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**REPORT OF THE OECD/IPCS WORKSHOP ON TOXICOGENOMICS**

**Kyoto, 13-15 October 2004**

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**REPORT OF THE OECD/IPCS WORKSHOP ON TOXICOGENOMICS**

**Environment Directorate  
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT**

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## Final Report of the OECD/IPCS Workshop on Toxicogenomics

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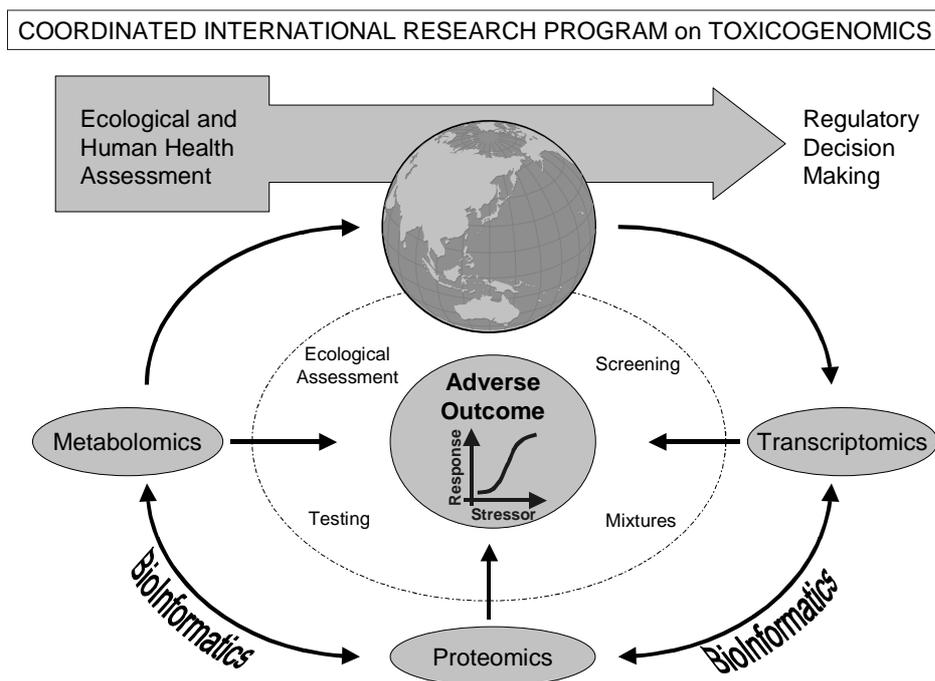
## EXECUTIVE SUMMARY

Molecular-based approaches, such as transcriptomics, proteomics and metabolomics for studying the impact of chemicals on human and wildlife populations will have an important role in hazard and risk assessment. As such, OECD and the International Programme on Chemical Safety (IPCS) co-operatively organised two workshops to explore the potential regulatory applications of toxicogenomics. This report summarises the outcome from the second workshop held in Kyoto, Japan on 13-15 October 2004, with a focus on environmental effects.

Key recommendations from the OECD/IPCS Workshop were:

1. Concerted efforts are required to conduct comprehensive (eco)toxicogenomic studies to differentiate compensatory and adaptive responses from adverse toxicological outcomes.
2. Concerted efforts are required to determine which “omics” technological platforms hold the most promise for studying diverse taxa and for cross-taxa comparisons, and to improve and develop platforms for use across species and taxa.
3. Quality assurance and quality control of all sequencing data and the individual “omics” techniques need to be coupled to good data management and good annotation. All sequencing data and clones should be publicly available.
4. A strategic plan is required to realise the transfer of genomic-based techniques from fundamental research to a potential regulatory use.
5. An OECD/IPCS Expert Group to implement these recommendations and coordinate future actions should be established. Responsibilities of such a group would include the development of an OECD initial policy for the evaluation and use of “omics” data for regulatory purposes.

In view of the above recommendations a primary responsibility of the Expert Group would be to initiate the development of a co-ordinated international research programme on toxicogenomics (Figure 1). The lack of national and international coordinated efforts in environmental genomics is in danger of delaying and compromising the integration of genomic techniques into ecotoxicology and their use in ecological and human health risk assessment.



A coordinated international programme would focus on efforts to address ecological and human health concerns such as:

- understanding how and why species and subgroups differ in sensitivity and response to chemical stress, and create a stronger scientific foundation for the use of safety factors. This will allow effective policies to be developed in order to protect endangered and important species.
- assessing the effects of chemical mixtures and combination of stressors. Previously, appropriate methods have been either non-existent, or had important limitations.
- reducing uncertainty in assessment of ecological conditions
- providing training in molecular-based techniques, data requirements and bioinformatics relating to ecotoxicogenomics. These training opportunities should be made available to risk assessors, similarly the scientific community need training and greater appreciation of the regulatory requirements in hazard and risk assessment.
- offering long-term possibilities to reduce, refine and replace costly animal intensive methods for chemical screening and testing

For these reasons, it is important that these new tools are evaluated and implemented for chemical risk and hazard assessment.

## 1. BACKGROUND AND OBJECTIVES

### *Background*

1. Toxicogenomics, in a broader sense, is defined as a study of the response of a genome to hazardous substances, using:

- Genomic-scale mRNA expression (transcriptomics);
- Cell and tissue wide protein expression (proteomics); and
- Cell and tissue wide metabolite profiling (metabolomics)

in combination with bioinformatic methods and conventional toxicology. (In a narrower sense, it refers to the use of transcriptomics.) In relation to chemical hazard/risk assessment, this emerging science could provide tools for improving the understanding of mechanisms of toxicity, identification of biomarkers of toxicity and exposure, and possibly alternative methods for chemical screening, hazard identification and characterisation.

2. The 33<sup>rd</sup> Joint Meeting in February 2002, at its half-day Special Session on Toxicogenomics, decided that an exploratory workshop should be held to develop a strategy concerning the future application of toxicogenomics in the Test Guidelines, Existing Chemicals, Pesticides and Biocides Programmes.

3. The Secretariat agreed with the International Programme on Chemical Safety (IPCS) to have twin workshops, one focusing on human health aspects with IPCS in the lead, and the other focusing on environmental aspects with OECD in the lead. The report of the first of these twin Workshops, held on 17-19 November 2003 in Berlin, will be published from IPCS. This document is the report from the second workshop.

### *Objectives*

4. The objectives of the Workshop were:

- to review the current status of the science relevant to the environmental effects;
- to identify the possible application of methods based on toxicogenomics in regulatory hazard assessment;
- to determine the current limitations to the use of toxicogenomics in regulatory assessment, and develop a plan to overcome such limitations.
- to identify the need for future OECD activities with regard to the use of these methods in Test Guidelines, New and Existing Chemicals, Pesticides and Biocides Programmes.

5. The Workshop focused on the environmental side of the science, while identifying the commonalities and differences between the human health and environmental sides.

***Organisation of the Workshop***

6. The Workshop was organised by the OECD in collaboration with IPCS, and hosted by the Ministry of the Environment of Japan.

7. A Steering Group for the Workshop was established based on the nominations from the OECD member countries, IPCS and Industry, Environmental NGOs. Through several teleconferences, the Steering Group developed the outline and the agenda for the Workshop.

8. In preparation for the Workshop, a questionnaire survey was conducted to collect information on the toxicogenomic tools existing or being developed in member countries. Japan, Korea, Sweden, Switzerland, the United States provided information on what “omics” technologies (microarrays, proteomics and metabolomics” have been or are being applied to what species on what chemicals. Overall, 54 cases were reported. A summary table of the questionnaire results were made available before the Workshop, but it was recognised that the information was incomplete and outdated. Follow-up to this questionnaire survey will be considered.

## 2. OVERVIEW OF THE WORKSHOP

9. The Workshop was held on 13-15 October 2004 in Kyoto, Japan, back to back with the Toxicogenomic International Forum 2004 on 12-13 October to benefit from the interaction between participants in both meetings. Fifty six participants nominated by member countries, IPCS and industry attended. The list of participants is attached as Annex 1. Mr. Taisen Iguchi of Japan chaired the plenary sessions of the Workshop.

10. The Workshop consisted of a series of plenary presentations and discussions in four breakout groups, with plenary feed-back and concluding sessions. Its agenda is attached as Annex 2.

11. The Workshop started with the welcoming remarks by Ms. Kazuko Kamiya (Japan). This was followed by an explanation by Mr. Eisaku Toda (OECD) about the background and objectives of the Workshop, in the OECD context, and remarks by Mr. Tim Meredith (IPCS) about the importance of the work on toxicogenomics in the context of SAICM and WHO policy.

12. The plenary presentations started with introductory speeches about the science and its possible use in regulatory framework. Outcomes from related workshops in the past – the IPCS/OECD Workshop in November 2003, ECVAM/ICCVAM Workshop in December 2003, and SETAC/SOT Workshop in July 2004, were also presented. These were followed by five presentations on the topics for the Workshop, and nine presentations on case studies in (eco)toxicogenomics. The list of speakers and presentation titles are found in Annex 2. The abstracts of the presentations are attached as Annex 3.

13. Then the participants were divided into the following four breakout groups:

- Group 1: biological issues (Chair: Mr. Sean Kennedy; Co-chair: Mr. Jun Kanno; Rapporteur: Mr. Joakim Larsson)
- Group 2: technological issues (Chair: Mr. Jason Snape)
- Group 3: regulatory issues (Chair: Mr. Maurice Zeeman; Co-chairs: Ms. Raffaella Corvi and Mr. Tohru Inoue)
- Group 4: bioinformatics issues (Co-chairs: Mr. George Douglas and Mr. Kazumi Kawahara; Rapporteurs: Ms. Susanna-Assunta Sansone and Mr. Weida Tong).

The list of breakout group members and questions for the discussion is found in Annex 2. The reports from these breakout groups are attached as Annexes 4-1 to 4-4.

14. The outcomes from the breakout discussion were reported to plenary sessions. Time for the concluding plenary session was not sufficient for agreeing on the consolidated overall conclusions and recommendations. Therefore, these were finalised by circulating written comments. The conclusions and recommendations explained in the following two chapters were agreed by the Workshop participants.

### 3. OVERALL CONCLUSIONS

15. This chapter summarises the conclusions from the Workshop, with regard to the questions listed as the objectives of the Workshop (See chapter 1, paragraph 4).

#### *Current status of the science relevant to the environmental effects*

##### *Available molecular-based tools*

16. A number of different molecular-based tools are available for studying changes in transcript, protein and metabolite composition (see Table 1 in Annex 4-2). It must be emphasised that each of the individual techniques are at different stages of development and as such their potential integration into ecotoxicological risk assessment will be realised at different points in time.

17. Present array-based approaches to studying transcripts seem closer to being applied to regulatory ecotoxicological issues than proteomics and metabolomics. Further analysis of individual tools is found in Annex 4-2.

##### *Available bioinformatics tools*

18. Due to the large amount of data that are generated from toxicogenomic studies, it is imperative to develop bioinformatics tools to handle and analyse the data. Currently available tools for ecotoxicogenomic studies are limited due to factors such as the limitation in genome sequences and annotations. Techniques such as the use of ESTs, comparative hybridization and SAGE would provide bridging capabilities to compare across species.

19. Within the MGED Society the toxicology and environmental communities have initiated the development of a reporting structure for describing “omics” based experiments, assisting in the identification of technical measures correlated with data interpretability (MIAME/Tox and MIAME/Env). Collaborative infrastructures in support of ecotox data requirements are being developed, such as Maxd (NERC), TIS (NCTR-FDA), CEBS (NIEHS-NCT), ArrayExpress (EMBL-EBI) (See Table 2 in Annex 4-4).

##### *Human and ecological linkage*

20. Human and ecological risk assessments are both about assessing risks to organisms, founded on biology. However, for the most parts, human risk assessment is based on the health of individuals, whereas in ecological risk assessment the focus is on traits that may have consequences for populations and species health. Also, ecological risk analysis covers the effects on many species. Because of these differences, ecological risk analyses are faced with more pronounced problem with extrapolations between physiologically and taxonomically diverse species.

21. With regard to toxicogenomic tools and data, the following differences were identified:

- Different type of experiment designs (e.g. use of controls)

- Larger number and greater variety of experimental parameters

22. In spite of these differences, the Workshop identified opportunities that toxicogenomic methods could provide for developing interconnections between the human and ecological risk paradigm, as explained in paragraph 29.

***Possible application of toxicogenomics-based methods in regulatory hazard assessment***

23. Toxicogenomic technologies have unique opportunities to address ecological and human health concerns, such as:

- Offering possibilities to reduce, refine and replace costly animal intensive methods for chemical screening and testing
- Understanding how and why species and subgroups differ in sensitivity and response to chemical stress.
- Assessing the effects of chemical mixtures and combination of stressors.
- Reducing uncertainty in assessment of ecological conditions

*Alternatives for animal testing*

24. Currently, toxicogenomic approaches are recognised as not yet developed enough for risk assessment decisions or replacement of existing approaches. However, they can be used as supportive evidence on a case by case basis. More promising uses of these approaches are to refine hazard identification and risk assessment in the following ways:

- Elucidate modes of action, support established toxic endpoints, and help to identify endpoints with improved predictive capability.
- Add confidence to the derivation of no observed adverse effect levels.
- Prioritising chemicals on a structural analogue basis or chemical class.

25. Furthermore, large scale analyses of genomic responses may be useful also for chemicals with largely known or suspected mechanisms of action, such as pharmaceuticals. For field studies, comprehensive profiling may allow diagnosis of the cause of adverse effects. Furthermore, multiple mechanisms of action may be elucidated, which will lead to the development of improved methods for hazard assessment.

26. In the long-term, it is likely that regulatory agencies will be faced with intellectual property rights issues in relation to the use of genomic tools. To the extent possible it will not be recommended to use patented methods as a regulatory requirement.

*Interspecies extrapolation*

27. Improved understanding of inter- and intra-species differences will allow effective policies to be developed in order to protect endangered and important species. It will also create a stronger scientific foundation for the use of safety factors. Defined gene expression profiling may help interspecies extrapolation even without dosage information (independent of species sensitivity).

28. There is value in identifying common pathways, allowing extrapolation between species regarding the mechanism-of-action of different chemicals. Developing a list of genes having a conserved function and expression profiles (phenotype) across species is one way of assisting in the comparisons of effect across different organisms used in ecotox studies. In addition, metabolomics offers great potential for investigating common pathways across multiple ecotox species since it does not require prior sequence information.

29. Interspecies extrapolations also provide opportunities for developing interconnections between the human and ecological risk paradigm. Thus, synergy should be promoted between the toxicology and ecotoxicology communities, including a dialogue between scientists, regulators and bioinformaticians on an international scale.

#### *Assessment of mixtures*

30. Toxicogenomic tools can help assessing the effects of exposure to mixtures by elucidating additive, synergistic or antagonistic effects.

#### *Assessment of ecological conditions*

31. There is a use of genomic techniques to assess effects of chemical stress on species composition (such as microbes, small invertebrates and algae) in environmental or laboratory samples (such as soil and water). Assays based on genomic DNA sequences specific for individual species or higher taxa will therefore be useful for ecological assessment. Similarly, transcript analyses for whole communities of microbes or other small organisms may provide further information on the effects of chemical stress on the ecosystem functioning. This approach requires that particularly conserved genes are studied.

#### ***Current limitations to the use of toxicogenomics in regulatory assessment and ways to overcome such limitations***

32. Although there has been significant progress in each of the “omics” techniques over the past few years, there are still a number of important biological, technical, and bioinformatic issues that need to be addressed before these tools are robust enough to apply them with confidence in regulatory decision making.

33. In order for each molecular-based parameter to be considered robust enough for regulatory use, a weight of evidence approach coupled to established biological endpoints is required. Table 1 in Annex 4-2 lists a number of transcriptomic, proteomic and metabolomic approaches together with some important and relevant technological and regulatory considerations. For routine use in regulatory assessment the following practical considerations should be made when identifying suitable “omics” platforms:

- A cross-species potential is desirable for array and non-array based systems.
- It must be quantitative and have a diagnostic potential.
- Good QA/QC is needed.
- The end point must be platform independent and (semi)quantitative.
- A standardised system for data capture, data processing and reporting.
- A potential for non-invasive or non-destructive sample collection is desirable.

- It should be scaleable from sample collection through to biological information.
- It should be cost effective (set-up and maintenance costs).
- The system should provide data that is fit for the intended purpose.

34. It is platforms with these essential criteria, particularly those with cross-species potential that should be prioritised for regulatory use. However, it must be recognised that the other 'research' platforms will provide important and relevant information.

*Need for international co-ordination and funding*

35. The Workshop recognised the lack of national and international coordinated efforts in environmental genomics that may seriously delay the integration genomic techniques into ecotoxicology and its use in environmental risk assessment.

36. Also, the lack of discrete or distinct funding available for ecotoxicogenomics was identified as a major barrier. Current funding is heavily biased towards molecular aspects of mammalian toxicology. Without a substantial increase in funds dedicated to ecotoxicogenomics, it will not be possible to make interconnections between ecotoxicology and mammalian toxicology in the immediate future.

*Recommendations for future OECD activities*

37. The recommendations for further activities are explained in the next chapter.

#### 4. OVERALL RECOMMENDATIONS

38. Key recommendations from the OECD/IPCS Workshop were:

- Concerted efforts to conduct comprehensive (eco)toxicogenomic studies to differentiate compensatory and adaptive responses from adverse toxicological outcomes are required.
- Concerted efforts are required to determine which “omics” technological platforms hold the most promise for studying diverse taxa and for cross-taxa comparisons, and to improve and develop platforms for use across species and taxa.
- Quality assurance and quality control of all sequencing data and the individual “omics” techniques need to be coupled to good data management and good annotation. All sequencing data and clones should be publicly available.
- A strategic plan is required to realise the transfer of genomic-based techniques from fundamental research to a potential regulatory use.
- An OECD/IPCS Expert Group to implement these recommendations and coordinate future actions should be established. Responsibilities of such a task force/ working group would include the development of an OECD initial policy for the evaluation and use of “omics” data for regulatory purposes.

These recommendations are further explained below.

##### *Differentiating compensatory/adaptive responses from adverse effects*

39. Not all changes at molecular levels are adverse. It is important to understand what is inherent biological variability (e.g. variability in control animals under laboratory or field circumstances). Compensatory changes have the potential to be useful in hazard identification. In short-term they may not be related to toxicity, but may in some cases give rise to adverse effects in the long-term (e.g. non-genotoxic carcinogens which might first lead to a loss of cell communication) and metabolic changes.

##### *Developing technological platforms for diverse taxa*

40. Toxicogenomic and bioinformatic approaches (methodologies and tools) should be developed to predict population effects (hazard and risk).

41. Priorities for sequencing and developing genomic tools depend on the questions asked, e.g.:

- Understanding core biological processes
- Performing ecological risk assessment
- Prediction of human health

- Field monitoring
- Assessing the health of wild species.

42. Initial efforts should be centred on a few species representative of the ecological complexity. Priority should be given to species currently used in the ecotox regulatory context (see Table 2 in Annex 4-3).

43. Apart from sequencing, creating EST databases may be very useful and possibly a more cost or time efficient approach. In addition, sequencing some representative species across the evolutionary tree, which also may be widely distributed and useful for field studies would be beneficial. Results from any cross-species arrays need to be evaluated carefully (as should homologous assays).

44. Proteomics need to be anchored in genomics. Available genome or EST databases therefore greatly facilitate the identification of regulated proteins.

#### ***Data quality and management***

45. If toxicogenomic data are required for regulatory assessment, strict QA/QC including GLP compliance would become necessary. In a shorter term, in the consideration of case-by-case based data, confidence will be supported by improved harmonized quality controls, and link to quality-assured data on known mechanisms and endpoints.

46. Stable and adequate funding at national and international levels should be provided for the following:

- Development of international standards for data communication format and data annotations (terminology, ontology) within ecotoxicogenomics technologies.
- Development of an integrated, modular data model combining “omics” with toxicological endpoints and chemical structures.
- Establishment of the infrastructure for data management (reporting, archiving, curation, user support and querying) and analysis. (Expanding suitable infrastructure in support of ecotox data requirements to avoid duplication of effort.) Database and analysis tools should be transparent. Data schema should be thoroughly documented and analysis methods should be peer-reviewed

47. Training should be provided to risk assessors in data requirements and bioinformatics issues relating to ecotoxicogenomics.

#### ***Bridging science and regulatory use***

##### *Exploring regulatory use*

48. A road map is required on how to move from research applications of these tools to their regulatory use. This road map needs to include strategies for moving from global studies to more targeted studies, training issues, linking molecular responses to biological endpoints, and data management and data analysis. These issues need to be addressed by an appropriate technical expert group with member of the regulatory, industrial and academic community.

49. Any strategies to move from global to targeted “omics” studies need case studies based on real data and should not be based purely on theory.

50. One potential barrier could be the initial capital costs associated with setting up “omics”-based techniques. Setting up national centres of excellence that serve both a research and regulatory purpose may reduce these costs. There is also a need for strong technical expertise; this may also be addressed through establishing technical centres.

51. There are some additional requirements for instrument manufacturers. These include a common import/ export formats for raw data and metadata. This will facilitate subsequent data analysis and integration with other relevant data sets.

52. At some point, validation is needed if tests are required by regulatory authorities.

#### *Developing policies on the use of toxicogenomic methods*

53. Present available policies [e.g. EPA (2004) Potential Implication of Genomics for Regulatory and Risk Assessment Applications at EPA – Draft] are based on a case by case basis evaluation. OECD and its member countries should consider development of initial guidance on the use of “omics” methods in chemical assessment.

#### *Next steps*

54. An OECD/IPCS Expert Group to implement these recommendations and coordinate future actions should be established. The Expert Group should integrate the conclusions and recommendations of both reports and divide next tasks.

**ANNEX 1: LIST OF PARTICIPANTS**

**Canada / Canada**

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- Ms. Caren HELBING** *Assistant Professor  
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- Mr. Sean KENNEDY** *National Wildlife Research Centre  
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**Germany / Allemagne**

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**Japan / Japon**

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- Mr. Ryolichi KIYAMA** *Principal Investigator & Group Leader  
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**Mr. Peter KILLE** *Cardiff School of Biosciences*

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*Director, Center for Toxicoinformatics  
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National Center for Toxicological Research  
US Food and Drug Administration*

**Mr. Maurice ZEEMAN**

*US National Coordinator for OECD Test  
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**EC / CE**

**Ms. Raffaella CORVI**

*DG Joint Research Center  
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**Business and Industry Advisory Committee (BIAC) / Comité consultatif économique et industriel (BIAC)**

**Mr. Robert HOKE**

*DuPont Haskell Laboratory*

**World Health Organization (WHO) / Organisation mondiale de la santé (OMS)**

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**Ms. Lesley ONYON**

*International Programme on Chemical Safety  
World Health Organization*

**OECD / OCDE**

**Mr. Eisaku TODA**

*ENVIRONMENT DIRECTORATE  
OECD*

## ANNEX 2: WORKSHOP AGENDA

### OECD/IPCS Workshop on Toxicogenomics

**Kyoto International Conference Hall  
Takaragaike, Sakyo-ku, Kyoto 606-0001, Japan  
13-15 October 2004**

#### DAY 1 (Wednesday 13<sup>th</sup> October)

##### **13h00 1. Welcome Introduction**

After the welcoming remarks by **Kazuko Kamiya** (Japan), **Eisaku Toda** (OECD) will explain the background, objectives, format and expected outcomes of the Workshop, from the perspectives of the OECD Environment, Health and Safety Programme. **Tim Meredith** (IPCS) will welcome the participants and mention the linkage between OECD and IPCS.

##### **13h20 2. Plenary presentations – setting the scene –**

*Five presentations, each maximum 20 minute, including questions and answers.*

(1) Ecotoxicogenomics - overview of the science.

**Jason Snape** (United Kingdom) will review the state of the art of this fast growing science. The outcome from the questionnaire will be referred to.

(2) Ecotoxicology - regulatory assessment of chemicals (& toxicogenomics).

**Maurice Zeeman** (United States) will explain the principles and approaches in assessing environmental effects/risks of chemicals. The topic will include species selection, effects on population and ecosystem, use of dose-response relationship, safety/uncertainty factors, etc.

(3) Lessons from mammalian toxicogenomics.

**Lesley Onyon** (IPCS) will present the overview of the use of genomics in human health effects/risk assessment, referring to the outcome from the IPCS Workshop in November 2003.

(4) Validation issues.

**Raffaella Corvi** (European Commission) will explain the crucial role of validation for toxicogenomics-based tests and will present the outcome of the ECVAM/ICCVAM Workshop on “Principles for validation of toxicogenomics-based test systems”, December 2003.

(5) Interspecies extrapolation.

**Bill Benson** (United States) will present the outcome from the SETAC/SOT Workshop on Emerging Molecular and Computational Approaches for Cross-Species Extrapolations, July 2004.

##### **15h00 Coffee break**

**15h30 3. Plenary presentations – introduction to the issues for the workshop -**

*Five presentations, each maximum 20 minute, including questions and answers.*

(1) Commonalities and differences between ecotoxicogenomics and mammalian toxicogenomics.

**Taisen Iguchi** will present some specific considerations in ecotoxicogenomics, such as the use of control animals, bioinformatics structures, data exchange, interspecies variety, possibility for testing environmental samples, etc

(2) How genomics could bring about revolution (or evolution) to ecotoxicology.

**Jason Snape** (United Kingdom) will explore different types of possible uses of genomic methods for chemical assessment. This will include: replacement and refinement of ecotoxicity tests, prioritisation/screening/classification, identifying chemical categories with similar ecotoxicological properties, identifying unknown mode of action, reducing uncertainty in interspecies extrapolation, testing environmental samples, etc.

(3) Functional Genomics

**Kenneth Ramos** (US) will present issues related to functional genomics, transcription factor interactions and nephrogenesis.

(4) Open Methods of Gene Expression - Applications to Ecotoxicology

**Sean Kennedy** (Canada) will present the results of some of his recent research that has used Serial Analysis of Gene Expression (SAGE) and Differential Display PCR for determining the effects of environmental contaminants on wildlife.

(5) (Eco)Toxicogenomics Data Management - Standards and implementations

**Susanna Sansone** (UK) will review community vetted- standardization activities and related, standard compliant database efforts . Focus would be on the Microarray Gene Expression Data (MGED) Society and its Reporting Structure for Biological Investigation (RSBI) Working Groups, acting as a “single point of focus” for Toxicogenomics, Nutrigenomics and Environmental genomics communities.

**17h30 Adjourn for the day**

**18h00 Welcome reception, at Banquet Room “Swan”**

**DAY 2 (Thursday 14<sup>th</sup> October)****09h00 4. Plenary presentations - case studies -**

*Six presentations, each maximum 15 minute, including questions and answers.*

The following speakers will present their experience in applying genomic methods to ecotoxicology, for different species, endpoints, exposure regimes and objectives, including issues related to bioinformatics.

- **Hajime Watanabe** (Japan): Toxicogenomic study with mouse, Medaka and Daphnia
- **Masaru Matsuda** (Japan): Development of Medaka microarray
- **Caren Helbing** (Canada): Ecotoxicogenomics work with amphibians
- **Peter Kille** (United Kingdom): Population consequences of molecular variation in terrestrial invertebrates
- **Richard Sibly** (United Kingdom): Molecular stress responses in Daphnia magna
- **Kevin Chipman** (United Kingdom): Deriving biomarkers from (eco)toxicogenomics

**10h30 Coffee break****11h00 4. Plenary presentations - case studies -**

*Continue with three presentations, each maximum 15 minute, including questions and answers.*

- **Mark Viant** (United Kingdom): Environmental Metabolomics
- **Jun Kanno** (Japan): MHLW Mouse Toxicogenomics Project for Chemical Safety
- **Weida Tong** (United States): Bioinformatic methods for Toxicogenomics

**12h00 5. Introduction to breakout sessions**

The secretariat will provide introduction to the breakout discussions. After the afternoon session, the Workshop will break into the following four groups:

- Breakout Group 1: Biological
- Breakout Group 2: Technical
- Breakout Group 3: Regulatory
- Breakout Group 4: Bioinformatics

Questions to be addressed at these breakout sessions are attached at the end of this agenda. Membership and chairpersons for these groups will be announced later.

**12h30 Lunch Break****14h00 6. Breakout Session 1**

Groups may take a coffee break as appropriate.

**17h00 7. Plenary feedback from Breakout Session 1****17h30 Adjourn for the day**

**DAY 3 (Friday 14<sup>th</sup> October)**

**09h00 8. Breakout session 2**

Groups may take a coffee break as appropriate.

**13h00 Lunch Break**

**14h00 9. Plenary feedback from Breakout Session 2 (Meeting Room C-1)**

**15h30 Coffee break**

**16h00 10. Overall conclusions and recommendations**

**17h30 Close**

## Questions for breakout groups

Each of the four breakout groups would approach the questions for the Workshop from a different perspective. While there are some questions that are unique to some groups, many of the questions distributed among the groups are the same, providing the opportunity for expression of ideas from different perspectives. Outcome from the breakout discussion will be reported back to plenary for exchange of different perspectives.

### 1. Biological

- a. What are common issues that face human and ecological risk analysis
- b. How can integration of cross-species genomic analyses be used to address common issues and provide solutions?
- c. What types of genomic and/or proteomic data will be most useful to develop interconnections between the human and ecological risk paradigm?
- d. What organisms should receive the greatest priority for sequencing? Will the availability of comparative genomics maps for model species assist future sequencing efforts? Will we need to sequence multiple species? How will other methods assist in bridging the knowledge gap until sequence data becomes available?
- e. What organisms should receive the greatest priority for sequencing? Will the availability of comparative genomics maps for model species assist future sequencing efforts?
- f. In the evaluation of genomic responses, will it be possible to distinguish toxic response from compensatory or adaptive responses? If so, how?
- g. What regulatory decisions could genomic-based methods support, and how? (Replacing existing toxicity tests? As an additional endpoint? Reducing uncertainty in inter-species extrapolation? Supporting QSARs and categorisation of chemicals?) What validation approaches are appropriate for these regulatory uses?

### 2. Technical

- a. What are common issues that face human and ecological risk analysis?
- b. How can integration of cross-species genomic analyses be used to address common issues and provide solutions?
- c. What needs to be done to apply molecular and computational toxicological information to human and environmental risk assessment?
- d. What are the different technologies available for ecotoxicogenomic studies (e.g. DNA microarrays, SAGE)? What will be the greatest use of genomic technologies in ecotoxicology? Why?
- e. In the evaluation of genomic responses, will it be possible to distinguish toxic response from compensatory or adaptive responses? If so, how?
- f. Will we need to sequence multiple species? How will other methods assist in bridging the knowledge gap until sequence data becomes available?
- g. What organisms should receive the greatest priority for sequencing? Will the availability of comparative genomics maps for model species assist future sequencing efforts?
- h. What regulatory decisions could genomic-based methods support, and how? (Replacing existing toxicity tests? As an additional endpoint? Reducing uncertainty in inter-species extrapolation? Supporting QSARs and categorisation of chemicals?) What validation approaches are appropriate for these regulatory uses?
- i. What types of genomic and/or proteomic data will be most useful to develop interconnections between the human and ecological risk paradigm?

**3. Regulatory**

- a. From the standpoint of ecological and human risk assessment, what genomics technologies (e.g., gene arrays, proteomics, etc.) will be of greatest value? Why?
- b. What are common issues that face human and ecological risk analysis?
- c. What types of genomic and/or proteomic data will be most useful to develop interconnections between the human and ecological risk paradigm?
- d. How can regulators use genomic information if submitted?
- e. What regulatory decisions could genomic-based methods support, and how? (Replacing existing toxicity tests? As an additional endpoint? Reducing uncertainty in inter-species extrapolation? Supporting QSARs and categorisation of chemicals?) What validation approaches are appropriate for these regulatory uses?
- f. In the evaluation of genomic responses, will it be possible to distinguish toxic response from compensatory or adaptive responses? If so, how?
- g. How can regulators have confidence in the data from genomic-based methods?
- h. How can the transparency in the technology be warranted? What can manufacturers of genomic instruments do to improve confidence in the technology?
- i. How should issues of intellectual property right be taken into consideration in the regulatory use of genomic-based methods?

**4. Bioinformatics**

- a. Are ecotoxicogenomics data unique from other toxicogenomic data?
- b. Are the bioinformatics structures set up to handle data for mammals sufficient to address the needs of scientists working in ecotoxicogenomics? Is the MIAME/ENV scheme suitable for ecotoxicity?
- c. What informatic structures are required to facilitate comparative genetics and comparative genomics?
- d. What bioinformatics tools have you found valuable? Why?
- e. What needs to be done to apply molecular and computational toxicological information to human and environmental risk assessment?
- f. What types of genomic and/or proteomic data will be most useful to develop interconnections between the human and ecological risk paradigm? How should issues of intellectual property right be taken into consideration in the regulatory use of genomic-based methods?
- g. How should issues of intellectual property right be taken into consideration in the regulatory use of genomic-based methods?
- h. How can integration of cross-species genomic analyses be used to address common issues and provide solutions?

### Breakout Group membership

The following is the initial list of Breakout Group membership. Participants wishing to move to another group should inform the Secretariat (Eisaku Toda) or one of the relevant Steering Group members. Each group should elect two or three co-chairs.

		1 biological	2 technical	3 regulatory	4 bio- Informatics	Note
<b>George R. DOUGLAS</b>	CA				Chair	Steering Group
<b>Caren HELBING</b>	CA		x			
<b>Sean KENNEDY</b>	CA	Chair				Steering Group
<b>Marianne RAPPOLDER</b>	DE			x		
<b>Taisen IGUCHI</b>	JP					Plenary chair
<b>Tohru INOUE</b>	JP			Co-chair		Steering Group
<b>Kazuko KAMIYA</b>	JP					Secretariat
<b>Jun KANNO</b>	JP	Co-chair				Steering Group
<b>Minako TAKAMIYA</b>	JP	x				
<b>Yasunobu AOKI</b>	JP	x				
<b>Koji ARIZONO</b>	JP	x				
<b>Chisumi ETO</b>	JP			x		
<b>Hiroshi GOHDA</b>	JP		x			
<b>Fumi IRIE</b>	JP			x		
<b>Hisato IWATA</b>	JP	x				
<b>Kazumi KAWAHARA</b>	JP				Co-chair	Breakout chair
<b>Ryoichi KIYAMA</b>	JP		x			
<b>Tomonari MATSUDA</b>	JP	x				
<b>Saburo MATSUI</b>	JP			x		
<b>Masaru MATSUDA</b>	JP				x	
<b>Yuji OSHIMA</b>	JP		x			
<b>Tetsuo SATOH</b>	JP	x				
<b>Masao TANJI</b>	JP				x	
<b>Norihisa TATARAZAKO</b>	JP	Thursday			Friday	
<b>Nobuaki TOMINAGA</b>	JP				x	
<b>Osamu TOOI</b>	JP		x			
<b>Hajime WATANABE</b>	JP		x			
<b>Hirofumi YOKOTA</b>	JP	Thursday			Friday	
<b>Ho-II KANG</b>	KR			x		
<b>Chul-Woo LEE</b>	KR				x	
<b>Jae-Chun RYU</b>	KR	x				
<b>Young-Rok SEO</b>	KR		x			
<b>Przemyslaw FOCHTMAN</b>	PL			x		
<b>Joakim LARSSON</b>	SE	Rapporteur				
<b>Bettina HITZFELD</b>	CH			x		

<b>Kevin CHIPMAN</b>	UK			x		
<b>Peter KILLE</b>	UK	x				
<b>Susanna SANSONE</b>	UK				Rapporteur	
<b>Richard SIBLY</b>	UK	x				
<b>Jason SNAPE</b>	UK		Chair			Steering Group
<b>David SPURGEON</b>	UK		x			
<b>Mark VIANT</b>	UK		x			
<b>Kerry WALSH</b>	UK			x		
<b>William BENSON</b>	US	x				Steering Group
<b>Bruce BLUMBERG</b>	US		x			
<b>Sigmund DEGITZ</b>	US			x		
<b>Michael HEMMER</b>	US	x				
<b>Alex MERRICK</b>	US				x	
<b>Kenneth RAMOS</b>	US				x	
<b>Weida TONG</b>	US				Rapporteur	
<b>Maurice ZEEMAN</b>	US			Chair		
<b>Raffaella CORVI</b>	EC			Co-chair		Steering Group
<b>Robert HOKE</b>	BIAC			x		
<b>Tim MEREDITH</b>	IPCS					Secretariat
<b>Lesley ONYON</b>	IPCS		x			Secretariat
<b>Eisaku TODA</b>	OECD					Secretariat

### ANNEX 3: PRESENTATION ABSTRACTS

#### Ecotoxicology – Regulatory Assessment of Chemicals (& Toxicogenomics)

Maurice Zeeman, Ph.D., US-EPA, Office of Pollution Prevention & Toxics, Washington, DC, USA

There are many different national, regional, and international organizations that have been established for the regulatory assessment of a wide diversity of chemicals that are to be used in commerce &/or are found in the environment. The methods for the hazard/risk assessment of the potential, or actual, effects of these chemicals on organisms in the environment are ultimately an area of particular relevance in this OECD/IPCS Workshop on EcoToxicogenomics.

Past examples of the adverse effects that chemicals have had on organisms in the environment (i.e., ecotoxicity) have provided us with useful signals with regard to having to improve our understanding of how such chemicals can end up affecting not only environmental organisms (and processes), but even us and our children. The assessment of the ecotoxicity of chemicals on organisms in the environment has a long history (e.g., miners using canaries as protection from bad air, finding out how and why fish die from wastes released into streams, etc.).

Several national and regional laws have resulted in many formal methods and approaches being established for assessing the adverse effects of chemicals on organisms in the environment. Many of these test methods and assessment approaches are designed to examine how toxic a variety of specific chemicals actually are, or could be, to a relatively small (but representative) number of the numerous living things that exist in various environments (aquatic, terrestrial, etc.). One ultimate common goal is to devise some manner of assuring that significant adverse effects of chemicals on organisms in the environment can be prevented (or that they can subsequently be remediated).

It appears that the potential utility of the application of toxicogenomic techniques to the field of ecotoxicological assessment is enormous - e.g., for species to species extrapolations, finding common mechanisms of toxicity, development of potential alternative methods, etc. However, there are also probably several potential obstacles to be appreciated and then dealt with before such new methods will prove to be truly useful within the existing regulatory approaches for the hazard/risk assessment of chemicals upon organisms in the environment. One basic example is that the model organisms of interest for sequencing by molecular biologists are typically not near the same assemblage of potential interest to ecologists, let alone those of interest to ecotoxicologists – who can often only assess a very limited number and type of representative species in the environment. Another is the natural focus of many human health toxicologists upon using a limited number of (typically) mammalian species to assess and forecast the effects of chemicals upon one species, humans – with the contrasting difficulty of ecotoxicologists being the need to extrapolate the chemical effects seen in one or a few species to a huge number of similar (&/or different) species in the environment. It could take time and effort to get to useful ecotoxicogenomics.

## **Lessons learnt from Mammalian Toxicogenomics**

Lesley Onyon, International Programme on Chemical Safety (IPCS), World Health Organization, 20 Avenue Appia, CH-1211 Geneva 27; Switzerland.

The purpose of this presentation is to summarize the lessons learnt from the organization, conduct and outcomes of the IPCS Workshop on Toxicogenomics and the Risk Assessment for the Protection of Human Health, supported and held 17-19 November 2003 at the Federal Institute of Risk Assessment of the German Ministry of Environment, Nature Conservation and Nuclear Safety. Thirty-eight experts participated in the Workshop from governments, public and private sector research institutes, and industry in both OECD and non-OECD countries.

The focus of the collaborative IPCS/OECD Workshop was on the potential use of toxicogenomic information at different stages of the risk assessment process for the protection of human health. This focus was agreed in the anticipation that further OECD/IPCS collaborative activities would be taking place, particularly with respect to environmental effects.

The objectives of the Workshop were to:

- establish a scientific forum and dialogue between relevant experts including those with expertise in molecular biochemistry, genetic toxicology, epidemiology, public health, risk assessment, computational toxicology and clinical medicine;
- share information about scientific-level activities involving toxicogenomics including any programmes at national, regional and international levels;
- discuss the potential of toxicogenomics to contribute to improvements in the risk assessment process for the protection of health from environmental exposure to chemicals, for understanding the mode-of-action of environmental toxicants and the relevance and scope of gene-environment interactions;
- identify the near-term needs and necessary steps for enhancing international cooperation to contribute to improving the scientific understanding and the potential contribution of toxicogenomic research for improving chemical safety; and
- identify and discuss any gaps in knowledge, issues and challenges that might hinder the enhancement of awareness and use of toxicogenomics for protecting human health from environmental chemical exposures.

The Workshop followed a format that involved both plenary presentations and break-out groups. The break-out groups focused on three aspects: predictive models for identifying human health hazards; human exposure and susceptibility and risk assessment.

The Workshop viewed the science of toxicogenomics as one which endeavoured to understand the modes-of-action of chemicals and the potential role of gene-environment interactions. To do this the knowledge from the fields of genetics, genomic-scale mRNA expression (transcriptomics), cell and tissue-wide protein expression (proteomics), metabolic profiling (metabonomics) and bioinformatics needed to be combined with that obtained through conventional toxicological research, testing and assessment.

There was a general consensus at the Workshop that toxicogenomics is quickly evolving. As risk assessment also continues to evolve there is scope to learn from the new scientific discipline. This would

need scientists to work in close collaboration and use data derived from the new technologies together with those from more conventional sources. As most experience seems to have been obtained in single organizations either in research or in the process of drug discovery there seemed to be a need to establish ways and means to encourage the sharing of data and experiences between the private and public sector.

A number of technological and biological challenges were recurring themes throughout the Workshop. Perhaps the most often referred to were the need for new concepts to help convey the biological impact of toxicogenomic effects measured at low doses, the need for work to address the necessary information technology to handle the large amounts of novel data likely to be generated and concerted efforts to reduce interlaboratory and other sources of variability.

The Workshop recommended further global collaboration be undertaken to develop case-studies involving chemicals with known modes-of-action and available toxicogenomic data; building up of associated knowledge bases such as by identifying sentinel genes or gene clusters involved in toxicological responses and investigating sources of human toxicology data including the potential of existing “bio-banks”. In the area of hazard identification, it was recommended that possibilities for the design and execution of research to add toxicogenomic endpoints to existing test guideline protocols should be investigated.

For risk assessment it was also recommended that support be given for the development of improved biomarkers of exposure and protocols for their validation and continuing to develop a scientific understanding

The many activities in the area of toxicogenomics and risk assessment both before and after the IPCS/OECD initial Workshop in Berlin, the publication of policies and guidance by some governments and the statements of others of the need for new and harmonized risk assessment methodologies is testimony to the need for continued global interdisciplinary collaboration.

In May 2004, the governing body of the World Health Organization (WHO) recognized the need to strengthen and establish centres of genomics research to address health problems and to promote WHO's role in collaboration with other intergovernmental organizations. The need to convene forums and establish partnerships to mobilise resources was also highlighted. The twinning of IPCS and OECD collaborative work in the areas of human health and ecotoxicology respectively provides the possibility for further joint discussions to cement and extend the recommendations from both reports and organize the work appropriately.

### **Toxicogenomics: validation issues**

Raffaella Corvi, ECVAM, Institute for Health and Consumer Protection, European Commission Joint Research Centre, 21020 Ispra (VA), Italy.

Toxicogenomics is an emerging field in molecular toxicology that has substantial promise to revolutionize both the medical and health science fields. Appropriately developed and applied, toxicogenomics can deliver new approaches to identify and characterize the biological activity of drugs and chemicals as well as enhance the overall field of risk assessment.

The role of validation is certainly crucial for these methods to enter the regulatory arena and be implemented. For that reason, the European Centre for the Validation of Alternative Methods (ECVAM), which coordinates and funds validation studies on alternative methods to reduce, refine and replace the use of laboratory animals for regulatory testing, has started to investigate the specific considerations necessary for adequate validation of toxicogenomics-based test methods. Experience in validation of conventional alternative test methods has led to an understanding that new and innovative approaches will likely be

necessary to evaluate the scientific validity and regulatory applicability of test methods based on toxicogenomics. It is foreseen that the entire validation process will have to be adapted to these novel types of tests and will be more complex than that for classical alternative methods because both the predictive test system and the applied complex technology will need to be validated as a whole.

Since data are already being generated using this technology, it is both timely and important to address this issue now with the aim of establishing the foundation that will facilitate future regulatory acceptance of scientifically valid toxicogenomics-based test methods. By addressing the critical validation issues early on, and in parallel with the evolution and technological advancement of toxicogenomics-based methods, it should be possible to pre-empt many potential pitfalls and data gaps encountered with retrospective method evaluations that could impede validation of this promising research and regulatory tool.

In consideration of these related issues, ECVAM in collaboration with ICCVAM/NICEATM1 held the first of a planned series of workshops to begin to address the validation principles that lend themselves to toxicogenomics-based methods. It is expected that the resulting conclusions and recommendations will serve as the foundation for developing a strategy for the validation and regulatory acceptance of toxicogenomics-based methods and the planning of future efforts that will help guide the progress of the validation process.

1 The US Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program (NTP) Interagency Centre for the Evaluation of Alternative Toxicological Methods.

### **Emerging Molecular and Computational Approaches for Cross-Species Extrapolations: A Workshop Summary Report.**

William H. Benson<sup>1</sup> and Richard T. Di Giulio<sup>2</sup>.

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Similarities and differences in genomic responses among organisms to environmental pressure reflect the most basic inter-species interconnectivity. Advances in molecular technology have led to the elucidation of full genomic sequences of several multicellular organisms, ranging from nematodes to man. The related molecular fields of proteomics and metabolomics are now beginning to advance rapidly as well. In addition, advances in bioinformatics and mathematical modelling provide powerful approaches for elucidating patterns of biological response imbedded in the massive data sets produced during genomics research. Thus, changes or differences in the expression patterns of entire genomes at the levels of mRNA, protein and metabolism can quickly be assessed. Collectively, these emerging approaches greatly enhance our ability to address many of the major issues in human and environmental toxicology. Specifically, they are uniquely qualified to address the issue of cross-species extrapolation in risk assessment in both human and environmental toxicology. Moreover, these approaches provide a powerful means for elucidating interconnections between human health and ecological integrity. Given the significance of this topic to human and environmental toxicology, it was clear that SETAC and SOT were the appropriate societies to be a leading force behind the organization of a Pellston style workshop as a means to provide the ideal vehicle for objective and balanced discussion of this topic among professionals from different yet highly inter-related disciplines. The overall goal of the workshop was to outline a research agenda utilizing emerging technologies in “omics” and computational biology in order to: 1) elucidate similarities and differences among species to stressors, and relate the responses to phenotype, 2) take advantage of “omics”,

approaches to develop interconnections between human health and ecological integrity paradigm, and 3) extend this science into innovative approaches to risk assessment and regulatory decision-making. This presentation will provide a summary of the workshop.

### **Commonalities and Differences between Ecotoxicogenomics and Mammalian Toxicogenomics**

Taisen Iguchi, Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, National Institutes of Natural Science, 5-1 Higashiyama, Myodaiji, Okazaki 444-8787, Japan and Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corporation

Developing organisms are sensitive to estrogenic chemicals. Exposure to estrogens or estrogenic chemicals during critical periods of development induces persistent changes in reproductive as well as nonreproductive organs, including persistent molecular alterations. Chemicals released into the environment potentially disrupt the endocrine system in wild animals and humans, some of which exhibit estrogenic activity by binding to estrogen receptors. Estrogen-responsive genes and critical developmental windows of various animal species, therefore, need to be identified to understand the molecular basis of estrogenic activity during embryonic development. In order to understand molecular mechanisms of estrogenic chemicals on developing organisms, we identified estrogen-responsive genes using cDNA microarray and quantitative reverse transcriptase-polymerase chain reaction, and genes related to estrogen-independent vaginal changes in mice induced by estrogens during a critical window. We also analyzed genes in *Xenopus laevis* embryos related to abnormalities induced by estrogenic chemicals using microarray. Estrogen receptors and other steroid hormone receptors were cloned in various species, including the American alligator (*Alligator mississippiensis*), Nile crocodile (*Crocodylus niloticus*), red-bellied turtle (*Pseudemys nelsoni*), *Xenopus tropicalis*, roach (*Rutilus rutilus*), *Fundulus* and mosquitofish (*Gambusia affinis affinis*). Environmental androgen, trenbolone, was found to be induced persistent changes in gonopodium and ovotestis in mosquitofish. Several species of Daphnids are sensitive to juvenile hormone agonists: reduction of reproduction and induction of male offspring. To study molecular mechanism of sex determination of *D. magna*, we are currently establishing micorarray of *Daphina magna*. For toxicological study of medaka (*Oryzias latipes*), we selected several hundred genes and established medaka array. Microarray technology is a powerful tool to understand molecular mechanism of receptor-mediated toxicology in various animal species. Based on these experiences, commonalities and differences between ecotoxicogenomics and mammalian toxicogenomics will be discussed.

### **How genomics could bring about revolution (or evolution) to ecotoxicology.**

Jason Snape, AstraZeneca Global SHE

Rapid progress in the field of genomics (the study of how an individual's entire genetic make-up, the genome, translates into biological functions) is beginning to provide tools that may assist our understanding of how chemicals can impact on human and ecosystem health. In many ways, if scientific and regulatory efforts in the 20th century have sought to establish which chemicals cause damage to ecosystems, then the challenge in ecotoxicology for the 21st century is to understand the mechanisms of toxicity to different wildlife species. In the human context, 'toxicogenomics' is the study of expression of genes important in adaptive responses to toxic exposures and a reflection of the toxic processes per se. Given the parallel implications for ecological (environmental) risk assessment, we propose the term 'ecotoxicogenomics' to describe the integration of genomics (transcriptomics, proteomics and metabolomics) into ecotoxicology. Ecotoxicogenomics is defined as the study of gene and protein expression in non-target organisms that is important in responses to environmental toxicant exposures. The potential of ecotoxicogenomic tools in ecological risk assessment seems great. Many of the standardized methods used to assess potential impact of chemicals on aquatic organisms rely on measuring whole-organism responses (e.g. mortality, growth, reproduction) of generally sensitive indicator species at

maintained concentrations, and deriving ‘endpoints’ based on these phenomena (e.g. median lethal concentrations, no observed effect concentrations, etc.). Whilst such phenomenological approaches are useful for identifying chemicals of potential concern they provide little understanding of the mechanism of chemical toxicity. Without this understanding, it will be difficult to address some of the key challenges that currently face aquatic ecotoxicology, e.g. predicting toxicant responses across the very broad diversity of the phylogenetic groups present in aquatic ecosystems; estimating how changes at one ecological level or organisation will affect other levels (e.g. predicting population-level effects); predicting the influence of time-varying exposure on toxicant responses. Ecotoxicogenomic tools may provide us with a better mechanistic understanding of aquatic ecotoxicology. For ecotoxicogenomics to fulfil its potential, collaborative efforts are necessary through the parallel use of model microorganisms (e.g. *Saccharomyces cerevisiae*) together with aquatic (e.g. *Danio rerio*, *Daphnia magna*, *Lemna minor* and *Xenopus tropicalis*) and terrestrial (e.g. *Arabidopsis thaliana*, *Caenorhabditis elegans* and *Eisenia foetida*) plants, animals and microorganisms.

### **(Eco)Toxicogenomics Data Management – Standards and Implementations**

Susanna-Assunta Sansone, EMBL The European Bioinformatics Institute (EBI), Cambridge, UK

Several organizations and committees are tackling data **standardization issues** in a wider context of using emerging highly parallel “omics” approaches. Independent initiatives are important because they target specific requirements for the particular “omics” technologies being used and they capture and reflect the particular context of the fields of application (e.g. pharmaceutical, medical or environmental). Some regulatory bodies, such as the FDA and EPA, have also published their policy or guidance on genomics data submissions. However, there will be a fundamental difference in both the design and objectives of the efforts focused on regulatory submission of data versus those focused on needs of the research community. The former aims to accelerate the review process, facilitate proprietary data submission and optimise data visualization in a way that does not impact the vocabulary used by the individual submitter. The latter aims to develop detailed, highly structured databases, to facilitate data exchange using a common vocabulary and to provide a fundamental biochemical understanding. However, there may be a value in developing a compatibility where possible between regulatory and research objectives in the design of these data standards. Duplication and incompatibility should be avoided where possible, maximizing synergy and optimizing harmonization. In particular, a unified approach to describe and report the biological component of an experiment that is common to different “omics” technologies (transcriptomics, proteomics and metabolomics/metabolomics) or disciplines (e.g. pharmacogenomics, toxicogenomics, environmental genomics) is strongly recommended. Undoubtedly, specialized information is needed by certain types of applications, but a unified model should be able to encompass them all.

The need for a coordinated, **bioinformatics infrastructure** for “omics” data worldwide is clearly significant. National and institutional initiatives are important but can result in unnecessary duplications, incompatible databases and problematic data exchange. In preference the creation of an internationally compatible informatics platform for omics data will enhance the impact of the individual datasets and provide the scientific community with easy access to integrated data in a structured standard format, facilitating data comparison and data analysis. Ultimately this knowledge will also serve as a reference for regulatory organizations to evaluate data submitted by registrants to those organizations.

The Microarray Gene Expression Data (MGED) Society ([www.mged.org](http://www.mged.org)) Working Groups have developed a set of open source ‘standards’, including the Minimum Information About Microarray Experiments (MIAME), the Microarray Gene Expression (MAGE) and the MGED Ontology (MO). The response from the scientific community to these ‘standards’ has been extremely positive. Currently, the MGED ‘standards’ have been adopted and implemented by public and institutional databases and several microarray informatics tools. Furthermore, most of the major scientific journals and some funding agencies

require publications describing microarray experiments to comply with MIAME and data submitted to public repositories, such as ArrayExpress, GEO and CIBEX. Following the very favorable response, MIAME has been extended to describe array-based toxicogenomic experiments. MIAME/Tox is the result of a collaborative undertaking with the Life Sciences Institute Health Environmental Sciences Institute (ILSI HESI), National Institute of Environmental Health Sciences (NIEHS), National Center for Toxicogenomics (NIEHS-NCT), FDA National Center for Toxicological Research (NCTR), Center for Toxicoinformatics and the EBI. Similarly, MIAME/Env has been developed by the UK Natural Environment Research Council (NERC) environmental genomics community to fulfill the specific requirements of this domain of application. Discipline-specific initiatives are important because they target 'real world' data capture requirements. A consequence of this however is that the knowledge can become fragmented, resulting in unnecessary duplications, problematic data exchange and ultimately incompatible databases. To maximize the synergy and optimise harmonization, a new working group has been formed within MGED recently, acting a 'single point of focus' for **Toxicogenomics**, **Environmental Genomics** and **Nutrigenomics** communities, where efforts are already underway to promote standardization and develop databases to facilitate data exchange. The **MGED Reporting Structure for Biological Investigations Working Groups (RSBI WGs)** aim to maintain collaboration between activities that relate to biological investigations in specific domains of application and technology-driven standardization efforts, such as the Human Proteomic Organization (HUPO)-Proteomics Standardization Initiative (PSI) and the Standard Metabolic Reporting Structure (SMRS) Group. The development of a reporting structure for describing information intensive investigations RSBI Tiered Checklist (RSBI TC) is under way. RSBI TC will be a modular context depended structure allowing to described the same concept unambiguously within and across the different communities.

### **Development of medaka microarray**

Masaru Matsuda and Yoshitaka Nagahama, Laboratory of Reproductive Biology, National Institute for Basic Biology

Sex determination and sexual differentiation are the important targets of sex steroid hormones and endocrine disrupters. For examining the mechanisms of exogenous sex steroids and endocrine disrupters actions, we established two goals. The first is, understanding normal development of sex determination and sexual differentiation. The second is, understanding the mechanism of sex steroids and endocrine disrupters actions on sex determination and sexual differentiation.

Exogenous sex steroids or endocrine disrupters affect phenotypic sexes in lower vertebrate species. Therefore, these vertebrates are good experimental models for this purpose. We use Japanese medaka as a model animal. Medaka is a small, egg-laying freshwater fish native to Asia, and is established as a model experimental animal in Japan.

In medaka, we have identified the sex-determining gene DMY (DM domain gene on the Y chromosome) by the positional cloning method. Therefore, we know that the sex determination of medaka starts with DMY expression. In sexual differentiation, we have also known that estrogen is important for female development and that DMRT1 is a useful gene marker for male development. Because exogenous sex steroids affect DMRT1 expression and not affect DMY expression, the target of sex steroids and endocrine disrupters is sexual differentiation.

We suppose that sex steroids and endocrine disrupters have various action and various targets, but we have scarce information a gonadal sex differentiation. Therefore, to understand the action of endocrine disrupters, we should understand the sexual differentiation deeply. To analyze gene expression exhaustively, we have used microarrays. To collect cDNAs for print microarrays, we have first constructed two cDNA libraries from whole embryos of 2 days before hatching, just hatching, 5, 10, 15 days after

hatching and ovary, and from subtraction between XX gonads and XY gonads of 5, 10, 20 days after hatching fry. From the first non-subtracted libraries, we sequenced total 24,960 clones. We picked up 7,512 clones as non-redundant clones. We printed these clones as two microarrays. On the other hand, from subtracted libraries, we sequenced 10,368 clones and made one microarray. Now, we are collecting samples for expression analysis using these microarrays. We soak fertilized eggs in water containing estrogen, androgen, or DES, and collect eggs in just hatching, 5 and 10 days after hatching. We hope that these microarrays will be useful for analyzing effects of sex steroids and endocrine disruptors on gene expression.

### **Ecotoxicogenomics work with amphibians**

Caren Helbing, University of Victoria, Victoria, British Columbia, Canada

Frogs are widely acknowledged as being excellent sentinel species to assess the impacts of human activity on the environment. Their association with aquatic environments, close proximity to population centres, and sensitive life stages make frogs ideal indicator species for water-borne contaminants. Over the recent decades, declines in frog populations and increased incidences of developmental abnormalities have raised the alarm with respect to the presence of chemicals that could have an adverse effect on human and wildlife health. Of particular concern are the effects of exposure to chemicals or chemical mixtures that are at concentrations much lower than the typical “toxicological” concentrations that evoke lethal or morbid responses. These sublethal, detrimental effects can substantially alter normal development including behaviour, sexual differentiation and fitness, which could have far-reaching impacts on populations.

There are ~5,000 species of frogs worldwide and a diverse subset of species have been used for traditional toxicological studies. A challenge for ecotoxicogenomics is to develop molecular tools that are applicable to multiple species. At the same time, genetic information from *Xenopus* species (*laevis* and *tropicalis*) provide exciting possibilities.

This presentation will highlight the genomics efforts that have been undertaken for native and laboratory species in Canada and the US using DNA arrays and quantitative real-time polymerase chain reaction approaches. Special focus will be on the development of genomics tools for incorporation into a tier 1 metamorphosis assay for the detection of thyroid hormone disruption.

### **The Population and Molecular Stress Responses of *Daphnia magna***

Richard Sibly<sup>1</sup>, Amanda Callaghan<sup>1</sup>, Richard Connon<sup>1</sup>, Helen Hooper<sup>1</sup>, Steve Maund<sup>2</sup>, George Orphanides<sup>3</sup>, Jonathan Moggs<sup>3</sup>, David Moore<sup>3</sup>, Fei Ling Lim<sup>3</sup>, Tom Hutchinson<sup>4</sup>

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All organisms respond to environmental stressors by regulating the expression of genes. In some instances this effectively means turning them on or off and often it means changing the intensity of the expression. Microarrays provide a powerful new technology that allows us to measure these changes. The products of gene expression (mRNA converted to cDNA) are measured by hybridising them to complementary sequences of DNA printed onto a glass slide or “microarray”. After processing, genes increased in their expression appear green and those decreased in expression appear red. We are using this new technology, known as transcript profiling, to identify genes that respond to specific environmental stressors.

To make sense of the molecular stress responses it is necessary to measure the population responses to stress. This can be appreciated by considering two examples. If a population is unaffected by a chemical, then there is no population stress. On the other hand if animals die or the ability to reproduce is impaired there will be a measurable population response. In our work we determine population stress responses of water fleas (*Daphnia magna*) by measuring population growth rate. This is achieved using image analysis of photographs of populations over a 14 day period. Our experiments are designed to measure population and gene responses simultaneously to a range of stressors, including toxicants (cadmium, Lufenuron) and physico-chemical aspects of an organism's environment (pH, water hardness).

To make our microarray, we obtained DNA from the *Daphnia* using Subtractive Suppression Hybridization, a technique to isolate the DNA of genes whose expression levels change when stressors are applied. Further genes were generously provided by our collaborators (see below). In total we have DNA from about 3000 genes on the current microarray. Preliminary results for cadmium, presented at the workshop, suggest that a number of genes, including chitinase, haemoglobin and ferritin, respond in similar ways to increasing concentrations of cadmium chloride. Eventually we hope to understand why *Daphnia magna* are found in some ponds and not others, and which man-made chemicals threaten their existence. We chose *Daphnia magna* because they are a widely-used ecotoxicology indicator species. It is already clear that this new technology will have many applications in the field of ecotoxicology.

We are grateful to our collaborators Dr T. Iguchi and Dr H. Watanabe, of the Center for Integrative Bioscience, Okazaki National Research Institutes, Japan; and Dr W. de Coen and Ms A. Soetaert of the Laboratory for Ecophysiology, Biochemistry and Toxicology, University of Antwerp, Belgium, for providing between them some 12000 ESTs, and to NERC, Syngenta and AstraZeneca for funding. For further information contact [r.m.sibly@reading.ac.uk](mailto:r.m.sibly@reading.ac.uk) or [a.callaghan@reading.ac.uk](mailto:a.callaghan@reading.ac.uk) or see <http://www.ams.rdg.ac.uk/zoology/daphnia/>

### **Development of biomarkers from (Eco) toxicogenomics**

J. Kevin Chipman, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

There is increasing interest in gaining information on altered gene expression as adaptive and toxic responses to chemical exposure. These studies will aid the identification of toxic mechanisms and the development of novel, sensitive and early biomarkers for use in chemical screening and in environmental monitoring. Information is relatively forthcoming in model organisms for which adequate sequences and databases are available. This will be demonstrated through gene expression changes observed in rats following exposure to the peroxisome proliferator (PP) DEHP and the discovery of PP-regulated processes through gene ontology (GO) and GenMAPP bioinformatic analyses. The situation is somewhat more difficult when studying toxicogenomics in organisms of environmental relevance. We initiated studies in the flounder by designing degenerate primers for a range of genes of toxicological interest; gene fragments were amplified from flounder cDNA by PCR. In addition, subtractive suppressive hybridisations (SSH) between flounder collected from the Alde (reference) and Tyne (polluted) estuaries provided clones of differentially expressed genes, as did SSH between laboratory-maintained flounder treated with benzo(a)-pyrene or cadmium in comparison with carrier controls. These clones have been combined with clones from a liver cDNA library to produce a 13,000 cDNA microarray for the flounder (EU-GENIPOL Project). Proof of principle has been achieved by comparing the differences in gene expression between flounder sampled from the two estuaries of different pollution status and also between flounder treated in the laboratory with model toxicants. We have detected induction of known biomarker genes, e.g. CYP1A with PAH treatments, metallothionein with cadmium, vitellogenin and choriogenin with estradiol and ethinyl-estradiol. Importantly, interactive effects have been discovered e.g. between PAH and metals. Results are beginning to clarify the processes that are activated and there are correlations emerging between laboratory

and field responses e.g. induction of CYP1A, Cu/Zn SOD. Potential novel biomarkers have been indicated such as HSP 30B and other, yet to be characterised, ESTs.

This work was funded by the CEFIC-LRI, NERC, CEFAS and EU and has involved Syngenta CTL (PP) and GENIPOL (Flounder) collaborations.

### **Environmental Metabolomics**

Mark Viant, NERC Advanced Fellow, School of Bioscience, University of Birmingham, B15 2TT, U.K.

Ecotoxicogenomics encompasses a range of “omics” methodologies, in particular genomics, transcriptomics and proteomics, for the study of DNA, mRNA expression and protein abundance, respectively. The complete characterisation of cellular processes, whether associated with normal homeostasis or as a result of toxic insult, also requires information on the metabolic status of the cell or organism. Indeed it can be argued that the metabolic status is the most functional measure of the cell’s phenotype. *Metabolomics*, the most recent of the “omics” approaches, addresses this issue and is defined as the comprehensive analysis of all metabolites within a cell under a defined physiological state.

To date, relatively few metabolomics studies have been published in comparison with the more mature omics techniques, and only a very small number of these have addressed environmental metabolomics. Metabolomics has simply had insufficient time to prove itself, and in fact considerable work remains in developing the bioanalytical and bioinformatic methods that underpin this science, and without which its full potential will not be realised. Studying the metabolome has, however, several key benefits, particularly for environmental studies. These include:

- *No a priori information, such as sequence data, is required for comparative toxicity studies across multiple non-model organisms.*
- *The analytical methods facilitate high throughput, inexpensive and tractable measurements.*
- *Within ecotoxicogenomics, the metabolomic measurements are potentially the most representative of organism physiology.*

Unlike DNA microarrays, which can measure all genes within a genome, no single analytical technique is capable of observing all the metabolites within a metabolome. The two leading platforms are <sup>1</sup>H NMR spectroscopy and mass spectrometry, each with benefits and disadvantages, and hence the choice of toolset should be based upon the question posed. For either method, the wealth of metabolic information derived from the spectra can be used to interpret hypothesis-driven research, as well as to support discovery-driven research and the development of novel biomarkers.

This presentation will introduce the field of metabolomics and will highlight the primary advantages of this approach in environmental toxicology. It will be illustrated by a number of recent studies on fish and aquatic invertebrates. Aspects of this emerging field that necessitate future development will also be addressed, in particular:

- *Standardisation of data acquisition, processing and reporting structures.*
- *Construction of metabolite libraries to facilitate biomarker identification.*
- *Methods to integrate and subsequently interrogate transcriptomic, proteomic and metabolomic datasets recorded from the same toxicant-exposed organism.*

## **Introduction of MHLW Toxicogenomics Projects and some thoughts about possible contribution of “Percellome” to EcoToxicogenomics**

Jun Kanno, Ken-ichi Aisaki, Katsuhide Igarashi, Noriyuki Nakatsu, Atsushi Ono, Yukio Kodama, Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences.

Our development, the Percellome method generates copy number of mRNA or probesets in a per one cell basis. This method displays its great ability in monitoring gene alterations when the whole repertoires of genes are drastically changing.

Here we display an example of gene expression analyses of uterotrophic responses. The rodent uterotrophic assay is an in vivo screening method for the detection of estrogenicity of the test chemical. All animals were ovariectomized 2 weeks prior, to disrupt the homeostatic system as well as to remove the source of intrinsic estrogen. The vehicle control uterus becomes severely atrophic due to loss of estrogenic stimuli, whereas, that of the treated groups becomes hypertrophic, suggesting drastic increase in gene transcription and translation compared to the vehicle control. In the globally normalized data, among 12,000 genes, 4600 genes showed more than 2 fold increase, 470 genes were reduced by 0.5 or less, and 7,400 remained unchanged (fold change between 0.5 and 2) by the treatment. By the Percellome, almost all 12,000 genes showed 2 fold or greater increase, and only 30 genes remained unchanged. It is also shown that house-keeping genes, such as beta-actin, were shown to alter by the treatment in a per one cell basis.

As shown above, the Percellome approach introduces different views of ups and downs from the global normalization approaches especially when the samples are significantly different in expression profiles. The merit of the Percellome expands to easier data comparison of different organ samples and different version or the make of the platforms.

Our possible contribution to the eco-toxicogenomics would be to facilitate direct comparison of transcriptome data between different species. It would also be biologically interesting if this “per one cell” comparison approach is applied to the developmental stages of the sentinel species ranging from nematoda to vertebrates. We anticipate the development of the phylogenic database for molecular developmental toxicology.

### **ArrayTrack - Supporting Toxicogenomics research at the US Food and Drug Administration National Center for Toxicological Research**

Weida Tong<sup>1\*</sup>, Stephen Harris<sup>2</sup>, Xiaoxi Cao<sup>2</sup>, Hong Fang<sup>2</sup>, Leming Shi<sup>1</sup>, Hongmei Sun<sup>2</sup>, James Fuscoe<sup>1</sup>, Huixiao Hong<sup>2</sup>, Qian Xie<sup>2</sup>, Roger Perkins<sup>2</sup>, Dan Casciano<sup>1</sup>

<sup>1</sup>FDA National Center for Toxicological Research (NCTR), <sup>2</sup>Z-Tech Corp.

The mapping of the human genome and the determination of corresponding gene functions, pathways, and biological mechanisms are driving the emergence of the new research fields of toxicogenomics and systems toxicology. Many technological advances such as microarrays are enabling this paradigm shift that indicates an unprecedented advancement in the methods of understanding the expression of toxicity at the molecular level. At the National Center for Toxicological Research (NCTR) of the U.S. Food and Drug Administration, core facilities for genomic, proteomic, and metabonomic technologies have been established that use standardized experimental procedures to support center-wide toxicogenomic research. Collectively, these facilities are continuously producing an unprecedented volume of data. NCTR is developing a toxicoinformatics integrated system (TIS) for the purpose of integrating genomic, proteomic, and metabonomic data with the data in public repositories as well as conventional in vitro and in vivo toxicology data. The TIS will enable data curation in accordance with standard ontology and provide or

interface a rich collection of tools for data analysis and knowledge mining. In this presentation, the design, practical issues, and functions of the TIS are discussed through presenting its prototype version, ArrayTrack, for management, analysis and interpretation of DNA microarray data. ArrayTrack is logically constructed of three linked components: a) a database (MicroarrayDB) that stores microarray experiment information; b) tools (TOOL) for data visualization and analysis; and c) libraries (LIB) that provide curated functional data from public databases for data interpretation. Specifically, ArrayTrack is MIAME (Minimum Information about a Microarray Experiment) supportive for storing both microarray data and experiment parameters associated with a toxicogenomics study. A quality control mechanism is implemented to assure the fidelity of entered expression data. ArrayTrack also provides a rich collection of functional information about genes, proteins and pathways drawn from various public biological databases for facilitating data interpretation. In addition, several data analysis and visualization tools are available with ArrayTrack, and more tools will be available in the next released version. Importantly, gene expression data, functional information and analysis methods are fully integrated so that the data analysis and interpretation process is simplified and enhanced. Using ArrayTrack, we can select an analysis method from the TOOL and apply the method to selected microarray data stored in the MicroarrayDB; the analysis results can be linked directly to gene information in the LIB. ArrayTrack is publicly available online (<http://www.fda.gov/nctr/science/centers/toxicoinformatics/ArrayTrack/index.htm>) and the prospective user can also request a local installation version by contacting the authors.

## **ANNEX 4-1: REPORT FROM THE BREAKOUT GROUP 1: BIOLOGICAL ISSUES**

Chair: Sean KENNEDY

Co-chair: Jun KANNO

Rapporteur: Joakim LARSSON

Other participants: Minako TAKAMIYA, Yasunobu AOKI (left at 16.00), Koji ARIZONO, Hisato IWATA, Tomonari MATSUDA, Tetsuo SATOH, Norihisa TATARAZAKO, Hirofumi YOKOTA, Jae-Chun RYU, Richard SIBLY, David SPURGEON, William BENSON, Michael HEMMER

### **Executive summary:**

Toxicogenomic technologies have unique opportunities to address ecological and human health concerns, such as:

- offering possibilities to reduce, refine and replace costly animal intensive methods for chemical screening and testing
- understanding how and why species and subgroups differ in sensitivity and response to chemical stress, and create a stronger scientific foundation for the use of safety factors. This will allow effective policies to be developed in order to protect endangered and important species.
- assessing the effects of chemical mixtures and combination of stressors. Previously, appropriate methods have been lacking.
- reduced uncertainty in assessment of ecological conditions

For these reasons, it is important that these new tools are evaluated and implemented for chemical risk assessment.

### **Key recommendations:**

1. It is imperative that there is a concerted effort to conduct comprehensive (eco)toxicogenomic studies to differentiate compensatory and adaptive responses from adverse toxicological outcomes.
2. Concerted efforts are required to extend technological platforms for diverse taxa and develop these for use across species.
3. Quality assurance and quality control of all sequencing data coupled to good data management and good annotation. All sequencing data and clones should be publicly available.

**Specific questions/answers:**

***1. What are the common (and different) issues that face human and ecological risk analysis?***

*Common issues:*

Both are about assessing risks to organisms – both are founded in biology

Both assess exposure and effects in a similar way, but there is a difference in what is considered an acceptable risk. The views of what is an acceptable risk boils down to a cost benefit analysis. These considerations are affected by social and economic issues.

Surrogate species are often used for both human and ecological risk assessments. Species extrapolation is a problem for both types of assessment, however this is most often a more pronounced problem with ecological risk analyses. The latter requires extrapolations between physiologically and taxonomically diverse species. However, it is sometimes possible to carry out ecological risk assessment using the specific species of concern.

*Differences:*

For the most parts, human risk assessment is based on the health of individuals, whereas in ecological risk assessment the focus is on traits that may have consequences for populations and species health. Also, in ecological risk analysis, we are interested in the effects on many species.

***2. How can integration of cross-species genomic analysis be used to address common issues and provide solutions? What types of genomic and/or proteomic data will be most useful to develop interconnections between the human and ecological risk paradigm?***

There is a value in identifying common pathways, allowing extrapolation between species regarding the mechanism-of-action of different chemicals. The “omics” techniques, including both transcriptomics, proteomics and metabolomics, may assist in elucidating such pathways.

***3. What organisms should receive the greatest priority for sequencing? Will the availability of comparative genomic maps for model species assist future sequencing efforts? Will we need to sequence multiple species? How will other methods assist in bridging the knowledge gap until sequence data become available?***

The needs depend on the questions asked:

- Understanding core biological processes?
- Performing ecological risk assessment?
- Prediction of human health?
- Field monitoring - assessing health of wild species?

Organisms used in standard ecotoxicological risk assessment should have priority, but creating EST databases may be very useful and possible a more cost or time efficient approach. In addition, sequencing some representative species across the evolutionary tree, which also may be widely distributed and useful for field studies would be beneficial. Results from any cross-species arrays need to be evaluated carefully (as should homologous assays).

Proteomics need to be anchored in genomics. Available genome or EST databases therefore greatly facilitate the identification of regulated proteins.

Quality assurance and quality control of all sequencing data coupled to good data management and good annotation. All sequencing data and clones should be publicly available.

***4. In the evaluation of genomic responses, will it be possible to distinguish between toxic responses from compensatory or adaptive responses?***

It is a strong hypothesis, which urgently needs evaluation. To distinguish such responses may be possible by an integrated “omics” approach. The outcome from such analyses should be assessed in relation to conserved compensatory responses. It is imperative that there is a concerted effort to conduct comprehensive (eco)toxicogenomic studies to differentiate compensatory and adaptive responses from adverse toxicological outcomes. What is considered “adverse” will be determined on a case-by-case basis.

***5. What regulatory decisions could genomics-based methods support, and how? (Replacing existing toxicity tests? As an additional endpoint? Reducing uncertainty and inter-species extrapolation? Supporting QSARs and categorisation of chemicals?). What validation approaches are appropriate for these regulatory issues?***

We believe there is a role for genomics-based methods in this context.

See executive summary

**Other issues raised:**

There is a use of genomic techniques to assess effects of chemical stress on species composition (such as microbes, small invertebrates and algae) in environmental or laboratory samples (such as soil and water). Assays based on genomic DNA sequences specific for individual species or higher taxa will therefore be useful for ecological assessment. Similarly, transcript analyses for whole communities of microbes or other small organisms may provide further information on the effects of chemical stress on the ecosystem functioning. This approach requires that particularly conserved genes are studied.

We would like to emphasize that large scale analyses of responses (omics) are useful also for chemicals with largely known or suspected mechanism of action, such as pharmaceuticals. For field studies, comprehensive profiling may allow diagnosis of the cause of adverse effects. Furthermore, multiple mechanisms of action may be elucidated, which will lead to the development of improved methods for hazard assessment.

## **ANNEX 4-2: REPORT FROM THE BREAKOUT GROUP 2: TECHNOLOGICAL ISSUES**

Chair: Jason SNAPE

Other participants: Caren HELBING, Hiroshi GOHDA, Tyoichi KIYAMA, Yuji OSHIMA, Osamu TOOI, Hajime WATANABE, Young-Rok SEO, Mark VIANT, Bruce BLUMBERG, Lesley ONYON

### **Questions/answers:**

#### ***1. What needs to be done to apply molecular and computational toxicological information to human and environmental risk assessment?***

Molecular-based approaches, such as transcriptomics, proteomics and metabolomics, to study the impact of chemicals on human and wildlife populations will have an important role to play in risk assessment practices in the future.

There has been significant progress in each of the 'omic' techniques over the past few years but there are still a number of important biological, technical, and bioinformatic issues that need to be addressed before these tools are robust enough to apply them with confidence in regulatory decision making.

The lack of national and international coordinated efforts in environmental genomics is in danger of seriously delaying the integration genomic techniques into ecotoxicology and its use in environmental risk assessment.

The lack of discrete or distinct funding available for ecotoxicogenomics is also a major barrier. Current funding is heavily biased towards molecular aspects of mammalian toxicology. Without a substantial increase in funds dedicated to ecotoxicogenomics it will not be possible to make interconnections between ecotoxicology and mammalian toxicology in the immediate future.

#### ***2. What are the different technologies available for ecotoxicogenomic studies (e.g. DNA microarrays, SAGE)? What will be the greatest use of genomic technologies in ecotoxicology? Why?***

A number of different molecular-based tools are available for studying changes in transcript, protein and metabolite composition (see Table 1). It must be emphasised that each of individual techniques are at different stages of development and as such their potential integration into ecotoxicology will be realised at different points in time. It is quite clear that at present array-based approaches to studying transcripts are closer to being applied to ecotoxicological issues than proteomics and metabolomics.

A weight of evidence approach coupled to established biological endpoints is required for each molecular-based parameter before it can be considered robust enough for regulatory use. Table 1 lists a number of transcriptomic, proteomic and metabolomic approaches together with some important and relevant technological and regulatory considerations. Some of these tools have very clear research advantages and others possess a diagnostic or regulatory potential. It should be recognised that the research platforms will identify endpoints that can be transferred to more diagnostic platforms (e.g. Q-PCR

and ELISA). Not all the platforms in Table 1 should be used for regulatory assessment. However for routine use in regulatory assessment the following practical considerations should be made when identifying suitable “omics” platforms:

- A cross-species potential is desirable for array and non-array based systems
- It must be quantitative and have a diagnostic potential
- Good QA/ QC
- The end point must be platform independent and (semi)quantitative
- A standardised system for data capture, data processing and reporting
- A potential for non-invasive or non-destructive sample collection
- Scalable from sample collection through to biological information
- Cost effectiveness (set-up and maintenance costs)
- The system should provide data that is fit for purpose

*Conclusion: It is platforms with these essential criteria, particularly those with cross-species potential that should be prioritised for regulatory use but it must be recognised that the other ‘research’ platforms will provide important and relevant information.*

*Conclusion: A road map is required on how to move from research applications of these tools to their regulatory use. This road map needs to include strategies for moving from global studies to more targeted studies, training issues, linking molecular responses to biological endpoints, and data management and data analysis. These issues need to be addressed by an appropriate technical working group with member of the regulatory, industrial and academic community.*

*Conclusion: Any strategies to move from global to targeted “omics” studies need case studies based on real data and should not be based purely on theory.*

One potential barrier could be the initial capital costs associated with setting up omic-based techniques. Setting up national centres of excellence that serve both a research and regulatory purpose may reduce these costs. There is also a need for strong technical expertise; this may also be addressed through establishing technical centres.

There are some additional requirements for instrument manufacturers. These include a common import/ export format for raw data and metadata. This will facilitate subsequent data analysis and integration with other relevant data sets.

### **3. What needs to be done to realise the integration of the “omics” into ecotoxicology?**

#### ***Generically***

- QA/ QC
- Performance criteria
- Common data capture, data outputs, and reporting standards

#### ***Transcriptomics***

- Need for quality standards,
- Observations must be platform independent.
- Relevant arrays are needed.
- Resource.

*Conclusion: Global arrays used in R&D are likely to be replaced by targeted arrays and Q-PCR-based assays for regulatory use*

#### ***Proteomics***

- 2-D gels are unlikely to have any regulatory role but will remain an essential research tool for the foreseeable future

*Conclusion: ELISAs and antibody protein arrays may be of considerable use for monitoring protein biomarkers and might have a role for regulatory purposes.*

#### ***Metabolomics***

- Chemical libraries for metabolite identification and quantification needed ASAP to realise potential of metabolomics, in particular for biomarker discovery and elucidating mechanisms of toxic action.
- Method development should emphasize need for quantification: NMR can be quantitative but is currently used in a qualitative manner; Global-MS based approaches cannot currently be used quantitatively; Targeted MS based approaches are quantitative

*Conclusion: Chemical libraries to facilitate metabolite identification are need urgently.*

#### ***4. Genomic techniques for species composition?***

- Phylogenetic arrays do exist for studying microbial community diversity.
- SNP arrays are also becoming available for a number of species.
- Intraspecies variability can also be studied by SNP and microsatellite analysis using non-invasive methods e.g. fin biopsies.

#### ***Informatic recommendations:***

Tools required for:

- Cross-species pathway comparison.
- Connectivity between different “omic” level data, mechanistic pathways and toxicological meta data.
- Requires establishment of system biology schema to deliver structure proposed by Suzan.
- Funding bodies should dictate compliance with standards.

If global array studies are being used and there are specific important mode(s) of action, from a regulatory perspective, then automated informatic analyses should be developed that focus on rapidly identifying whether these mode(s) or pathways are important of analysis. A secondary investigation of other responses in the global study should then be assessed.

Table 1: Summary of relevant “omic” techniques and issue related to there application to (Eco)toxicology.

Technique	Cross Species	Prior sequence	Utility	QC/QA	Measurement	Data Capture Standards	Scalability	Sampling	Cost	Training Requirements	Regulatory Interpretation
<i>Transcriptomics</i>											
Affy	N	Y	R&D	Y	SQ	Y	L	I&D	H	M	D
Global Oligo Arrays	N	Y	R/D++	N/Y+	SQ	Y	M	I&D	M	M	D
Global cDNA Arrays	Y	N	R/D++	N/Y++	SQ	Y	M	I&D	M	M	D
Targeted Oligo	N	Y	R/D+	Y	SQ	Y	H	I&D	M	M	E
Targeted cDNA	Y	Y	R/D+	Y	SQ	Y	H	I&D	M	M	E
Q-PCR	N	Y	R/D	Y	QT	Y	M	I&D	L	M	E
SAGE	N	N	R	N	SQ	N	L	I&D	H	H	D
<i>Proteomics</i>											
2D	Y	N	R	N	SQ	N/Y+	L	I&D&NI	M	M	D
2D-Maldi	N(Y)	Y	R	N	SQ	N/Y+	L	I&D&NI	M	H	D
2D-MS/MS	Y	N	R	N	SQ	N/Y+	L	I&D&NI	H	H	D
Ciphergen-Maldi	N	N	R/D?	N/Y++	SQ	N/Y+	H	I&D&NI	M	H	D
Protein Arrays	Y	N	R/D++	N/Y++	QT	N/Y++	L	I&D&NI	M	H	M
LC-MS/MS	Y	N	R/D+++	N/Y+++	QT	N/Y+++	L	I&D&NI	H	H	D
ICAT-Maldi	N(Y)	Y	R/D+++	N/Y+++	QT	N/Y+++	M	I&D&NI	H	H	D
ICAT-MS/MS	Y	N	R/D++	N/Y++	QT	N/Y++	M	I&D&NI	H	H	D
Elisa ( +others)	Y	N	R&D	Y	QT	Y	H	I&D&NI	L	L	E
<i>Metabolomics</i>											
NMR	Y	N	R/D+	N/Y+	SQ/QT+	N/Y+	H	I&D&NI	L	H	D
Global MS-based	Y	N	R/D++	N/Y++	SQ/QT++	N/Y+	M	I&D&NI	M	H	D
Targeted MS-based	Y	N	R/D++	N/Y++	QT	N/Y+	M	I&D&NI	M	H	D
LC-Electrochemical	Y	N	R/D?	N/Y+++	SQ/QT+++	N/Y+++	M	I&D&NI	M	H	D

Note: Issues include the following. Ability to utilise tools developed for a specific organism to different species i.e. perform “cross species” experiments(Y, yes; N, no; N(Y), indicates technique can be transferred to closely related species). Requirement for prior detailed **sequence** knowledge is indicated. **Utility** in respect to application to research (R) and/or diagnostics (D) is provided. Availability of **QC/QA** standardisation is indicated together with whether the technique generates semi-quantitative (SQ), quantitative (QT) or qualitative (QL) data. Availability of community agreed **data capture standards** is indicated. Throughput or **scalability** of the technique is indicated as high (H; >100 samples / day), medium (M; 10-100 / day) and low (L; <10 / day). **Sampling** methods as either invasive (I), destructive (D) or non-invasive (NI) is also indicated. **Costs** are given as low (L; <\$10 / sample), medium (M; \$10-100 / sample) or high (H; >\$100 / sample). **Training requirements** are listed as low (L), medium (M) and high (H), and the transparency of the results for **regulatory** bodies given as easy (E), medium (M) or difficult (D). The development path of each category is indicated after the present status separated by a “?” with the time for development indicated as 1-2 years (+), 3-5 years (++), and 6-10 years (+++).

### **ANNEX 4-3: REPORT FROM THE BREAKOUT GROUP 3: REGULATORY ISSUES**

Chair: Maurice ZEEMAN

Co-chairs: Tohru INOUE, Raffaella CORVI

Other participants: Marianne RAPPOLDER, Chisumi ETO, Fumi IRIE, Saburo MATSUI, Ho-II KANG, Przemyslaw FOCHTMAN, Bettina HITZFELD, Kevin CHIPMAN, Kerry WALSH, Sigmund DIGITZ, Robert HOKE

#### **Conclusion and recommendations:**

- Further OECD/IPCS discussion to 1) integrate (conclusions) recommendations of both reports and 2) divide next tasks. (IPCS/OECD Berlin, Nov. 2003)
- Ecotoxicogenomics not yet developed enough for risk assessment decisions or replacement of existing approaches, but may be useful to provide supportive evidence on a case by case basis. (modified from IPCS/OECD Berlin, Nov. 2003)
- Priority for sequencing should be given to species currently used in the ecotox regulatory context (see Table 2) and should also include Algae, Lemna, Japanese quail, Honey bee and Chironomids. (modified from SETAC/SOT, Oregon, July 2004)
- Design (and use) additional “omic” endpoints into existing ecotox studies (methods). (SETAC/SOT, Oregon, July 2004)
- OECD should develop an initial policy for the evaluation and use of “omics” data for regulatory purposes.
- Health and ecotox regulators need to interact closely in the future.
- The scientific and regulatory communities must acknowledge the need for suitable workforce (e.g. education in “omics”).

#### **Specific questions/answers:**

The group decided to focus on ecotoxicology (rather than mammalian toxicology) and hazard assessment (rather than risk assessment). The group focused on what can be achieved in the next years.

Definition: toxicogenomics includes transcriptomics, proteomics and metabolomics.

#### ***1. How can regulators use genomic information if submitted?***

- Used to support or refute mode of action.
- Present available policies (EPA, FDA) are based on a case by case basis evaluation.

- EU has no real interim policy on toxicogenomics. For pharmaceutical, policy on a case by case basis, but data will only be used if a clear interpretation of response can be made.
- For chemicals (EU and Japan) assessment is not carried out on a case by case basis.
- Need for training of regulators in correct understanding of toxicogenomics in safety assessment, and also for training and greater appreciation on the part of scientific communities of the regulatory requirements in hazard and risk assessment.
- Health and ecotox regulators need to interact stronger than in the past.
- Validation is definitely needed if tests are required by regulatory authorities. Maybe not if data submitted on voluntary basis.

***2. What regulatory decisions could genomic-based methods support, and how? (Replacing existing toxicity tests? As an additional endpoint? Reducing uncertainty in inter-species extrapolation? Supporting QSARs and categorisation of chemicals?) What validation approaches are appropriate for these regulatory uses?***

- This approach will add confidence to the derivation of NOAELs.

***3. In the evaluation of genomic responses, what are the steps necessary to distinguish toxic response from compensatory or adaptive responses? If so, how?***

- Not all changes are adverse. Understand what is inherent, biological variability (e.g. control animals under laboratory or field circumstances). Compensatory changes have the potential to be useful in hazard identification. In short-term may not be related to toxicity, but may in some cases give rise to adverse effects in the long-term (e.g. non-genotoxic carcinogens which might lead first in loss of cell communication) and metabolic changes.

***4. How can regulators have confidence in the data from genomic-based methods?***

- QA, GLP-like, best practice for toxicogenomics
- Standardization more difficult in ecotox.
- At present consider these data as supportive data linked to functional markers for which we have GLP data
- How to convince scepticism of regulators if test is not validated?
- Validation is needed if tests are required by regulatory authorities. Maybe not if data submitted on voluntary basis.
- Considering case by case based data, confidence will be supported by: 1) improved harmonized quality controls, 2) link response to those that are known mechanisms and endpoints.
- In long-term an understanding of pattern of changes will be used in a predictive way.

**5. How should issues of intellectual property right be taken into consideration in the regulatory use of genomic-based methods?**

- In the short-term we don't envision regulatory bodies to require "omics" data on a routine basis.
- In the long-term it is likely that regulatory agencies will be faced with trade mark issues. We have precedent that thus far regulatory agencies have not required the use of patented/trademark methods.
- To the extent possible it will not be recommended to use trade mark methods.

**6. Why do we need toxicogenomics for hazard and risk assessment?**

- In the short term, useful as supportive to established endpoints.
- In the long term, may provide improved predictivity and a better understanding of mechanisms. Thus, they may help to refine hazard identification and risk assessment.

**7. Will ecotoxicogenomics assist us in assessment of chemicals and mixtures?**

- e.g. Elucidate if single mechanism or multiple mechanisms.
- Use to prioritize chemicals (eg. REACH assessment) on structure analogues basis or chemical class.
- Global patterns of change may indicate mode of action

**8. Ecotoxicogenomics and interspecies extrapolation?**

- What are the situations where it could be possible in a "short-term"? Use data to help with threatened species
- Defined gene expression profiling may help interspecies extrapolation even without dosage information (independent of species sensitivity).
- Knowing mechanisms and/or modes of action it would reduce uncertainties and allow better interspecies extrapolation. Consider all "omics".

**9. Prioritization of ecotox species for sequencing and use in ecotoxicogenomics.**

- Consider guidance from SETAC-SOT (North America biased).
- Selection criteria: choose existing ecotox models
- Identify closely related species with high homology and world wide distribution. Species have already been chosen by US, EU and Asian national regulators.

Table 2: Modified list for priority species for sequencing and use in ecotoxicogenomics

	Common name	Scientific name	Sequencing
Invertebrates	Nematode	<i>Caenorhabditis elegans</i>	sequenced
	Earthworm	<i>Eisenia fetida</i>	
	Water flea	<i>Daphnia pulex</i>	sequencing in progress
	Water flea	<i>Daphnia magna</i>	EST sequencing
	Mysid species		
Fish	Zebrafish	<i>Danio rerio</i>	sequenced
	Medaka	<i>Ozyrias latipes</i>	sequenced
	Three-spined stickleback	<i>Gasterosteus aculeatus</i>	in progress
	Fathead minnow	<i>Pimephales promelas</i>	EST
	Rainbow trout	<i>Oncorhynchus mykiss</i>	
Amphibians	African frog	<i>Xenopus tropicalis</i>	near complete sequence
Birds	Mallard	<i>Anas platyrhynchos</i>	
In addition:			
	Algae		
	Lemna		
	Japanese quail		
	Honey bee		
	Chironomids		

#### **ANNEX 4-4: REPORT FROM THE BREAKOUT GROUP 4: BIOINFORMATIC ISSUES**

Chair: George DOUGLAS

Co-chair: Kazumi KAWAHARA

Rapporteurs: Susanna-Assunta SANSONE, Weida TONG

Other participants: Yuji OSHIMA, Masao TANJI, Nobuaki TOMINAGA, Tomonari MATSUDA,  
Hajime WATANABE, Norihisa TATARAZAKO

The following recommendations provide the framework for achieving the benefit of –omics technologies for the assessment of ecological hazard and risk. Not implementing them would have far-reaching, serious consequences for improving global ecosystem health.

##### **General recommendations:**

1. Stable funding and adequate funding must be provided to a national and international level for standardization initiatives, database and tool development and long-term maintenance.
2. Provide training in data requirements and bioinformatics issues relating to ecotoxicogenomics for risk assessors.
3. Governments should promote synergy between the toxicology and ecotoxicology communities, including a dialogue between scientists, regulators and bioinformaticians on an international scale.
4. In the broader context of ecotoxicogenomics studies, initial efforts should be centered on a few species representative of the ecological complexity.
5. Establish a task force/working group to implement these recommendations and coordinate future actions.

##### **Specific recommendations:**

1. Governments should promote and financially support development of international standards for data communication format and data annotations (terminology, ontology) within eco-omics technologies.
  - 1.1. Initiatives such as the Microarray Gene Expression Data (MGED) Society ([www.mged.org](http://www.mged.org)), the Human Proteome Organisation (HUPO) Proteomics Standardization Initiative (PSI) ([psidev.sourceforge.net](http://psidev.sourceforge.net)) and the Standard Metabolic Reporting Structure (SMRS) ([www.smrsgroup.org](http://www.smrsgroup.org)) are critical for building internationally compatible infrastructure and allow a meaningful data sharing. There should be a value in encouraging a compatibility (where possible) between regulatory and research requirements in the design of these standards.

2. Develop an integrated, modular data model combining -omics with toxicological endpoints and chemical structures.
  - 2.1. Within the MGED Society and the RSBI WGs the toxicology and environmental communities have initiated the development of a reporting structure for describing –omics based experiments, assisting in the identification technical measures correlated with data interpretability.
  - 2.2 The integration of microarray data with conventional toxicological data and chemical structure is demonstrated in ArrayTrack (Table 3), a toxicogenomics software developed by NCTR/FDA.
3. Infrastructure for data management (reporting, archiving, curation, user support and querying) and analysis are pivotal to the data interpretation. Governments should promote and provide long-term support for the development and maintenance of (flexible, expandable, public, centralized, standard supportive) databases and analysis tools.
4. Expand suitable infrastructure in support of ecotox data requirements to avoid duplication of effort, maximizing compatibility and optimizing use of funds.
  - 4.1. A list of available standard-compliant and highly collaborative bioinformatics infrastructures (to date) for environmental and mammalian toxicogenomic data is summarised in table 3.
5. Database and analysis tools should be transparent, data schema should be thoroughly documented and analysis methods should be peer-reviewed.
6. Bioinformatics approaches (methodologies and tools) should be developed to predict population effects (hazard and risk) from ecotoxicogenomics data, including the effect of mixtures.
7. Biological pathway and regulatory network analysis should be emphasized to describe ecotoxicological effects.
  - 7.1 Such efforts should be placed in an international context and synergize with existing initiatives (Reactome, GO etc)
8. Define core biological processes across species
  - 8.1 Developing a list of genes having a conserved function (phenotype) across species is one way of assisting in the comparisons of effect across different organisms used in ecotox studies

### **Definitions:**

This section defines general bioinformatics tasks involved in the field of “omics” research with emphasis on the array-based technology.

- Experimental design – This is the first bioinformatics step towards a valid “omics” experiment. In this step, the questions such as the number of samples and replicates need to be addressed to ensure a statistically sound experiment. The choice of a design method is largely dependent on research hypothesis and availability of resources (e.g., animals and chips) for conducting the experiment.
- Data management – This step is focused on data capturing and archiving. A validated database should permit meaningful comparison across experiments, labs, platforms. But most importantly, data in the database should be allowed easily communicated with other databases. This requires appropriate annotation to capture experimental metadata and genes/proteins sequences and chemical structure of metabolites when possible.

- QCs – Quality control metrics and threshold are essential for regulatory acceptance of the “omics” data. The QC metrics determines the minimal level of performance that the biological significance in the experiment could be identified.
- Normalization – This removes systematic error associated with an individual experiment and allows cross-experiment comparison. Different “omics” technologies might use different normalization methods.
- Analysis and data interpretation – There are a number of analysis methods important for this task, including methods for the significant genes/proteins/metabolites identification, clustering and classification and data interpretation. “Omics” data integration, network identification and pathway analysis should be emphasized.

**Questions/answers:**

***1. Are ecotoxicogenomics data unique from other toxicogenomic data?***

YES

Recognizing uniqueness of ecotoxicity

- Different type of experiment designs
  - Field experiments (uncontrolled)
  - Conditioned field experiments (uncontrolled)
  - Lab experiments (controlled)
- Data capturing is more difficult
  - Larger number of experimental parameters
  - Challenge to describe organisms and variable at sampling sites
  - Downstream analysis maybe be hampered
- Challenge to design the experiments

***2. What informatics tools and approaches are required to facilitate comparative genetics and comparative genomics?***

Currently limited systems available to compare across species used in ecotox studies

- e.g. limited genome sequences, annotations
- Techniques such use of ESTs, comparative hybridization and SAGE would provide bridging capabilities to compare across species.

Develop methods to weight ‘omic’ data based on phylogenetic tree

Track biological changes (post-translational changes) of specific isoforms/splice variants

**3. Are the bioinformatics structures set up to handle data for mammals sufficient to address the needs of scientists working in ecotoxicogenomics?**

Table 3: Available standard-compliant bioinformatics systems (to date) for environmental and mammalian toxicogenomic data are summarised in the following table.

Systems	Strengths	Limitations (as of October 04)
<b>Environmental genomics system</b>		
UK Natural Environment Research Council (NERC): Maxd	<ul style="list-style-type: none"> <li>• Data management and visualization environment</li> <li>• MIAME and MIAME/Env compliant</li> <li>• MAGE-ML export</li> <li>• Extendable/customable</li> <li>• Freely available software</li> <li>• Freely available source code</li> <li>• Pipeline to ArrayExpress</li> </ul>	<ul style="list-style-type: none"> <li>• Currently only for array-based environmental experiments</li> <li>• Currently only supporting NERC community</li> <li>• Not a public database (but pipelined with ArrayExpress)</li> </ul>
<b>Mammalian toxicogenomics systems</b>		
FDA National Center for Toxicological Research (NCTR): ArrayTrack	<ul style="list-style-type: none"> <li>• Data management and analysis environment</li> <li>• Tools and libraries</li> <li>• MIAME and MIAME/Tox compliant</li> <li>• MAGE-ML export</li> <li>• Freely available software</li> </ul>	<ul style="list-style-type: none"> <li>• Currently only for array-based experiments</li> <li>• Source code not yet available</li> <li>• Only supporting NCTR-FDA community/users</li> <li>• Not a public database</li> </ul>
NIEHS National Center for Toxicogenomics (NCT): CEBS	<ul style="list-style-type: none"> <li>• Public infrastructure</li> <li>• MIAME and MIAME/Tox compliant</li> <li>• MAGE-ML export</li> <li>• Pipeline to ArrayExpress</li> </ul>	<ul style="list-style-type: none"> <li>• Currently under development (10 years dev cycle 2002-2012)</li> </ul>
European Bioinformatics Institute (EBI): ArrayExpress	<ul style="list-style-type: none"> <li>• Public infrastructure</li> <li>• MIAME and MIAME/Tox compliant</li> <li>• MAGE-ML export</li> <li>• Closely collaborative with other MIAME-compliant systems GEO (at NCBI, USA), CIBEX (at DDBJ, Japan) and other systems as listed above</li> </ul>	<ul style="list-style-type: none"> <li>• Only for array-based experiments</li> </ul>

General issues on bioinformatics structures are:

- Interoperability
- Limited funds
- Users support
- Long term maintenance

**4. Is the MIAME/Env scheme suitable for ecotoxicity?**

YES

- Good attempt to capture the information consistently
  - Defined a structured framework
  - Identify controlled vocabulary to annotate the information
- Has been tested by NERC on ‘real data’
  - Help to harmonize the description but do not prescribe how the experiment is done
- Caveats:
  - Only for array-based experiments
  - Scheme is suitable and can be extended if needed
  - MIAME/Env is a structured framework but the list terms is not exhaustive
- Currently under the MGED RSBI WGs this framework is being revisited and extended.

**5. What bioinformatics (analysis) tools have you found valuable? Why?**

**6. What needs to be done to apply molecular and computational toxicological information to human and environmental risk assessment?**

- Train risk assessors to the use of the technologies

**7. What types of genomic and/or proteomic data will be most useful to develop interconnections between the human and ecological risk paradigm?**

- Ecological data connected to human genome, proteomics and metabolites
  - Genotype-phenotypic anchoring
  - Parallelogram with humans
- Use well-defined datasets, such as rodent tox data, to phenotypically anchor ‘omic’ data and refine algorithms aimed at predicting toxicological outcomes

**8. How can integration of cross-species genomic analyses be used to address common issues and provide solutions?**

- Computational methods for ortholog identification
  - Developing a list of genes having a conserved function (phenotype) across species is one way of assisting in the comparisons of effect across different organisms used in ecotox studies
  - Define core biological processes across species