 000 industrial chemicals on market today could be as high as 2.4 billion euros. Cosmetics legislations will not allow animal experiments after 2013 for testing of cosmetic ingredients, and at present no animal testing is allowed for the testing of final products. There are numerous examples that animals are not a relevant model to prove a chemical's toxicity or efficacy in man. Alternative methods and alternative approaches are desperately needed.

Good Intelligent Testing Strategy (ITS) approaches can reduce the cost and use of animals while providing the best quality data for risk assessment purposes. The basic principle in ITS is to utilize the existing data maximally, to use (Q)SARs for predicting effects on different endpoints, to perform read-across analyses and then to fill the data gaps by animal experiments. The animal experiments should be used as last resort. Integration of data from animal toxicity and ecotoxicity studies might diminish the need of animal experiments even more, and certainly be very valuable in total risk assessment.

This symposium discusses ITS from the point of view of pharmaceuticals, industrial and environmental chemicals with special reference to QSAR models and nanoparticles.

New trends in non-clinical safety assessment in pharmaceutical industry

Marja-Leena Toivonen

R&D Research, Orion Pharma, Espoo, Finland

Drug safety remains a high profile issue in Pharmaceutical Industry at a time when the cost and time required to develop a new drug are increasingly high. Historically, little preclinical safety assessment was done on lead candidates before the package of nonclinical studies to support the First-In-Man trials was started. As safety-related problems continue to be one of the leading causes of drug-candidate attrition during all stages of drug development, many companies are increasingly integrating toxicity prediction technologies into earlier phases of the drug discovery process. The early application of preclinical safety assessment principles includes both high and low-to-intermediate throughput *in vitro* assays as well as short-term *in vivo* toxicity assays. Prospective assays to predict development-limiting toxicities which are often missed in short-term *in vivo* assays are used for early prioritisation of compounds. Retrospective assays are used for issue management and compound prioritisation after target-organ toxicities have been identified in early *in vivo* assays. Early knowledge of the safety liabilities will enable project teams to build customized testing strategies to select superior molecules for development. Understanding the mechanism of toxicity helps extrapolate the relevance of the preclinical data to human. Emerging technologies offer new opportunities to improve throughput, lower the cost or increase the translatability. However, new technology can only be adopted when there is sufficient understanding of both their advantages and limitations.

Integrated Testing Strategies as a challenge for industrial chemicals

Kimmo Louekari

European Chemicals Agency

Specific information requirements have been set for the registration of chemical substances according to REACH Regulation. The requirements concern e.g. physical-chemical, ecotoxicological and toxicological properties of the substances. Depending on the amount of the substance manufactured or imported annually, the information requirements increase. At the highest tonnage (>1000 tpa), the complete information required by REACH for the effect endpoints has to be submitted by the registrant.

In the Integrated Testing Strategies (ITS), provided in the Regulation and in the relevant guidance, the step-wise consideration of all relevant data is described. This may lead to identification of testing needs in order to characterise the inherent toxic properties of a substance and to meet the information requirements. ITSs cover both general and specific rules of adaptation.

As pointed out in article 13 of the REACH Regulation “Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions of Annex XI are met.” These other means, i.e. general rules of adaptation are

- Use of existing data (not GLP/ non standard tests)
- Historical Human data
- (Q)SAR
- Grouping of substances and read-across approach
- In vitro methods
- Weight of evidence

Information requirements of REACH are listed as specific endpoints to be covered, i.e. “standard information required”. For example on skin sensitisation the standard requirements in sequence are: (1) an assessment of available human, animal and alternative data and (2) *in vivo* testing (LLNA as the first-choice method). The specific rule for adaptation for this endpoint is that the *in vivo* study is not required if the substance is classified as corrosive or sensitizer or when the substance is a strong acid or base or flammable at room temperature. In the ITS for sensitisation, these “data elements” plus QSAR and read-across are placed in a sequence and furthermore, the decision logic is specified. The ITSs for all toxicological endpoints to be applied by the registrant are specified in the “Guidance on information requirements and chemical safety assessment.”

Application of different elements of the ITSs will be explained and as an example, the outline of the ITS on skin irritation/corrosion is presented. Some first practical experiences are discussed at a general level.

QSAR(S) models for different end points?

Antti Poso

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Quantitative Structure Activity Relationship (QSAR) analysis has been used in pharmaceutical discovery and development to predict the affinity of new molecules to target proteins. In this situation the 'end points' have been clearly determined although that is not always the best way. In principle, QSAR is simple: affinity (or any property chosen as the target) is defined in clear numbers, and, correspondingly, the different properties of the molecule are also defined in numbers. A correlation between these numbers is created by using either a regression analysis or, more often at present different multifunctional methods.

Are QSAR methods really useful in toxicology, and how do these methods comply with REACH? The answer to the first question is short (but sometimes not simple at all). The more specific and of "*in vitro* type" the target to be modeled is, the better the QSAR methods can show a correlation, which possibly is based on causality. The answer to the other question is more difficult, because REACH indeed encourages the use of mathematical models but none of the models are validated in a way to fulfill the quality requirements of REACH.

In my presentation I will give several examples of the right and the wrong use of QSAR models. My purpose is also to give rough guidelines about how to assess the relevance of QSAR models.

International development and harmonization of methods for testing chemicals for regulatory purposes – roles of OECD, ISO, EU and Nordic co-operation

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The OECD test guidelines for testing chemicals have been widely used for regulatory purposes all over the world since the establishment of the MAD principle 1981. This Mutual Acceptance of Data ensures that, if a chemical or a substance is tested under the GLP (Good Laboratory Practise) conditions accordingly to an OECD test guideline, the data should be accepted in all OECD countries. The rationale behind this agreement has been to save resources and avoid duplicate testing, especially with vertebrate animals. The test guidelines have been developed and validated to be used for the hazard identification and risk assessment of a broad variety of chemicals, e.g. industrial chemicals, pesticides, biocides and veterinary drugs.

The test guideline development at OECD level should include initial scientific work, identification of relevant "endpoints" for regulatory purposes, adequate validation of the method and an international agreement on the test protocol based on the validation data. The initiative for developing a new test guideline usually comes from the OECD member country, which should also take the responsibility to finalize it. There seems to be a constant need to develop new test guidelines and update existing ones. One of the current challenges is the applicability of OECD test methods for testing nanomaterials. In the worst case a test guideline has to be adapted for testing each single nanomaterial. This will raise a question that how far a test guideline can be modified and still the data would be covered by the principle of mutual acceptance of data.

Historically the ISO standard methods for ecotoxicology were developed based on the protocols for testing chemicals. However, the scope of the biological methods developed in ISO Technical Committees for Soil Quality (ISO TC 190) and Water quality (ISO TC 147) has been now revised towards testing environmental samples e.g. soils, sediments and waters. Hence the future applicability of these methods for testing individual chemicals might be more limited, but some of the ISO standards are still referred in the technical guidance on how to generate data for the information requirements of the REACH regulation.

Before the REACH regulation, EU had already own test methods under the decision on the existing substances in its Annex V. Today these EU methods are taken under the new test guideline regulation which should provide the compilation of testing tools for REACH. Luckily, there has been a clear view to develop also these EU methods firstly at the OECD level in order to achieve broader global harmonization, which would then save costs and experiments. Hence the EU methods should be identical to the OECD methods. Only if there is an urgent regulatory need for a method in EU, and there is an unduly delay in the OECD process, the method can be adopted only as an EU test method.

The Nordic countries co-operate in the development of test guidelines for testing chemicals at OECD and EU level. The Nordic coordination group Nord-UTTE has been established under the Nordic Chemicals Group for this purpose. Nord-UTTE also support experimental research for the development of methods needed for our regulatory needs.

Risks of nanoparticles to the environment

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Synthetic nanoparticles (NPs) are increasingly used in various (consumer) products and some of them are already produced in high production volumes. Thus, the risk of environmental contamination by these NPs continuously increases. The toxicological information on synthetic NPs is already considerable but ecotoxicological data are just emerging. Since past five years we have been studying the (eco)toxicological effects of metal oxide NPs (CuO, ZnO, TiO₂) using various model organisms on different trophic level of the food-web: algae *Pseudokirchneriella subcapitata*, protozoa *Tetrahymena thermophila*, crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, yeast *Saccharomyces cerevisiae* and bacteria *Vibrio fischeri*. Recombinant luminescent sensor bacteria were used to study the solubilisation and bioavailability of metals from metal oxide (nano)particles. The used test battery involves organisms *a priori* not ingesting particles (bacteria, yeasts, algae) and particle-feeding organisms (crustaceans, protozoa). Our studies (references 1-4) showed that the least harmful from the tested compounds were TiO₂ NPs and most toxic ZnO NPs. Algae were the most sensitive and yeasts the least sensitive organisms under our test conditions. As a rule, the toxic effects of metal oxide (nano)particles correlated with their solubility and the toxicity to certain organism was usually explained by solubilised metal ions (Cu²⁺ in case of CuO-and Zn²⁺ in case of ZnO) The toxic effect of CuO (nano)particles was significantly reduced in natural water containing dissolved organic matter due to the decrease of copper bioavailability.

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RESEARCH TISSUE BANK FINLAND

Immo Rantala

FINTiB Research TISSUE BANK FINLAND collects and administers tissue archives together with the related patient data.

FINTiB's mission is to improve the impact of healthcare, to develop new therapies and diagnostic methods, and to generate new research and business.

FINTiB provides two services from one organization. We offer a research tissue bank with patient data and clinical laboratory tests. This duality is unique worldwide. FINTiB is part of the Pirkanmaa Hospital District Laboratory Centre, the first accredited clinical laboratory in the Nordic countries.

The specimen base consists of archives collected in connection with healthcare, and collections gathered for specific studies. FINTiB also collects new targeted patient materials for the purposes of study using the latest tissue engineering methods. Services that require specialist expertise are produced through an expert tissue engineering subcontracting network.

FINTiB provides services

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- digital microscopy and image archives
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- specimen sampling, analysis, storage and transportation

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- new therapy development and improvement, public health, drugs and diagnostic methods as well as the efficiency of healthcare systems
- healthcare applications development
- international competitiveness in the field of health

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FINTiB's activity in Finland offers key strengths

- a genetically and culturally uniform population
- high-standard healthcare
- systematic specimen archives

- excellent patient registers
- diverse background data
- internationally high-standard research and information technology
- scientific computing resources
- uniform procedures of a high quality

FINTiB seeks to build a future with research institutions and companies oriented toward the development of new therapies and diagnostic methods and fundamental research. In addition to more than one million existing specimens, FINTiB has the capacity to collect tens of thousands of new specimens every year.

FINTiB TISSUE BANK FINLAND is located in the city of Tampere, Finland, in the health and life sciences cluster on the Finn-Medi campus.

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- pharmaceutical companies
- companies in the field of diagnostics

FINTiB is an independent service organisation which does not participate in the research activities itself. Instead, we offer patient samples and the data related equally to both academia and industry.

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In all its activities, FINTiB emphasizes ethics, openness and trust. The research use of specimens is at all times subject to licence. Patients have the right to know what the specimens are used for, what the studies aim to achieve and the kind of results that have been gained.

Specimens are collected in connection with various research projects and patient treatment, based on the patients' consent. Their use is regulated by legislation and international ethical guidelines. Ethical considerations are evaluated in connection with every study, by the Pirkanmaa Hospital District Ethics Committee

The Finnish Centre for Alternative Methods, FICAM

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The Finnish Centre for Alternative Methods (FICAM) was established in December 2008. FICAM is located in the Medical School at the University of Tampere.

FICAM focuses on development and validation of cell and tissue culture 3D-models to complement and to replace animal experiments. In addition, FICAM's role is to be the centre of excellence for alternative (replacement) methods in Finland, to share information locally and to implement education and training on alternative methods into the advanced training courses. FICAM also provides GLP (Good Laboratory Practice) and validation expertise on alternative methods.

Medical School of Tampere University possesses a long experience in human cell and tissue research and development of related technology. Therefore, the majority of the tests developed in FICAM apply the use of human tissues and cells. The human tissue material is obtained from the Tissue Bank Finland (FinTiB) located in the Tampere University Hospital next to FICAM. FICAM applies GCP (Good Clinical Practice) principles for human tissue material. The routine testing in FICAM is performed under GLP. The developed validated test models can be used in safety studies (e.g. pharmaceuticals, industrial chemicals, cosmetics) and efficacy (pharmaceuticals) testing. Because of the gained expertise and GLP, FICAM is a competent reference laboratory e.g. for the purposes of method validations for ECVAM (European Center for Validation of Alternative Methods).

Poster Abstracts

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Fatty acid composition and gene expression profiles are altered in aryl hydrocarbon receptor-1 mutant *Caenorhabditis elegans*

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Background: Aryl hydrocarbon receptor (AHR) is a modulator of transcription with roles in development and metabolism. The *C. elegans* AHR ortholog AHR-1 likely shares many similar physiologic functions with the mammalian version.

Objectives: To understand the physiologic role of AHR in *C. elegans* by combining lipidomics and transcriptomics approaches.

Methods: Fatty acid profiles in *ahr-1* mutant and wild type *C. elegans* were measured with Mass Spectrometry. Gene expression profiles were measured with quantitative PCR and whole-genome microarrays. The promoters in the differentially expressed genes were inspected using motif searching tools.

Results: The *ahr-1* mutant contained lower expression levels of fatty acid synthesis genes. Also fatty acid compositions had higher proportions of lower chain fatty acids in the *ahr-1* mutant. Microarray analysis revealed dysregulation of genes associated with development, growth, and carbohydrate metabolism. The core AHR binding site was observed among the regulated genes.

Conclusion: The evolutionarily conserved functions of AHR are important physiologic processes such as growth, development and metabolism. Understanding of these processes would gain insight to the toxic effects caused by AHR activation by toxins.

Boar spermatozoa as biosensors for mitochondrial toxins in the context of intact eukariotic cells.

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Mitochondrial dysfunction can lead to neurodegenerative conditions and metabolic diseases. Substances like herbicides, insecticides, disinfectants and antimicrobials as well as microbial secondary metabolites have been suspected of mitochondrial toxicity. No rapid screening test is available for detection of environmental mitochondrial toxicants.

Boar spermatozoa have several properties of relevance for their use in mitochondrial toxicity assays. These cells are simple, consisting of condensed nuclear DNA in the sperm head, 70 mitochondria in the midpiece, and the axial filament complex in the sperm tail. They lack endoplasmic reticulum and golgi apparatus, and have a low cytosol content in relation to the cell surface. Physiological processes in spermatozoa are regulated by membrane potentials and ion fluxes. Lack of the pentose phosphate cycle and inability to generate NADPH limits the ability to repair cellular damages. No transcription or translation of the condensed nuclear DNA occurs in the spermatozoa rendering them insensitive to toxins affecting the synthesis of nucleic acids or their regulation.

In boar sperm, glycolysis provides only low cellular ATP levels insufficient for progressive motility. Progressive motility is exclusively dependent on oxygen and the pyruvate degradation pathway which generates high cellular ATP levels by the TCA cycle and the oxidative phosphorylation in the mitochondria. Metabolism in boar sperm cells have a limited ability to modulate ATP consumption, even transitory loss of ATP leading to rapid loss of motility and viability. In absence of damage to the plasma membrane integrity barrier, mitochondrial toxicity in exposed boar spermatozoa is indicated by: loss of motility, mitochondrial depolarisation, depletion of cellular ATP and preservation or accumulation of the cellular NADH contents. Methods for measuring these toxicity parameters have been in routine use for fertility analysis concerning storage and preservation of commercial boar semen.

With boar sperm, motility inhibiting toxins targeting different mitochondrial functions may be identified. Many ionophoric toxins (i.e. valinomycin) inhibits motility by rapid mitochondrial depolarisation without depletion of the cellular ATP or NADH. Inhibitors of ATPases (i.e. oligomycin) inhibit motility in absence of any mitochondrial depolarisation and without depletion on cellular ATP or NADH. Inhibitors of the electron transport chain (antimycin and myxothiazol) inhibit motility by depletion of cellular ATP, mitochondrial depolarisation occurs after a lag period and cellular NADH levels are preserved or increased. This toxicity pattern distinguishes mitochondrial toxins from toxins acting by forming channels through the plasma membrane. The toxins affecting such channels inhibit motility, depolarise mitochondria, and deplete cellular ATP and NADH simultaneously.

We developed a bioassay using boar spermatozoa as biosensors for mitochondrial toxins in the context of intact eukariotic cells. We detected health stable mitochondrial toxins in extracts prepared from vinyl gloves, perfumes, respirable indoor dust, contaminated indoor building material and animal feed. We have detected bacterial mitochondrial toxins in foods and identified the mitochondria as the biological target of several toxins produced by fungi and bacteria from water damaged buildings.

Our research group will evaluate boar spermatozoa as target cells in toxicological research in order to replace and reduce the usage of laboratory animals in toxicological exposure studies and also reduce the use of cultured cell lines needing foetal calf serum in the growth medium. Extended boar semen intended for pig breeding are commercially available from AI stations in most developed countries.

Evaluation of blood-brain barrier properties of ARPE-19 cell line in comparison to Caco-2 cell line and primary porcine microvessel endothelial cells

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The brain is protected by the blood-brain barrier (BBB), which is composed of brain microvessel endothelial cells. The integrity of BBB *in vivo* is assured by tight junctions, which restrict the paracellular passage of molecules through the barrier. *In vitro* models that maintain transport mechanisms and structural properties associated with the BBB *in vivo* would be of great importance in studying to study the ability of candidate drug molecules to pass the BBB, and in evaluating . An *in vitro* model for BBB is needed also to evaluate the neurotoxic risks of environmental chemicals.

Due to the poor availability of commercial human brain microvessel endothelial cell lines, we studied a human retinal pigment epithelial (RPE) cell line (ARPE-19) that has functional characteristics comparable to the brain endothelial barrier, could possibly be used for the BBB model. Retinal pigment epithelial (RPE) cells have several functional similarities to the BBB, e.g. efflux systems and intercellular tight junctions. To evaluate the suitability of a human RPE cell line ARPE-19 cell line for a BBB model, we have compared to each other certain barrier properties of ARPE-19 cell line, human colonic adenocarcinoma cell line Caco-2, and primary porcine microvessel endothelial cells (PMECs). The paracellular ionic permeability was evaluated by measuring the trans-epithelial or trans-endothelial electric resistance (TEER). The functionality of an efflux transporter P-glycoprotein was studied by means of rhodamine123 accumulation. The tight junction proteins occludin and ZO-1 were stained for immunocytochemistry.

All the cell types in this study stained positively for occludin and ZO-1. However, the TEER of ARPE-19 cells was low when compared to PMEC and Caco-2 cells. In addition, the P-glycoprotein activity in ARPE-19 cells was much lower than in PMEC and Caco-2 cells. In conclusion, the characteristics of ARPE-19 cell line are were not satisfactory for a BBB model. Our future plans include studies with the human brain endothelial cell line hCMEC/D3 in a co-culture with a human astrocytoma cell line U-87 MG.

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Benzalkonium Chloride Homologs in Ocular Surface Cells *in Vitro* and *in Vivo*

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Purpose Benzalkonium chloride (BAC) is the most commonly used preservative in commercial ophthalmic preparations. There are numerous studies reporting detrimental, concentration-dependent effects of BAC on ocular surface tissues. The commercial BAC is a mixture of at least three homologous N-alkyldimethylbenzylammonium chlorides with N-alkyl groups varying from 6 to 18 carbon atoms in length. The proportions of these homologs in the mixture determine its effectiveness as a preservative and disinfectant. The cytotoxic effect of different BAC homologs (BAC-C₁₂, BAC-C₁₄, BAC-C₁₆) and BAC mixture (containing 64.1% C₁₂, 33.8% C₁₄, and 2.1% C₁₆) was studied *in vitro* in corneal and conjunctival epithelial cell cultures. To study correlation between cytotoxicity and ocular absorption of BAC homologs, their distribution in the ocular surface tissues of rabbits was examined.

Methods Human corneal epithelial (HCE) and human conjunctival epithelial (IOBA-NHC) cell cultures were exposed to different BAC homologs or mixture for one hour. Cytotoxicity was assessed with the WST-1 assay for cellular growth and viability. BAC mixture as 0.02% (v/v) aqueous solution was applied into rabbit eyes once a day for 14 days and homologs in corneal and conjunctival tissues were analyzed using liquid chromatography-tandem mass spectrometry.

Results *In vitro*, conjunctival cells appeared to be more sensitive to BAC exposure than corneal cells. In corneal cells, the cytotoxicity for tested preservatives did not make much difference, with the EC₅₀ values 0.00130% for C₁₆, 0.00127% for BAC mixture, 0.00101% for C₁₂, and 0.00097% for C₁₄. In conjunctival cells, the EC₅₀ values were 0.00065% for C₁₆, 0.00047% for BAC mixture, 0.00041% for C₁₄, and 0.00038% for C₁₂. *In vivo*, the amounts of C₁₂, C₁₄ and C₁₆ in corneal and conjunctival tissues were respectively (mean ± SEM, n=5): 0.37 ± 0.08 and 2.64 ± 0.27 ng/mg, 0.42 ± 0.07 and 4.77 ± 0.43 ng/mg, 0.04 ± 0.01 and 0.54 ± 0.05 ng/mg.

Conclusions Within the studied BAC homologs, those with longer alkyl chain length and less aqueous solubility had a better absorption into rabbit ocular tissues. BAC-C₁₄, reported to have the most potent antimicrobial activity, possess the favorable combination of penetration into ocular surface tissues and tolerability on corneal and conjunctival cells.

The Cytotoxic Effects of Preserved Prostaglandin Analogues and Preservative-free Tafluprost on Human Corneal and Human Conjunctival Epithelial Cells *in Vitro*

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Purpose Glaucoma is one of the most common eye diseases in the elderly people. In Finland, there are more than 75 000 glaucoma patients and every year another 2 500 new cases are found. Prostaglandin analogues are the most commonly used drug group for the treatment of glaucoma because they reduce the increased eye pressure efficiently and they are easy to use as once a day application. However, in the long term usage they can induce local eye irritation. Most commercial prostaglandin analogues contain as a preservative benzalkonium chloride (BAC) which is a well known eye irritant. In this study, the possible adverse effects of the commercial preparations of BAC-preserved prostaglandin analogues; Xalatan® (0.005% latanoprost & 0.02% BAC, from Pfizer) Travatan® (0.004% travoprost & 0.015% BAC, from Alcon) and Lumigan® (0.03% bimatoprost & 0.005% BAC, from Allergan) and a new preservative-free Taflotan® (0.0015% tafluprost, from Santen Oy), and the effects of the appropriate concentrations of BAC were evaluated on human corneal epithelial (HCE) and human conjunctival epithelial (IOBA-NHC) cell cultures.

Methods Ocular cells were exposed to 0.1%-10% eye drop concentrations in culture medium without serum for one hour. Correspondingly, the cells were exposed to 0.00008%-0.005% BAC. Cytotoxicity was assessed with the WST-1 assay as an index of cell viability/proliferation and the lactate dehydrogenase (LDH) assay as an index of cell membrane integrity.

Results The order of decreasing toxicity was latanoprost \geq travoprost $>$ bimatoprost \geq tafluprost. IOBA-NHC cells were more sensitive than HCE cells. The EC₅₀ value of BAC was 0.0013% in HCE cells and 0.00047% in IOBA-NHC cells. The tested drugs had no effects on LDH leakage, except for 10% latanoprost in HCE cells and 10% latanoprost and 3-10% travoprost in IOBA-NHC cells.

Conclusions The cytotoxic effects of commercial preparations of latanoprost, travoprost, and bimatoprost were dependent on the BAC concentration of the drug. The *in vitro* toxicity of BAC was highly concentration dependent and appeared at the concentrations above those corresponding to 0.001% of BAC in ophthalmic medications. Preservative-free tafluprost had the least toxic effects in the culture conditions used.

Primary Placental Trophoblast Cells as a Research Model

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Studies with different human cell types are in most cases restricted to secondary cell lines. Secondary cell lines are transformed and therefore express altered cellular functions. Healthy human primary cells are quite difficult to obtain. However, there is one tissue which is readily available: the human term placenta. The use of term human placental tissue for research purposes is ethically quite acceptable because placentas are usually discarded after delivery.

Isolation and culturing of term human trophoblast cells was done according to Petroff *et al.* 2006. Briefly, fresh villous placental cotyledons (approx. 50 g) were cut, washed, and homogenized with scissors and scalpel to small pieces. Homogenized tissue was digested enzymatically to separate the individual cells. After digestion cell homogenate was centrifuged through Percoll's liquid gradient for separation of the trophoblasts. Finally, isolated trophoblast cells went through immunomagnetic bead purification. Purity and amount of the cells were secured with immunocytochemistry or flow cytometry.

In our last study (Huuskonen *et al.* 2008) we found that maternal cigarette smoking induced cytochrome P450 1A1 enzyme (CYP1A1) activity and conversely repressed aromatase (CYP19A1) activity in the placenta. Therefore, we started to test this enzyme divergence with primary cell culturing methods.

We treated primary trophoblasts with β -naphthoflavone, a CYP1A1 enzyme inducer. After induction ethoxyresorufin O-deethylase (EROD) activity and CYP1A1 gene expression were measured. Both EROD and CYP1A1 gene expression were 10 times higher in treated samples compared with controls. Our preliminary aromatase measurements showed decreased aromatase activity in the CYP1A1 induced cells.

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Recombinant luminescent bacterial sensors for bioavailable heavy metals: performance and applications

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Recombinant whole-cell sensors allow the assessment of the bioavailability of environmental pollutants like heavy metals and organic compounds. In the National Institute of Chemical Physics and Biophysics in Tallinn (in co-operation with University of Turku) 17 luminescent recombinant Gram-negative (*Escherichia coli*, *Pseudomonas fluorescens*) and –positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacterial sensors for bioavailable metals have been constructed. These sensors report on the presence of bioavailable sub-toxic metal concentrations by the increase in bioluminescence the induction of which is controlled by certain metal-response elements (specific for each metal): a metal-binding regulatory protein and its regulated promoter.

The set of luminescent sensor strains constructed by us includes sensor bacteria for Hg, organic compounds of Hg, Cr, Cu, Ag, Cd, Pb and Zn. Comparison of the performance of these sensor strains showed that their bioluminescence is mostly determined by the metal-response elements and depends on the tightness of their control over the bioluminescence-encoding genes. Also, the sensitivity and specificity of the sensor bacteria was primarily defined by the metal-response elements and not by the host cell. On the other hand, the toxicity of metals to these bacteria was primarily dependent on the type of bacterial host. In terms of toxicity, Gram-positive strains were more sensitive towards heavy metals than Gram-negative ones.

As currently the most challenging field of biosensor applications is the evaluation of bioavailable amounts of heavy metals in complex environmental matrices for risk assessment purposes, the constructed sensor bacteria were used to analyse bioavailable Hg, Cd and Zn from contaminated soil samples. All used sensor bacteria (Gram-negative and –positive strains and containing different metal-response elements) responded similarly to the metals in soil-water extracts demonstrating that for the evaluation of bioavailable metals in aqueous samples any type of bacterial metal sensor could be used. Interestingly, for all the sensor bacteria the bioavailable fraction of Cd in soil suspension assay (direct contact between the bacteria and soil particles) was about 14-fold higher than in soil-water extract indicating that a fraction of particle-associated Cd was also bioavailable to these bacteria. About 14-fold higher bioavailability of Hg in soil suspension assay compared with that in soil-water extract was also measured with Gram-negative sensor bacteria. However, for Gram-positive cells the bioavailability of Hg was similar in soil-water suspension and extract showing that the bioavailability of heavy metals to different bacterial groups may differ. Thus, for bioavailability studies of heavy metals in soils Gram-positive and Gram-negative sensor strains should be used in parallel.

The searchable database where the sensor bacteria constructed in the National Institute of Chemical Physics and Biophysics can be found is available upon registration in the web: <http://kbfi-databases.eu/ecotox/>.

Tributyltin Modulates Osteoblast Differentiation

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Organotins, such as tributyltin (TBT), have been widely used in agriculture and industry as antifoulants, wood preservatives and biocides. They are ubiquitous, persistent organic pollutants, which have many toxic effects, including endocrine-disrupting effects. However, their effects on bone formation are poorly known. Therefore, in this study, we utilized differentiation of bone marrow stem cells to osteoblasts as a model system to study the effects of TBT on bone formation. Stem cells were isolated from rat and mouse bone marrow, and differentiated to bone forming osteoblasts. Cells were exposed to 10^{-10} – 10^{-8} M TBT and samples were collected for analyses 2-13 days after starting the exposure. Cell proliferation was first measured in order to verify that TBT doses used did not affect the viability of cells. mRNA levels of the differentiation markers alkaline phosphatase, expressed at matrix maturation, and osteocalcin, expressed in the onset of mineralization, were determined using quantitative RT-PCR. TBT significantly and dose-dependently decreased the expression of alkaline phosphatase in both rat and mouse cells. In rat cells, this inhibitory effect was seen even with the lowest concentration of TBT (10^{-10} M). Also the activity of alkaline phosphatase was significantly inhibited in rat cells. The mRNA levels of osteocalcin, however, were decreased only in rat cells after treatment with the highest dose of TBT (10^{-8} M). The results indicate that differentiating osteoblasts are sensitive to TBT-exposure, and that this model system is useful to study effects of chemicals on bone formation.

Biotransformation of pharmaceuticals by aerobic and anaerobic microbes

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Pharmaceuticals used by human are mainly headed into sewage. Removal in sewage treatment plant (STP) has been found to be incomplete and pharmaceuticals are widely detected in surface waters (Ternes 1998). Microbial transformation in STP as well as in the aquatic nature is crucial in determining their fate and ecotoxicity. Certain pharmaceuticals are known to biotransform effectively (e.g. ketoprofen) whereas others are recalcitrant (e.g. carbamazepine) (Carballa et al. 2007). Overall, however, knowledge on microbial modifications is scarce, in particular under hypoxic and anoxic conditions.

The aim of this study was to investigate both aerobic and anaerobic biotransformation of three pharmaceuticals: anti-inflammatory drugs diclofenac and naproxen, and β -blocker bisoprolol. Aerobic biotransformation was evaluated by decay rate and manometrically, by measuring oxygen consumption. Anaerobic biotransformation was evaluated with the aid of methane production and concentration analysis.

In aerobic conditions diclofenac did not decline during the 75 days' incubation with or without additional carbon source, this conclusion supported both by the concentration and oxygen measurements. Bisoprolol concentration diminished to about half during 75 days, without difference with and without additional carbon source. Oxygen consumption suggested that this transformation was oxygen-demanding. Naproxen biotransformed quickly, and after 21 days it was undetectable from the experiments.

In anaerobic incubations, diclofenac and bisoprolol concentration declined about 25%. However, concentration of diclofenac in sterile treatments also declined by 23%, i.e. the transformation was abiotic. After 161 days, only trace amounts of naproxen were detected: over 97% of it was biotransformed. There was no difference in methane evolution between control, diclofenac, naproxen and bisoprolol treatments.

Results from the both aerobic and anaerobic experiments indicated, that naproxen is the most readily biotransformed and that diclofenac is the most recalcitrant of the studied pharmaceuticals. Transformation of naproxen and bisoprolol was faster under aerobic than anaerobic conditions.

Carballa, M, Omil, F., Ternes, T. and Lema, J.M. 2007. Fate of pharmaceuticals and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Res.* 41:2139-2150.

Ternes, T.A. 1998. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res.* 32:3245-3260.

Photoinduced lethal and sublethal toxicity of polyaromatic hydrocarbons to eleutheroembryos of whitefish (*Coregonus lavaretus*) – outdoor experiments

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Ultraviolet radiation (UVR) is directly or indirectly (phototoxicity) harmful to aquatic organisms, e.g. by causing mortality and neurobehavioral disorders (e.g. spiral swimming). Retene (7-isopropyl-1-methyl-phenanthrene) is produced from dehydroabiatic acid, a component present in wood resins and pulp mill effluents, by anaerobic microbial processes. It has been found at few micrograms to over one milligram per liter dry weight concentrations in pulp mill sludges and sediments downstream of wastewater discharges. It was found that retene is dissolved from sediment and is bioavailable to aquatic organisms. Retene is not necessarily acutely lethal under visible light conditions but it can be phototoxic to aquatic organisms due to UVR, e.g. fish embryos. Pyrene (benzo-phenanthrene) is generally considered to be representative model of many other PAHs. It has been found to be toxic to a number of aquatic organisms, including microbes, plants, invertebrates, and vertebrates. Pyrene is strongly phototoxic in the presence of UVR.

The aim of this research was to study the phototoxic effects of retene (RET) and pyrene (PYR) to the post-hatched embryos of whitefish. The study was done outdoors to assure full realism of UVR as a part of solar radiation.

The experiments were done in Jyväskylä, Finland, in May 2008. Post-hatched, less than one day old whitefish (*Coregonus lavaretus*), called eleutheroembryos, were used for experiments. Forty animals were placed in one-liter Pyrex bowl with the four centimeter depth of water. Whitefish were exposed to PAHs as nominal concentrations 1, 3.2 and 10 µg/l for RET and, equimolally, to 0.87, 2.9 and 8.7 µg/l for PYR. PAHs were first dissolved in DMSO (stock solutions one mg/ml). Half of the water was changed daily.

Fish were exposed to substances for 72 hours in an outdoors setting. Experiments were replicated three times. The photoperiod was 16 h light and 8 h dark. Bowls were moved outdoors once a day, and the incidental sunlight was let to radiate on two consecutive days, starting 24 h and 48 h after the beginning of the exposures to chemicals, three hours each time. Also dark control exposure was made outdoors. Radiation results were obtained from the lake Päijänne research platform 2008, the average radiation amount per day per total exposure time was 527 W/m². After 72 hours, the mortality was monitored and the live animals were sampled a whole for later biochemical analyses, sublethal studies. Glutathione reductase activity was measured.

The mortality between retene exposures and controls did not differ from each others. Pyrene was extremely phototoxic to whitefish in all concentrations. The glutathione reductase activity was increasing due to concentration in pyrene light and dark exposures. There were no changes between concentrations in retene dark exposures but there was 15 % less glutathione reductase activity in retene 10 µg/l light exposure when comparing to the control. In conclusion, in the concentrations of pyrene used to be lethally phototoxic to whitefish eleutheroembryos. Results also show that both pyrene and retene are sublethally phototoxic to whitefish eleutheroembryos.

Culturing zebrafish for post hatch embryonic and reproductive toxicity assays

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The zebrafish (*Danio rerio*) has emerged as a practical and sensitive whole-animal vertebrate model for toxicological research; appropriately termed the “aquatic mouse”. Besides favourable characteristics for husbandry including size, adaptability, high fecundity and short generation interval, zebrafish have a relatively short period of development. Optically as well, transparent embryonic stages facilitate ecotoxicological investigations during embryogenesis. Statutory requirements of animal toxicity testing to replace testing standard subjects (mouse) and reduce live animal usage could be addressed with employment of embryonic assays with post-hatch zebrafish stages. Embryonic assays are clearly more realistic than *in vitro* systems in studying cell differentiation and morphogenetic responses to chemical exposures. The combination of manipulated breeding with high fecundity contributes to reproductive assays while studying toxicological endpoints, i.e. spawning success, fecundity, fertility, and endocrinic status among others.

The maintenance of zebrafish was carried out in a semi-static system that involved manipulation of ambient conditions in the holding facility, and management of fish health and nutritional status. Ambient conditions were managed to ensure a relatively pathogen-free environment with temperature and photoperiod regulation to 24 °C ±1 and 14 h light + 10 h dark, respectively. Pathogen management in the holding facility included regular screening for symptomatic fish for physical and behavioural anomalies. High nutrition diet included brine shrimp in flake as well as freeze dried mixtures. The optimal water quality of the animal holding tanks was managed through routine checks and adjustments. Fish tanks were regularly monitored for suitable ammonia levels (< 0.02 mg L⁻¹). Zebrafish being batch spawners responded well to breeding stimuli such as minor increase in temperature, spawning setup design and spawning water quality. Successful spawning was managed by the combination of an effective sex ratio (three females and four males) as well as the growth conditions of males in the breeding pairs, essential for spawning success. The variation in water quality, such as hardness, affected the spawning potential of zebrafish. The effect of increased water quality on reproductive success resulted in higher fecundity and fertility of spawning episodes with system water adjusted for optimal general (21 °dH) and carbonate (10 °dH) hardness. Embryos (posthatch; 90 dpf) were maintained in a semi-static system with regular renewal of preaerated water. In comparison to soft water, larval rearing water with added salts resulted in higher larval survival, ultimately contributing to normal embryonic development.

There is now nearly four-month experience in our laboratory to settle and maintain stock culture of zebrafish. Next, in this process, focus is laid on maintenance and rearing of F₁ generation of the zebrafish stock and their use in research involving endocrinic and reproductive toxicity studies. Different developmental stages (embryos, juveniles, and adults) of zebrafish would be exposed to environmental chemicals to assess their estrogenic responses. Additionally, embryonic assays would be carried out in sediment toxicity assessments.

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