NORMAN WORKSHOP Emerging Chemicals, Oct. 2007

# The pros and cons of using genomics to assess emerging chemicals

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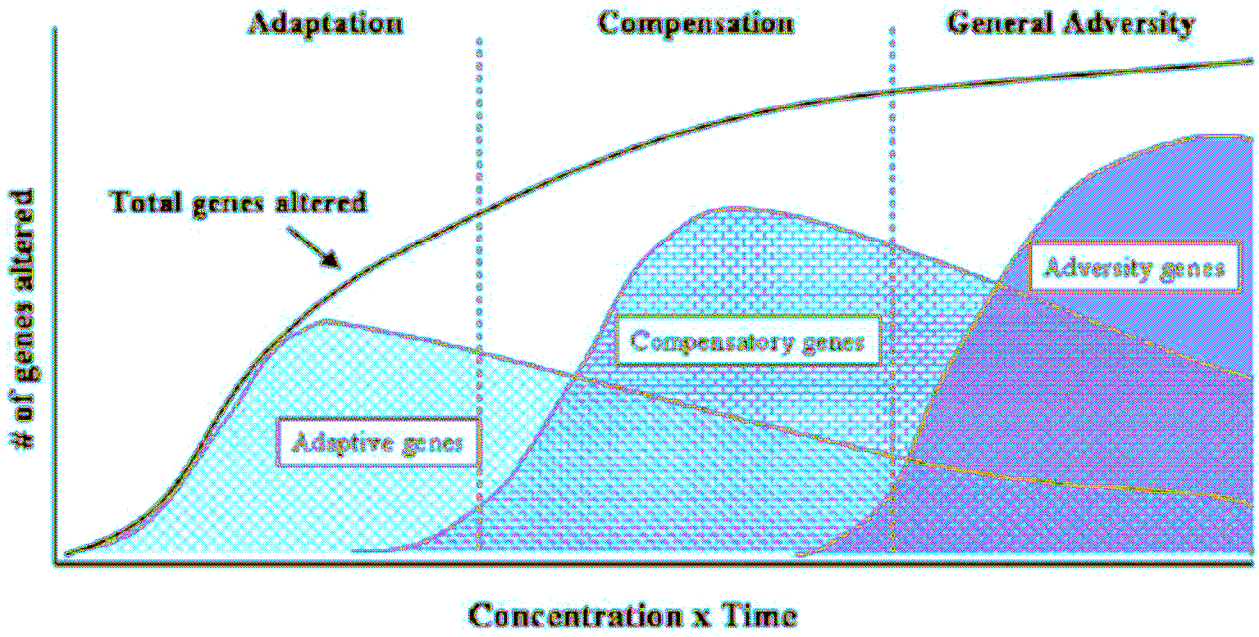
vrije Universiteit amsterdam

# Genomics in toxicology

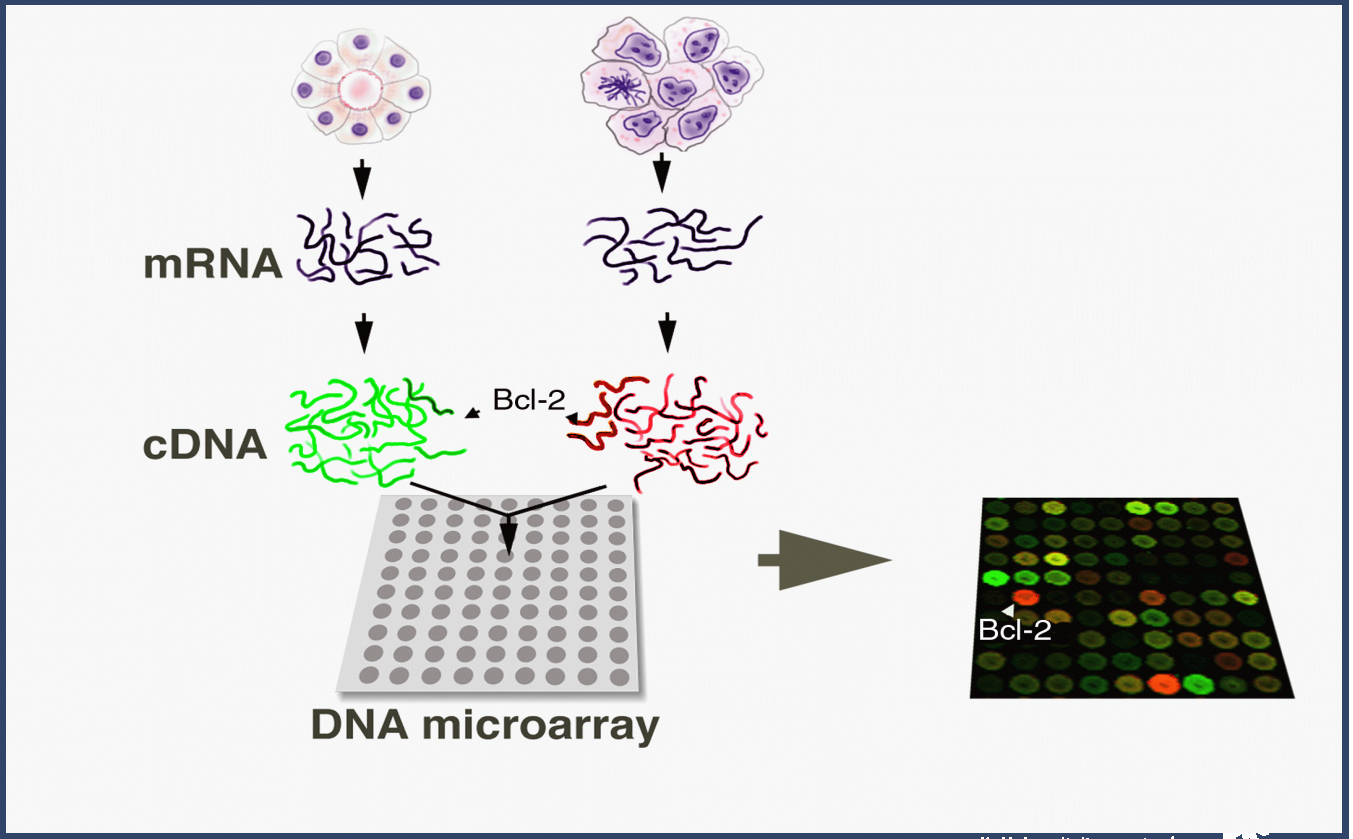
- Toxicogenomics: the “study of the relationship between the structure and activity of the genome and the adverse biological effects of exogenous agents”
- characterize changes in gene expression in cells or tissues after exposure to toxicants
- toxicologically relevant outcomes are based on differential gene expression



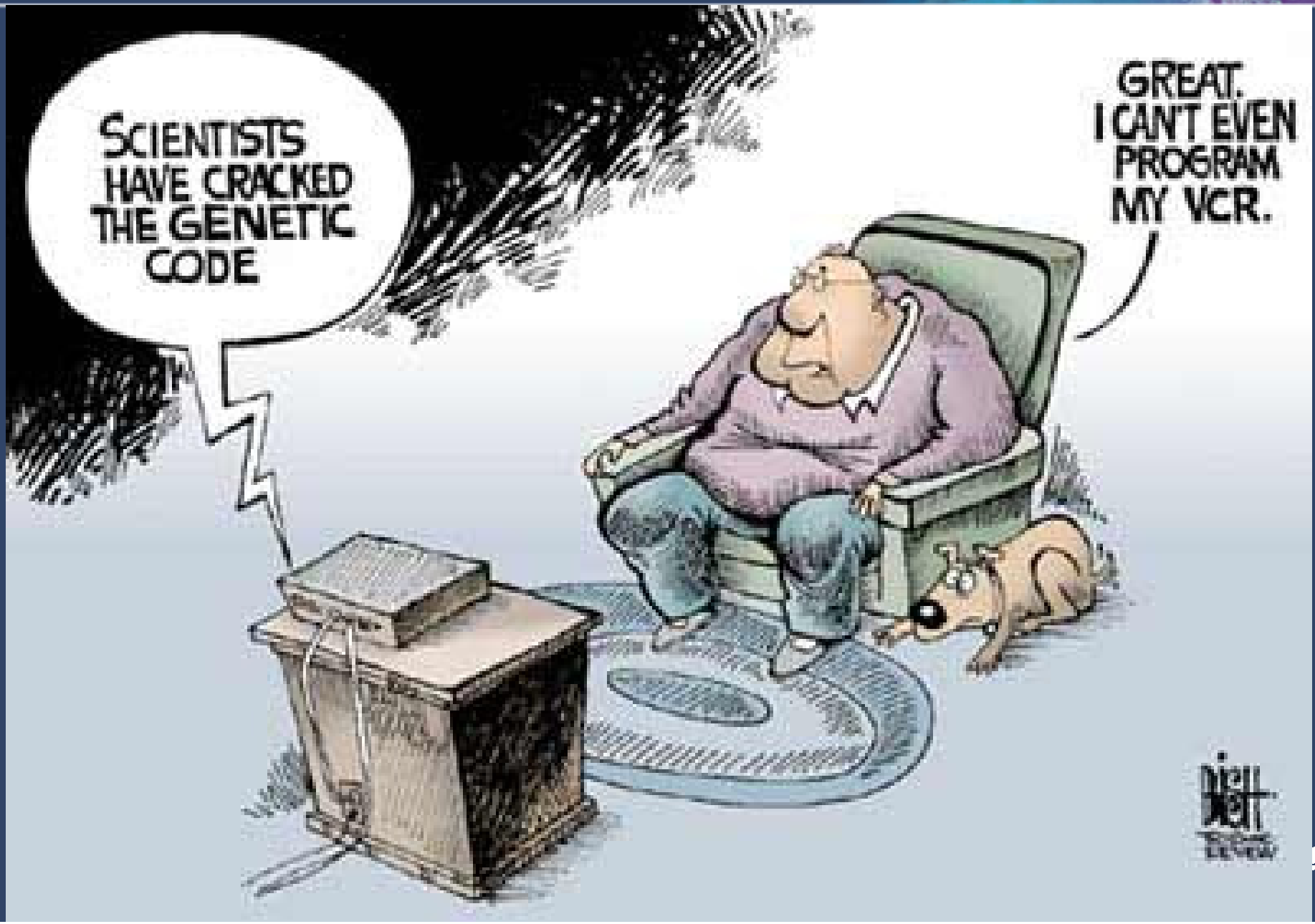
# Genomics in toxicology: microarray technology

- instantaneous and simultaneous genome-wide detection of the expression of thousand of genes, even if the function of some of the genes is unknown.

"a revolution in our ability to characterize simultaneously an unprecedented number of biological endpoints"

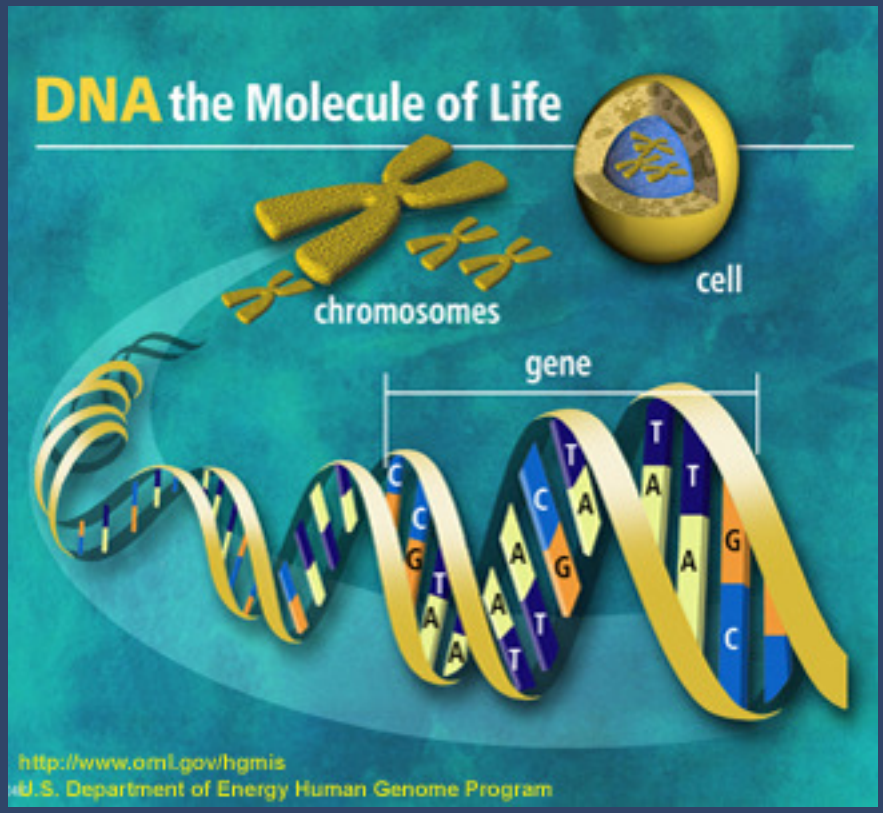


# How can we use genomics to assess emerging chemicals?



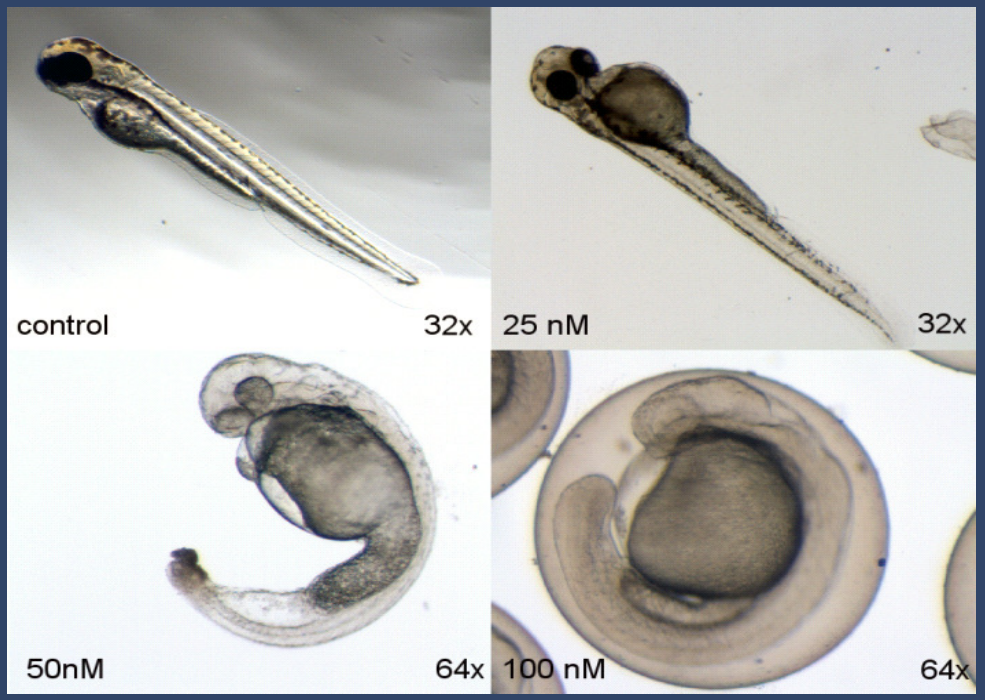
# Genomics to assess emerging chemicals

PROS



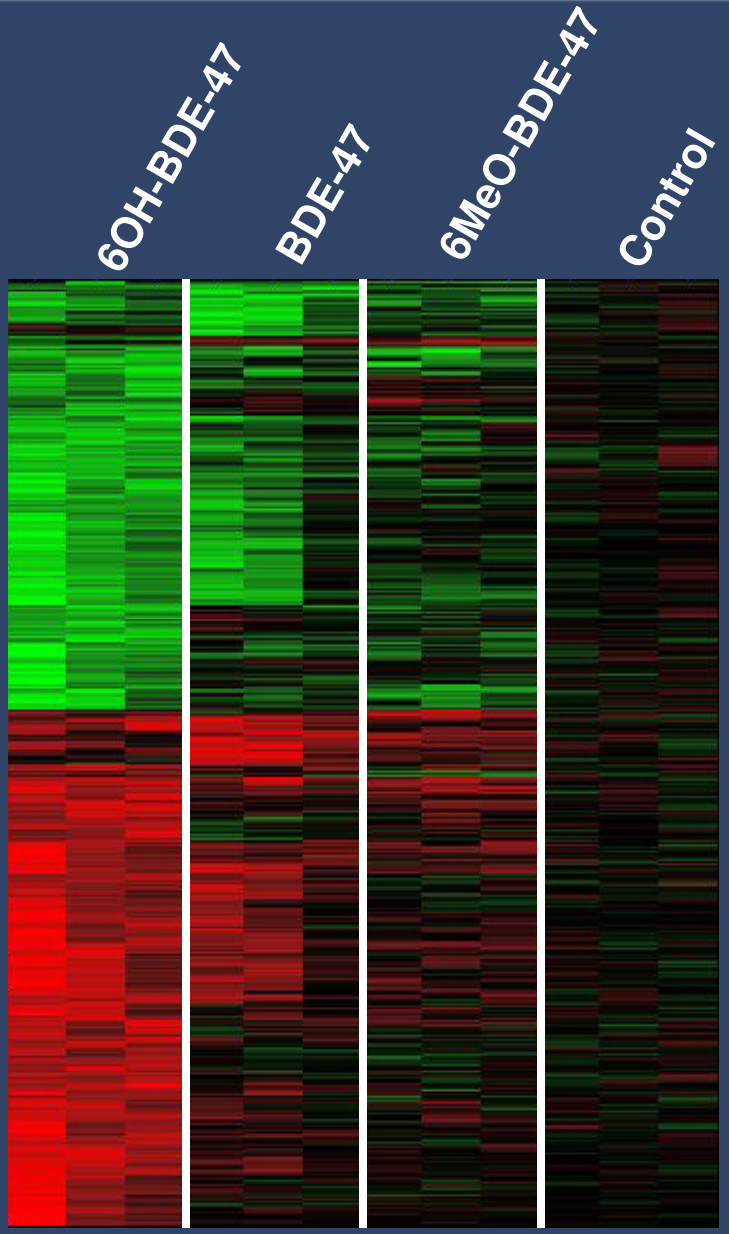
# Genomics can help reveal mechanisms of action

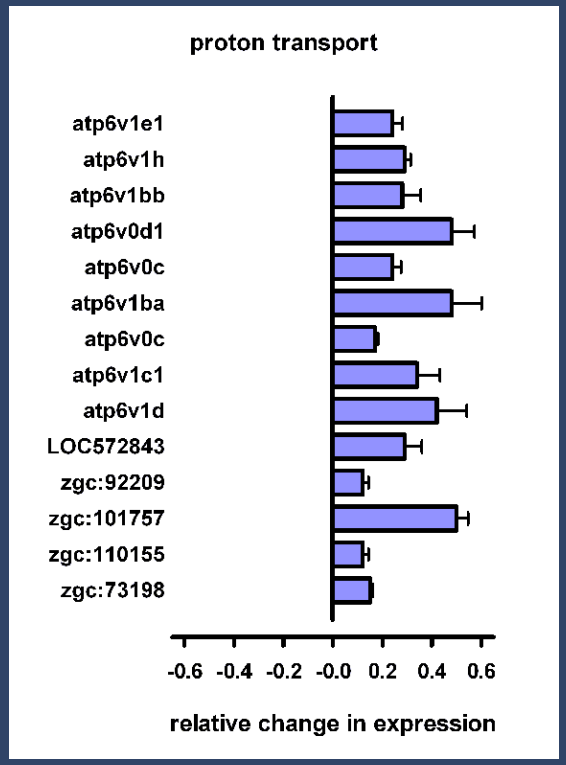
*T. Van Boxtel et al, 2007*

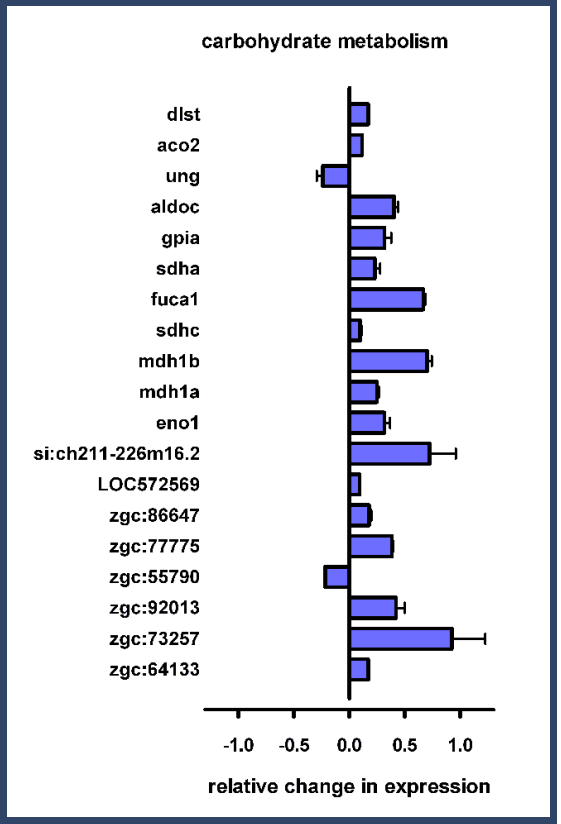



*Environmentally relevant metabolite of BDE 47 (6-OH BDE 47) is toxic to developing zebrafish embryo*

# Genomics can help to reveal mechanisms of action



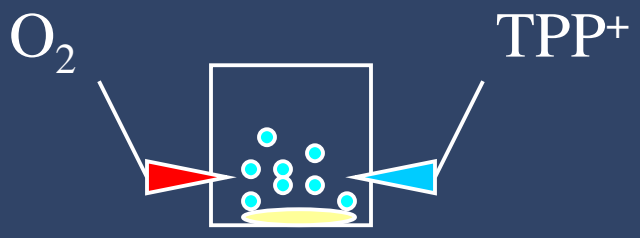



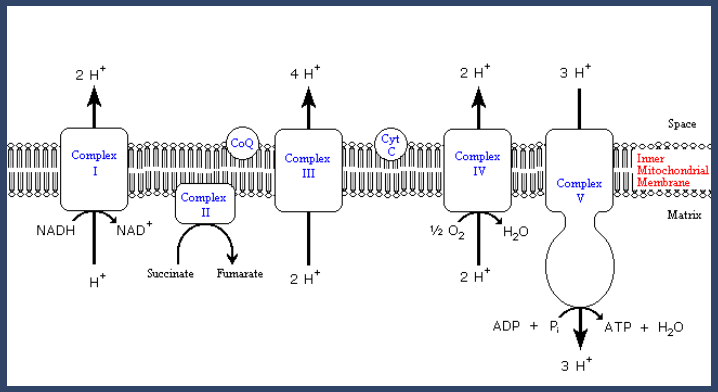



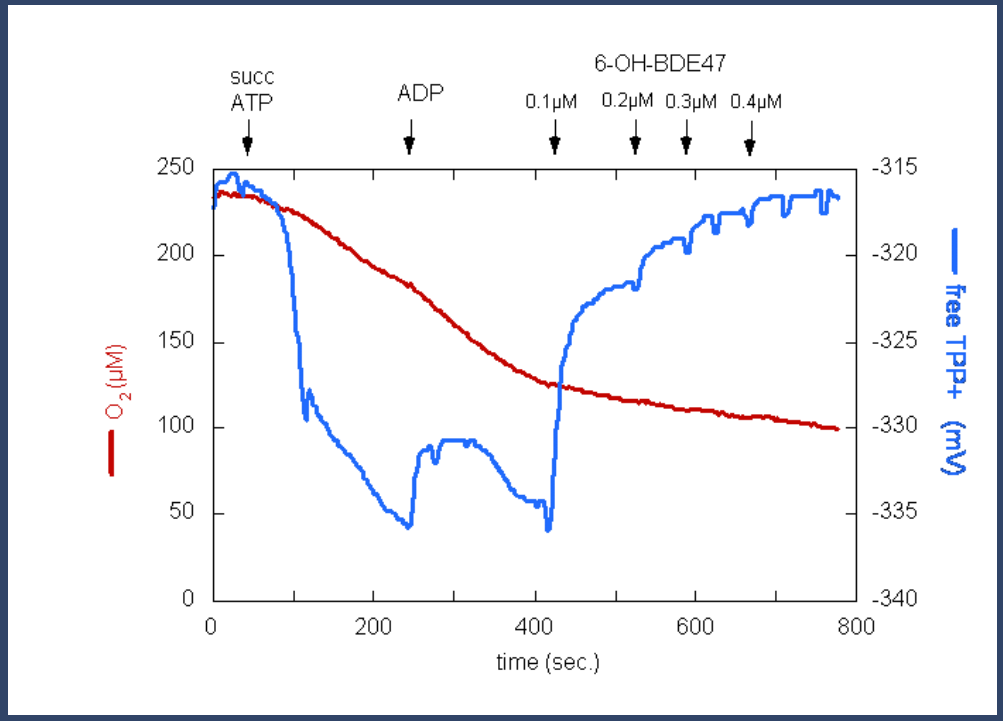

*Van Boxtel et al, 2007*

vrije Universiteit amsterdam

# Genomics can help to reveal mechanisms of action




*Van Boxtel et al, 2007*

6-OH-BDE47 is an uncoupler of oxidative phosphorylaton

# Genomics can reveal 'signatures' of toxicity

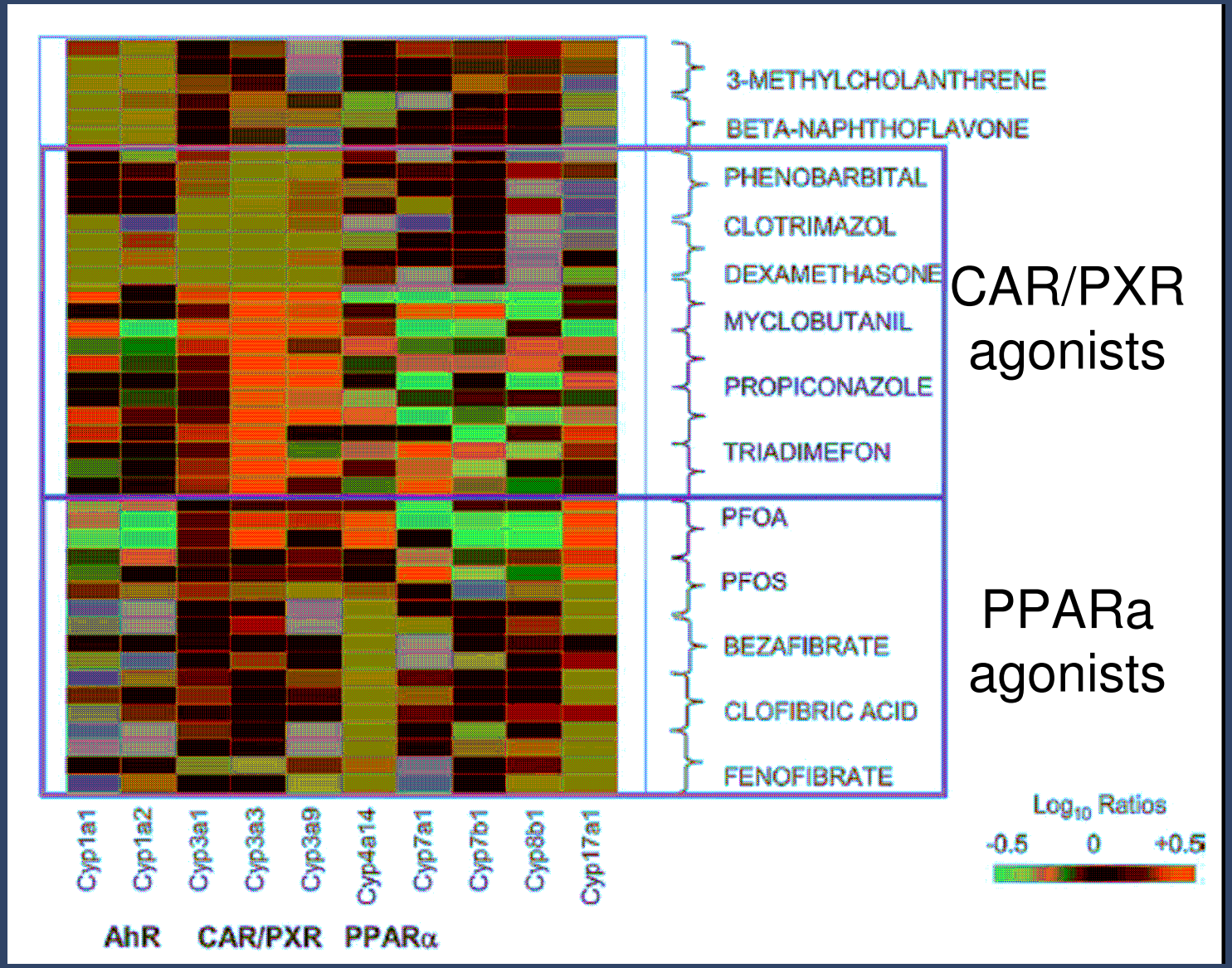
TOXICOLOGICAL SCIENCES 97(2), 595–613 (2007)
doi:10.1093/toxsci/kfm065
Advance Access publication March 22, 2007

## Toxicogenomic Study of Triazole Fungicides and Perfluoroalkyl Acids in Rat Livers Predicts Toxicity and Categorizes Chemicals Based on Mechanisms of Toxicity

Matthew T. Martin,* Richard J. Brennan,‡ Wenyue Hu,‡ Eser Ayanoglu,‡ Christopher Lau,‡ Hongzu Ren,† Carmen R. Wood,† J. Christopher Corton,† Robert J. Kavlock,* and David J. Dix*,1

- Gene expression profiles in rats exposed to three triazole antifungals (myclobutanil, propiconazole, and triadimefon) and two perfluorinated chemicals [perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS)]
- Compare gene expression profile to database of 630 chemicals

# Genomics can reveal ‘signatures’ of toxicity



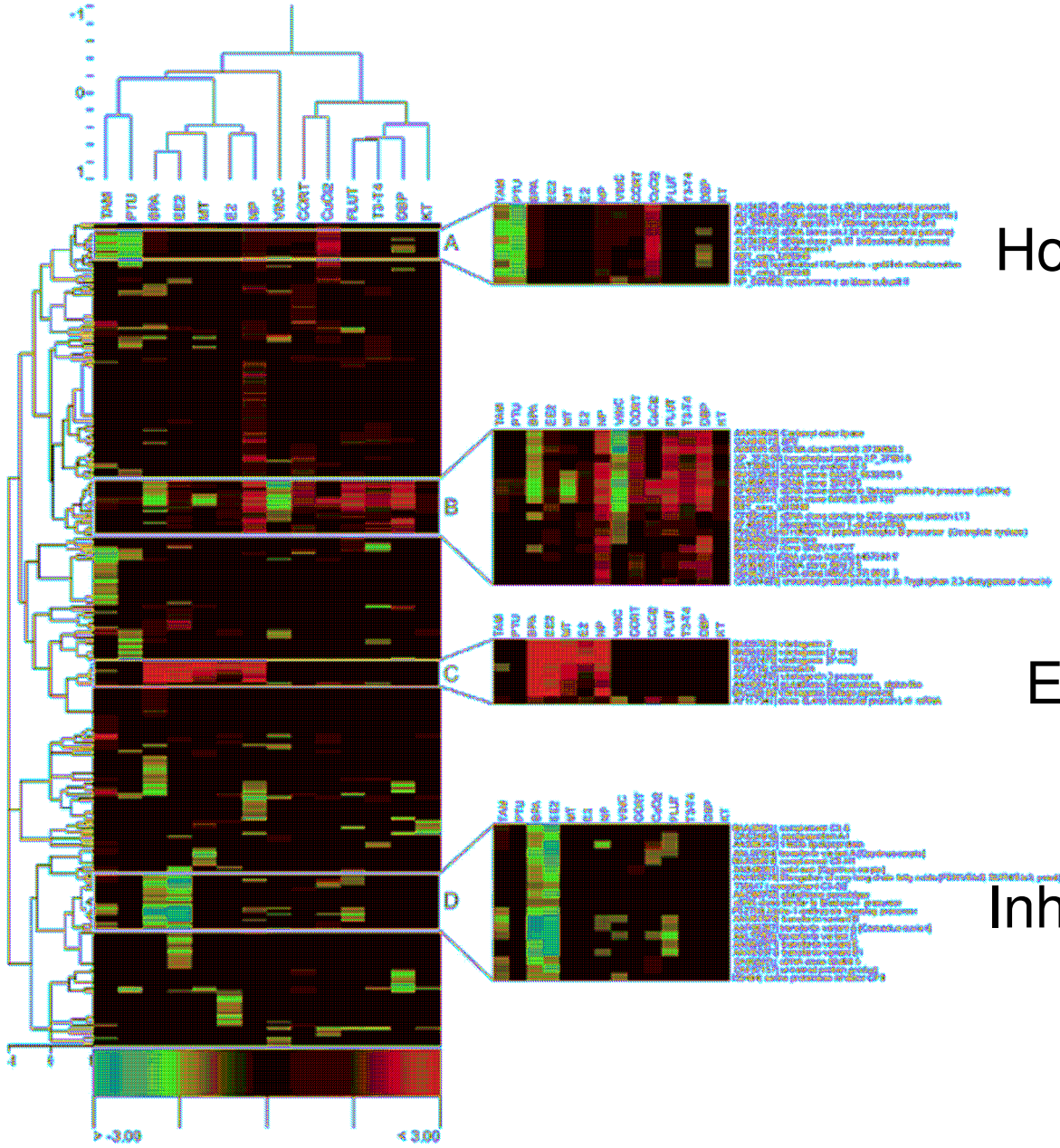
# Genomics can identify biomarkers/classifiers of toxicity

TOXICOLOGICAL SCIENCES 93(2), 298–310 (2006)
doi:10.1093/toxsci/kfl057
Advance Access publication July 11, 2006

## Expression Profiling of Endocrine-Disrupting Compounds Using a Customized *Cyprinus carpio* cDNA Microarray

Lotte N. Moens,*,[1] Karlijn van der Ven,* Piet Van Remortel,† Jurgen Del-Favero,‡ and Wim M. De Coen*

- 17beta-estradiol, 17alpha-ethinylestradiol, 4-nonylphenol, bisphenol A, tamoxifen, 17alpha-methyltestosterone, 11-ketotestosterone,dibutyl phthalate, flutamide, vinclozolin, hydrocortisone, CuCl2, propylthiouracil, and a mixture of L-triiodothyronine and Lthyroxine

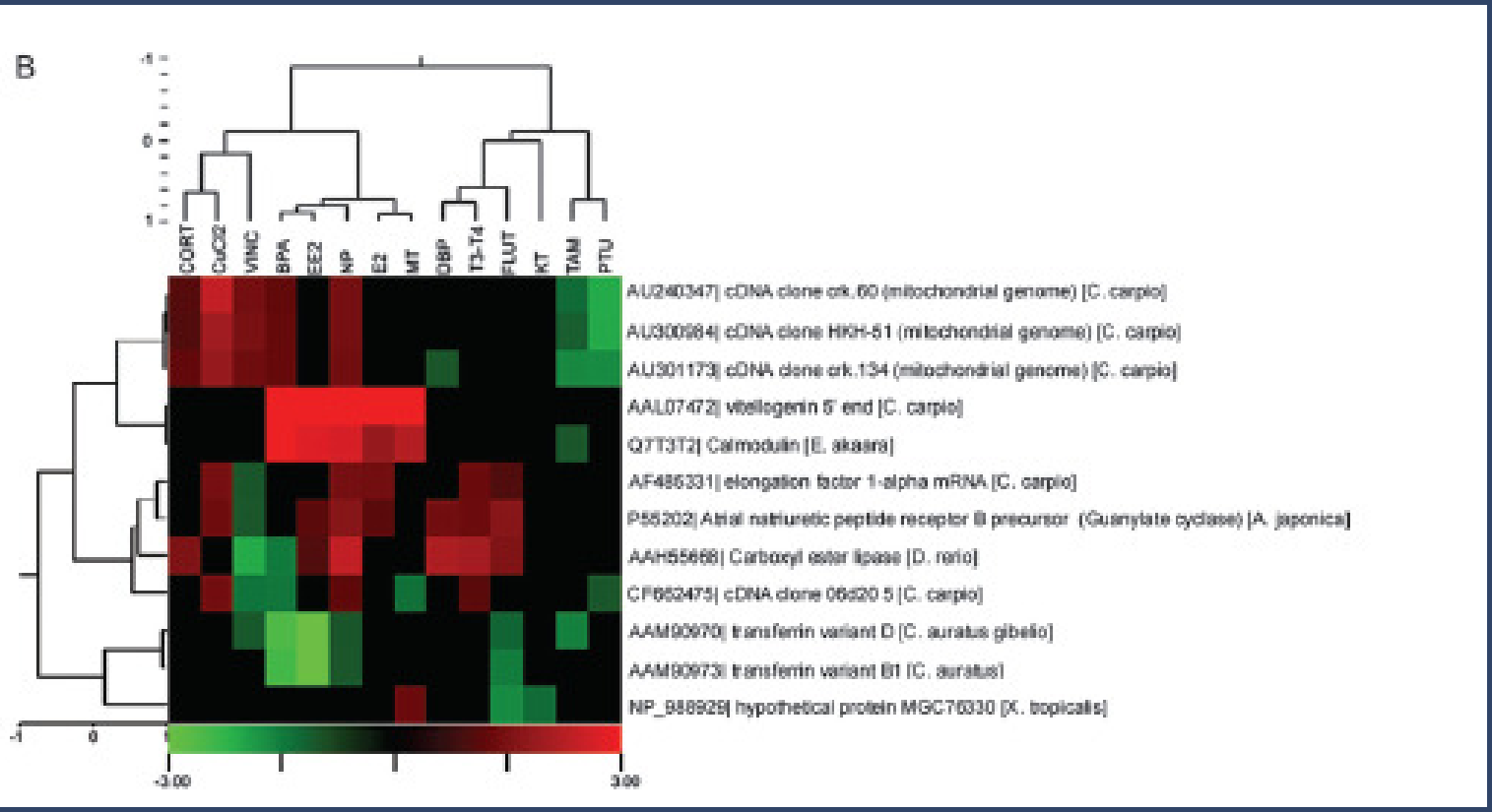



Hormone antagonists

Estrogen agonists

Inhibition transferrin genes

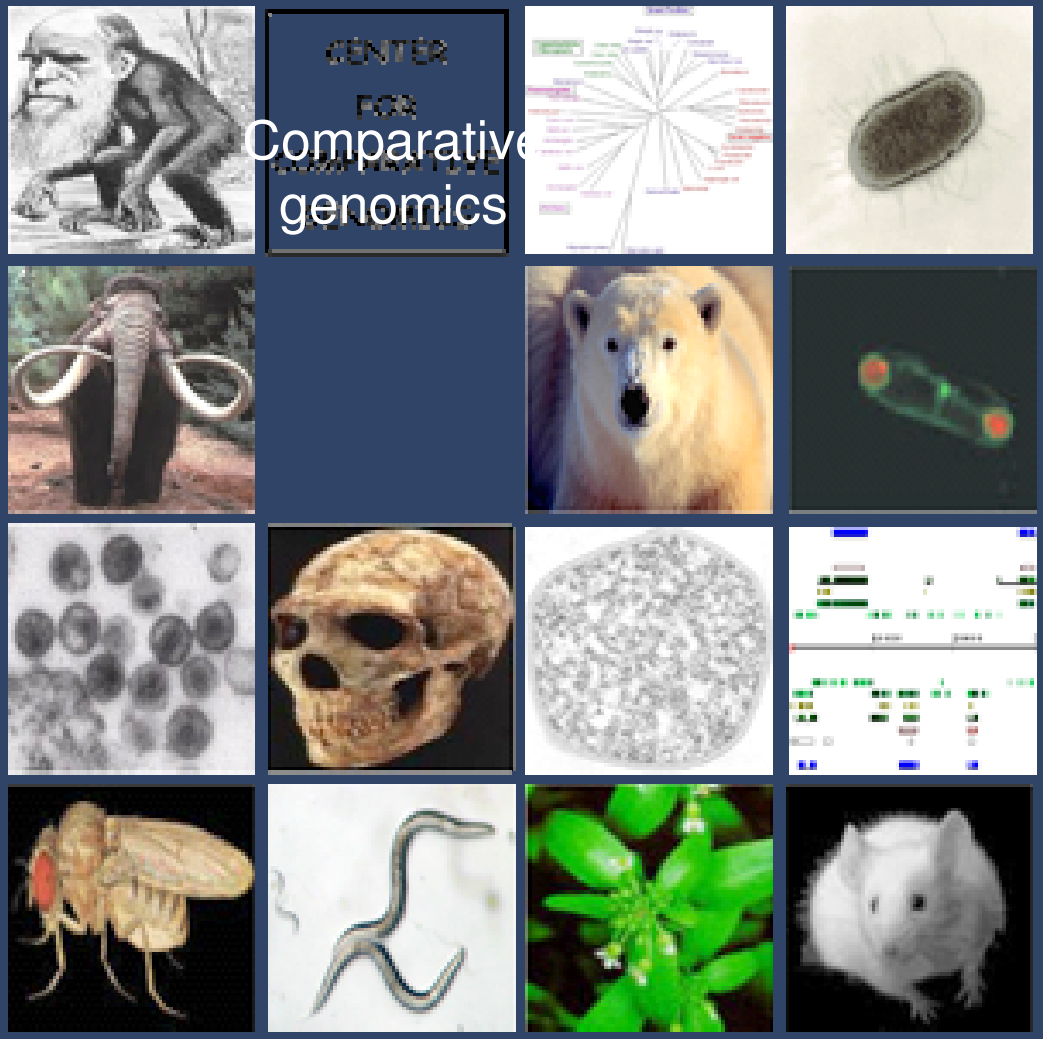
# Genomics can identify biomarkers/classifiers of toxicity




- Generational genetic algorithm: revealed a subset of genes that separated the compounds in an optimal way
- Subset of 12 genes discriminates endocrine disrupting chemicals

# Comparative genomics between species:

# predicting toxicity in humans?



# Comparative genomics between species: predicting toxicity in humans?

TOXICOLOGICAL SCIENCES 94(1), 71–82 (2006)
doi:10.1093/toxsci/kfl080
Advance Access publication August 17, 2006

## Gene Expression Profiles in Fathead Minnow Exposed to 2,4-DNT: Correlation with Toxicity in Mammals

Henri Wintz,*,[1] Leslie J. Yoo,† Alex Loguinov,* Ying-Ying Wu,* Jeffrey A. Steevens,† Ricky D. Holland,‡ Richard D. Beger,‡ Edward J. Perkins,† Owen Hughes,§ and Chris D. Vulpe*

*Department of Nutritional Sciences and Toxicology, Morgan Hall and Berkeley Institute of the Environment, University of California, Berkeley, California 94720; †US Army Corps of Engineer Research and Development Center, Environmental Laboratory, Vicksburg, Mississippi 39180; ‡Division of Systems Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079; and §Eon Corporation, Davis, California 95616

# Comparative genomics between species:

## predicting toxicity

Fish and rodents: similar metabolic pathways affected by 2,4 DNT

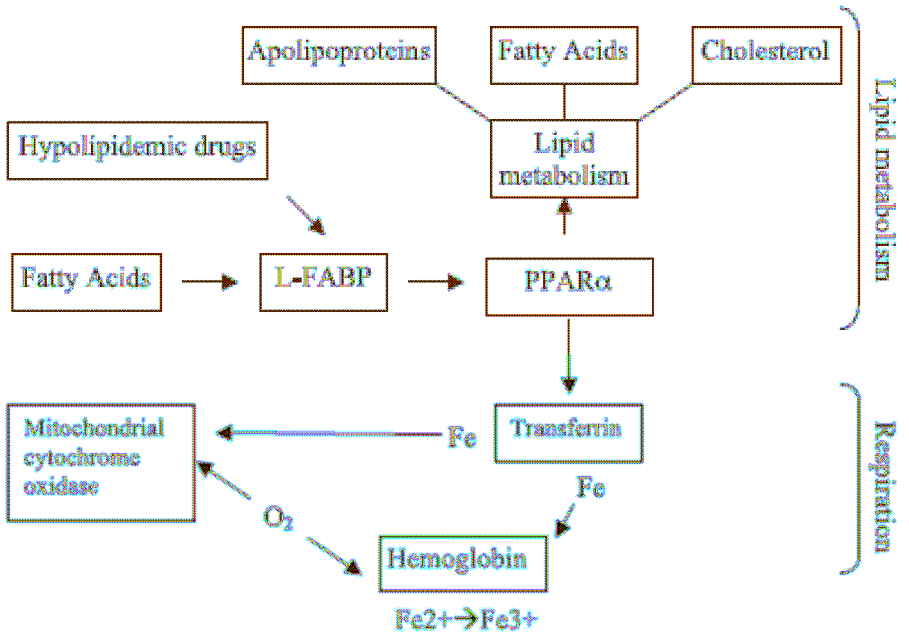



FIG. 2. Metabolic pathways affected by 2,4-DNT in fathead minnow liver. Genes affected by 2,4-DNT and how they relate to each other within known pathways are represented. Fatty acids or hypolipidemic drugs signals are relayed to the nucleus via the L-FABP, where it activates PPARα, which controls expression of lipid metabolism genes (apolipoproteins and fatty acid metabolism genes) as well as Tf gene. Transferrin carries iron, which is an essential cofactor of hemoglobin and of the mitochondrial respiratory chain. Oxygen is a substrate of cytochrome oxidase and hemoglobin. 2,4-DNT is known to affect oxygen transport by oxidizing hemoglobin ferrous iron to its ferric state.

# Application of genomics to “ecotox” species/models

Ecotoxicogenomics: gene expression in non-target organisms in response to environmental toxicant exposures

# Application of genomics to “ecotox”

*Environ. Sci. Technol.* **2007**, *41*, 1044–1050

## *Daphnia magna* Ecotoxicogenomics Provides Mechanistic Insights into Metal Toxicity

HELEN C. POYNTON,† JULIA R. VARSHAVSKY,† BONNIE CHANG,† GIORGIO CAVIGIOLIO,‡ SARAH CHAN,† PATRICIA S. HOLMAN,† ALEXANDRE V. LOGUINOV,† DARREN J. BAUER,§ KELLY KOMACHI,⊥ ELIZABETH C. THEIL,‡ EDWARD J. PERKINS,# OWEN HUGHES,⊥ AND CHRIS D. VULPE*,†

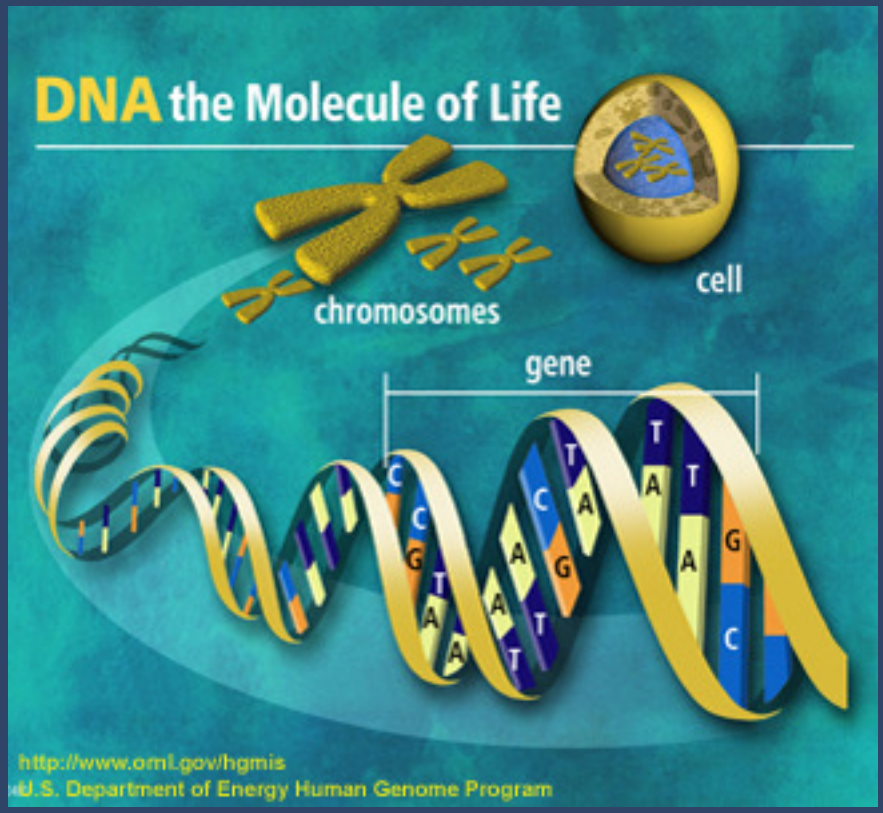
*Nutritional Sciences and Toxicology, University of California, Berkeley, California 94720, Center for BioIron at CHORI (Children's Hospital Oakland Research Institute), Oakland, California 94609, Hubbard Center for Genome Studies, University of New Hampshire, Durham, New Hampshire 03824, Eon/Terragenomics, Davis, California 95616, and Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, Mississippi 39180*

TABLE 1. Predicted Function of Differentially Expressed Genes after Exposure to Copper, Cadmium, or Zinc at the 1/10 LC50

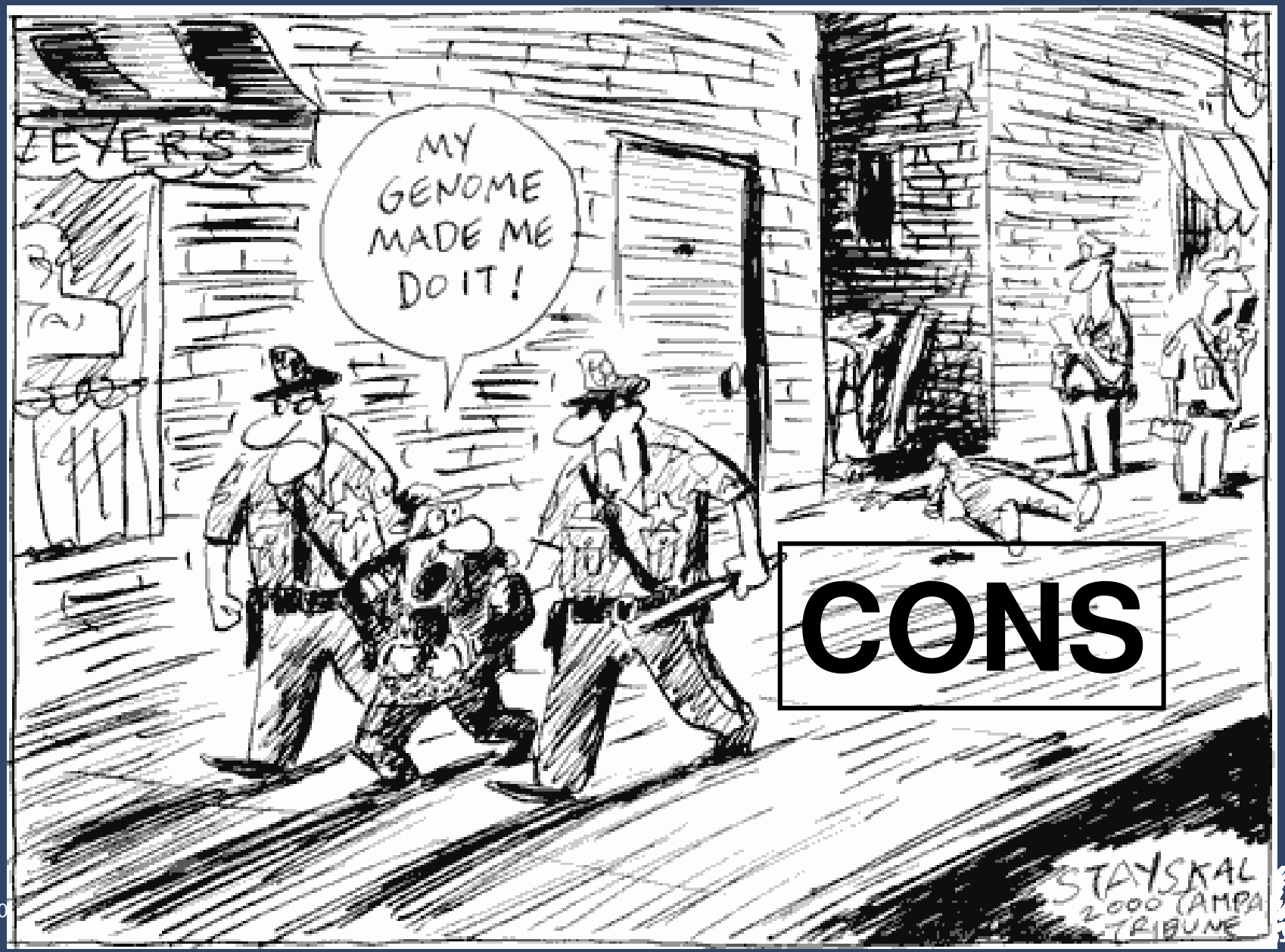
| Exp. level Cu Cd Zn | Acc # | Predicted Protein Class |
|---|---|---|
| **METAL BINDING AND TRANSPORT** | | |
| | AJ292559 | Ferritin |
| | DV437799 | Metallothionein |
| | DV437826 | Metallothionein |
| | DV437852 | Ferritin |
| | DV437849 | Ferritin subunit |
| | DV437835 | Heavy metal binding protein, Collagen α chain |
| **DIGESTION AND NUTRIENT ABSORPTION** | | |
| | DV437797 | Cellulase |
| | DV437798 | Endo-β-1,4-glucanase precursor |
| | DV437795 | Endo-β-1,4-mannanase |
| | DV437794 | α- amylase |
| | DV437819 | Cuticle |
| | DV437815 | Prealnylase precursor |
| **EXOSKELETON RELATED PROTEINS** | | |
| | DV437807 | Chitin binding and metabolism |
| | DV437809 | Chitinase |
| | DV437810 | Chitin binding and metabolism |
| | DV437850 | Chitin binding and metabolism |
| | DV437857 | Chitinase |
| | DV437858 | Chitinase precursor |
| | DV437836 | Cuticle protein |
| **CELL SIGNALING** | | |
| | DV437805 | Inositol monophosphatase |
| | DV437805 | Leucine-rich protein phosphatase |
| | DV437825 | Protein kinase |
| | DV437832 | Ras-related protein |
| **IMMUNE FUNCTION** | | |
| | DV437821 | Interferon γ-inducible protein |
| | DV437813 | Lectin-like protein |
| | DV437823 | β-1,3-glucan binding protein, Glycoside hydrolase 16 |
| | DV437848 | Carebellin precursor-like protein |
| **OXIDATIVE STRESS RESPONSE** | | |
| | DV437830 | Glutathione-S-transferase |
| | DV437833 | Glutathione-S-transferase |
| | DV437829 | Peroxiredoxin V protein |
| **MONOOXYGENASES** | | |
| | DV437798 | Monooxygenase |
| | DV437827 | Dopamine β-hydroxylase |
| | DV437820 | Dopamine β-hydroxylase |
| | DV437838 | Copper type II, ascorbate-dependent monooxygenase |
| **PROTEASES** | | |
| | DV437812 | Aminopeptidase |
| | DV437825 | Trypsin precursor |
| | DV437854 | Carboxypeptidase A1 precursor |
| | DV437853 | Trypsin |
| | DV437805 | Chymotrypsin BII precursor |
| | DV437822 | Zinc metallopeptidase |
| | DV437841 | Serine collagenase precursor |
| | DV437834 | Chymotrypsin precursor |
| | DV437842 | Serine protease |
| | DV437843 | Transmembrane serine protease |
| | DV437839 | Transmembrane serine protease |
| | DV437839 | Trypsin |
| | DV437840 | Trypsin |
| | DV437846 | Trypsin, cytoskeleton related |
| **SULFOTRANSFERASES** | | |
| | DV437811 | Sulfotransferase |
| | DV437801 | Retinol dehydratase |
| | DV437802 | Sulfotransferase, retinol dehydratase |
| **DEVELOPMENTALLY RELATED PROTEINS** | | |
| | DV437824 | posterior end mark |
| | DV437847 | GLG and sushi domain containing protein |
| | AB114859 | Vitellogenin SOD |
| | DV437844 | Anionic trypsin II precursor, nudel |
| **OTHER FUNCTIONS** | | |
| | DV437810 | Mitochondrial import inner membrane translocase |
| | DV437817 | Fatty acid binding protein |
| | DV437837 | GM2 activator protein |
| | DV437845 | NADH dehydrogenase |
| | DV437804 | Fatty acid binding protein |

# Genomics to assess emerging chemicals

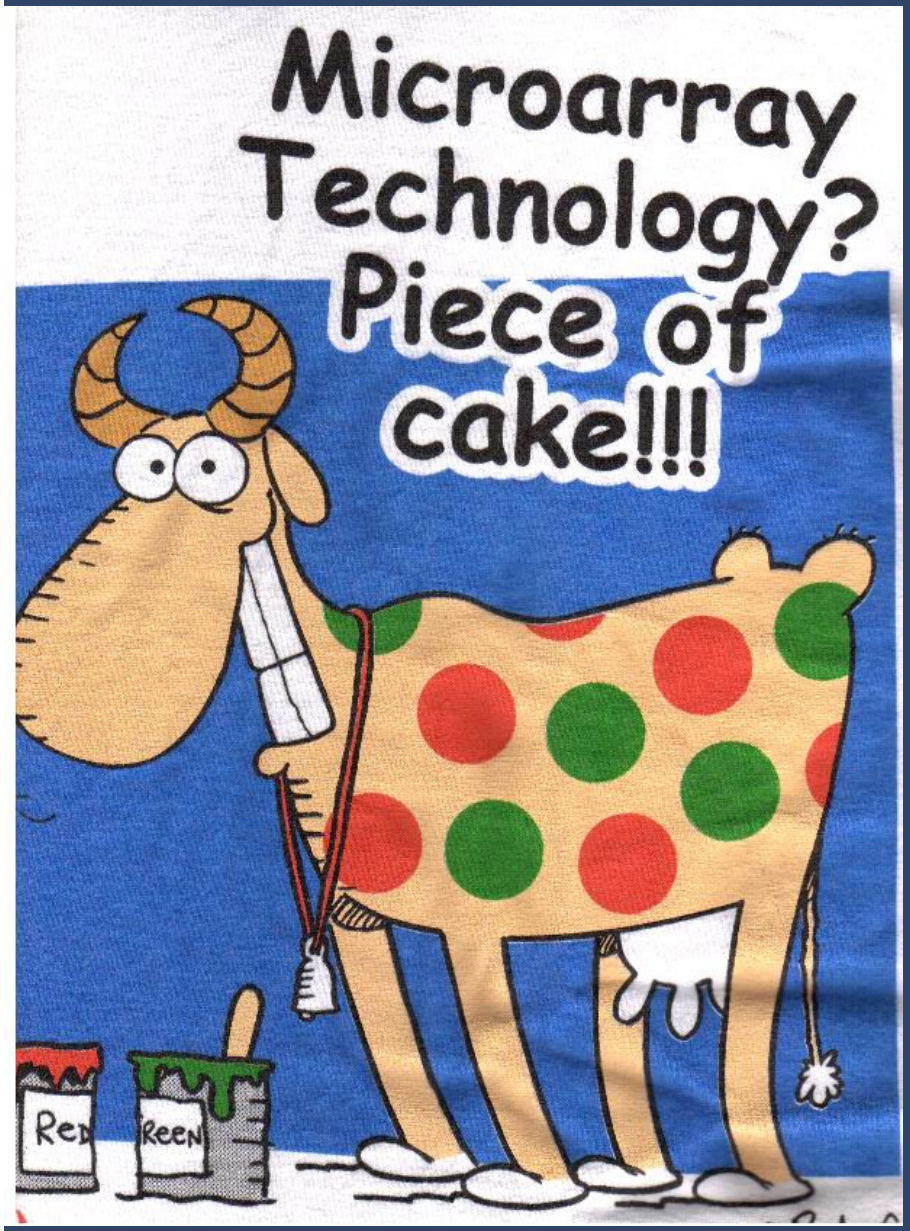
PROS



# Genomics to assess emerging chemicals



# Experimental design, data analysis and interpretation




- Experimental design
  - Number (biological) replicates
  - Dose response relationships
  - Temporal dynamics

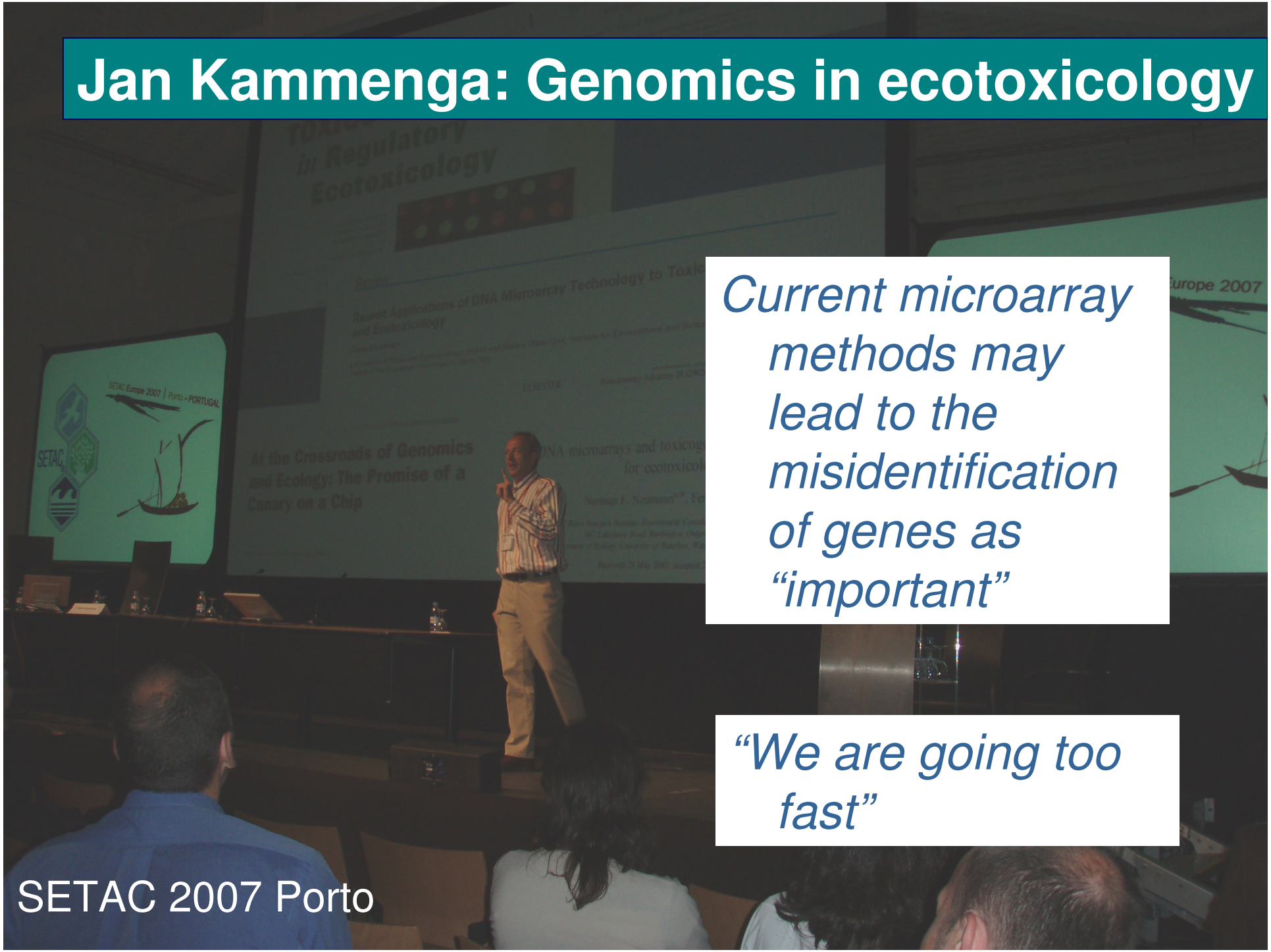
- Sources of variation
  - Technical
  - Biological
  - Ecotoxicogenomics: internal and external environmental variables

# Experimental design, data analysis and interpretation

- Data analysis
  - Huge numbers of data: multiple testing problem/false discovery
  - Normalization
  - Statistical analysis

- Interpretation
  - Gene function: many unknowns, differences in gene ontology allocations
  - Comparison with existing gene expression databases
  - Validation: RT-PCR sufficient?

# Jan Kammenga: Genomics in ecotoxicology




*Current microarray methods may lead to the misidentification of genes as "important"*

*"We are going too fast"*

SETAC 2007 Porto

# Can genomics be used to assess mixture effects?

ELSEVIER

Aquatic Toxicology 81 (2007) 293–303

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

## Toxicogenomic responses in rainbow trout (*Oncorhynchus mykiss*) hepatocytes exposed to model chemicals and a synthetic mixture

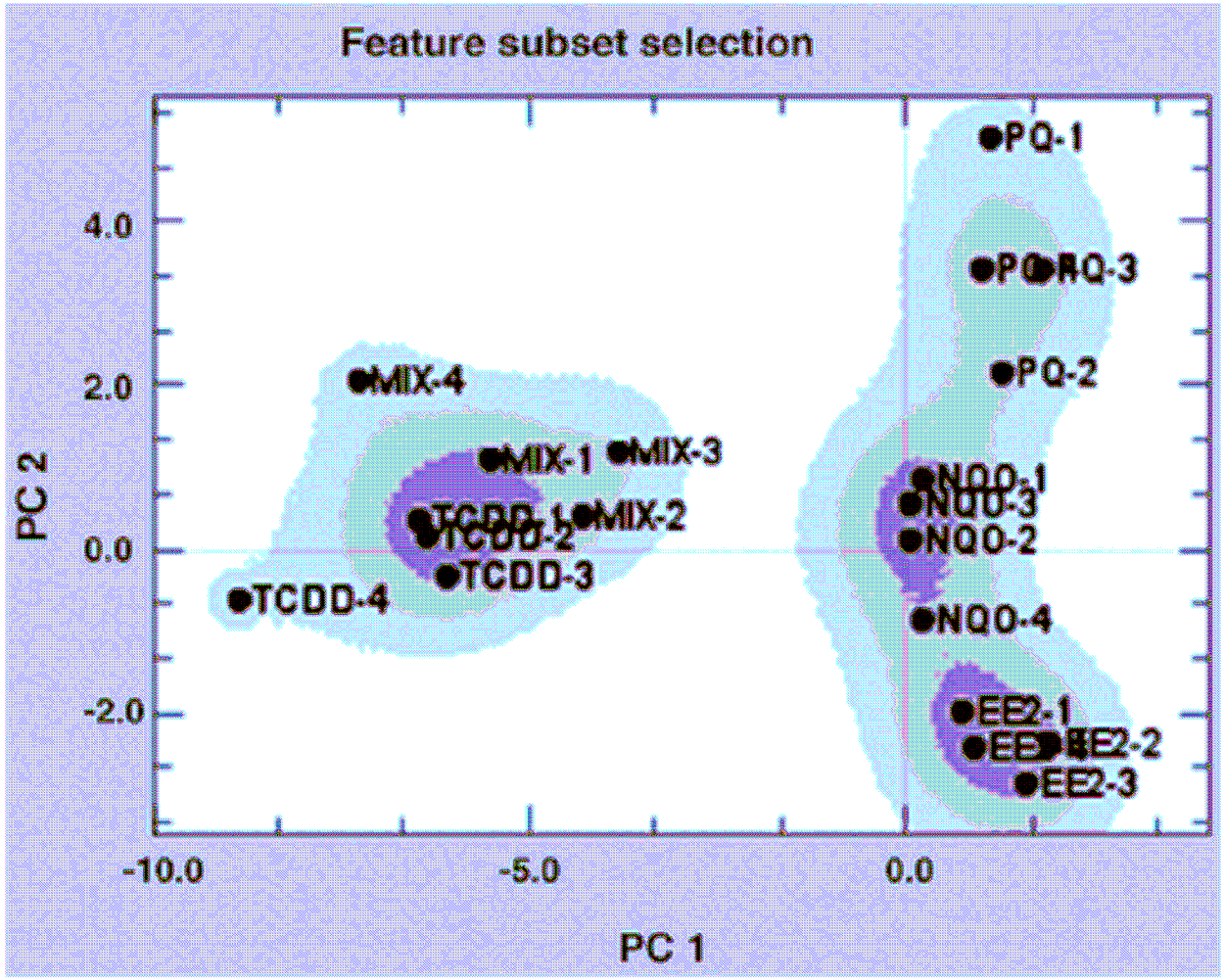
E.F. Finne[a,b,*], G.A. Cooper[c], B.F. Koop[c], K. Hylland[a,b], K.E. Tollefsen[a]

[a] *Norwegian Institute for Water Research, Gaustadallèen 21, N-0349 Oslo, Norway*
[b] *University of Oslo, Department of Biology, P.O. Box 1066, Blindern, N-0316 Oslo, Norway*
[c] *Centre for Biomedical Research, University of Victoria, BC V8P5C2, Canada*

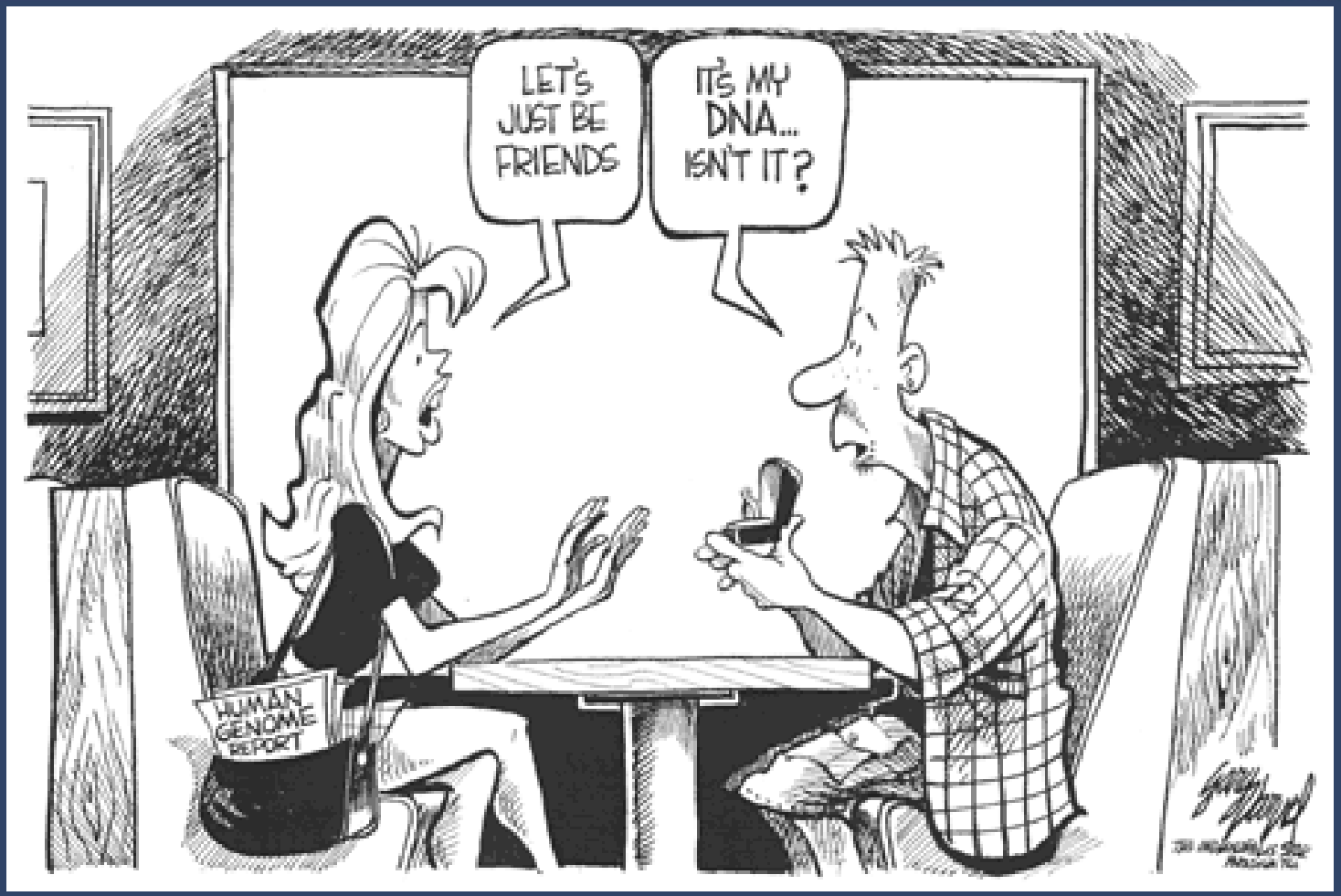
Received 11 September 2006; received in revised form 15 December 2006; accepted 18 December 2006

- 17-ethinylestradiol (EE2), 2,3,7,8-tetrachloro-di-benzodioxin (TCDD), paraquat (PQ) and 4-nitroquinoline-1-oxide (NQO)
- Tested as individual chemicals and as mixtures

# Can genomics be used to assess mixture effects?

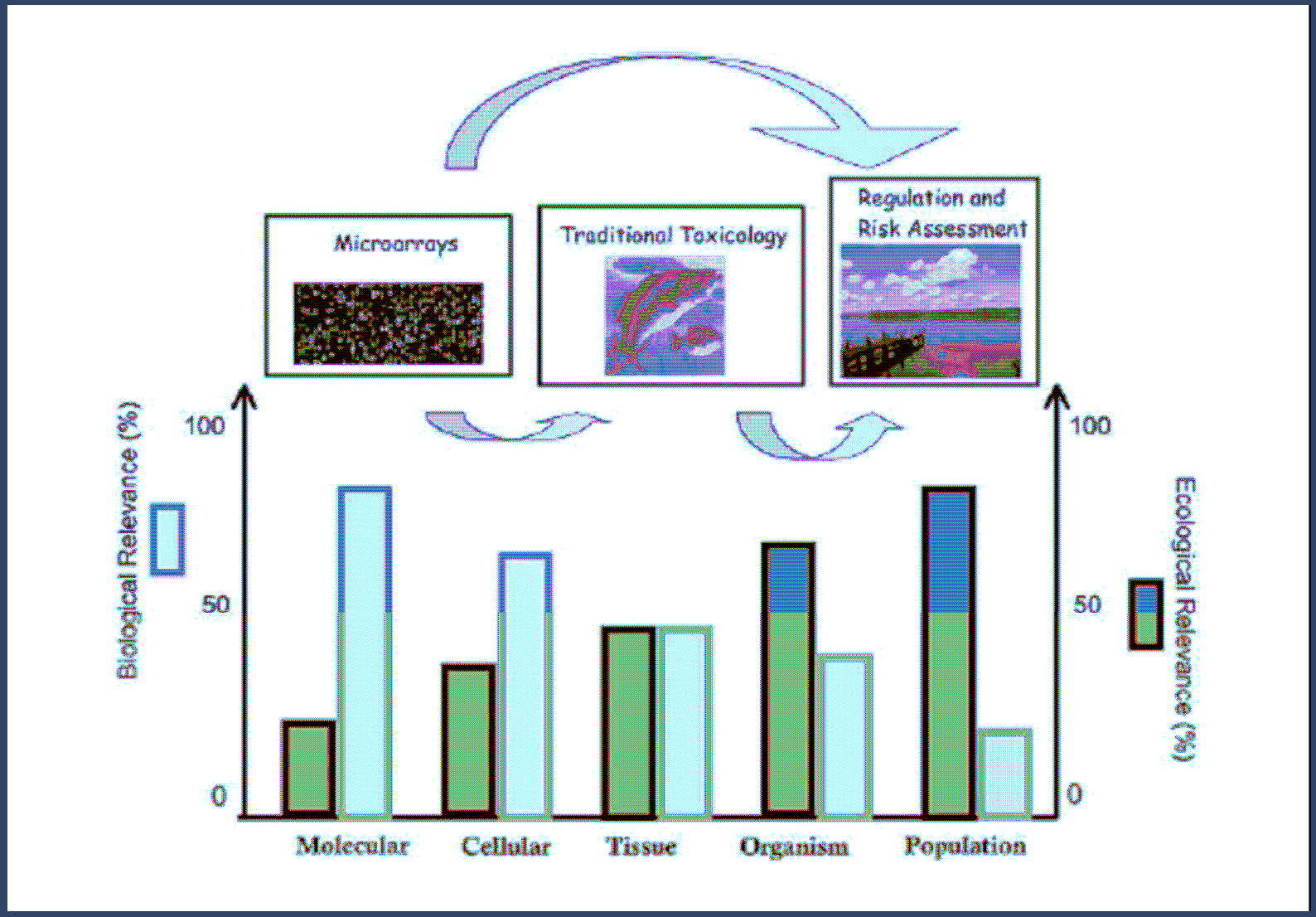


# High costs




"No microarrays on a budget less that $100,000"
Expensive to repeat experiments → limited experimental data available

# Linking gene expression with ecological effects




Denslow et al., 2007, Mol. BioSyst., 2007, 3, 172–177

## Advantages

-elucidate mechanisms of action

-identify biomarkers/classifiers of toxicity

-identify signatures of gene expression

-comparative toxicogenomics

-apply to environmentally relevant species

## Limitations

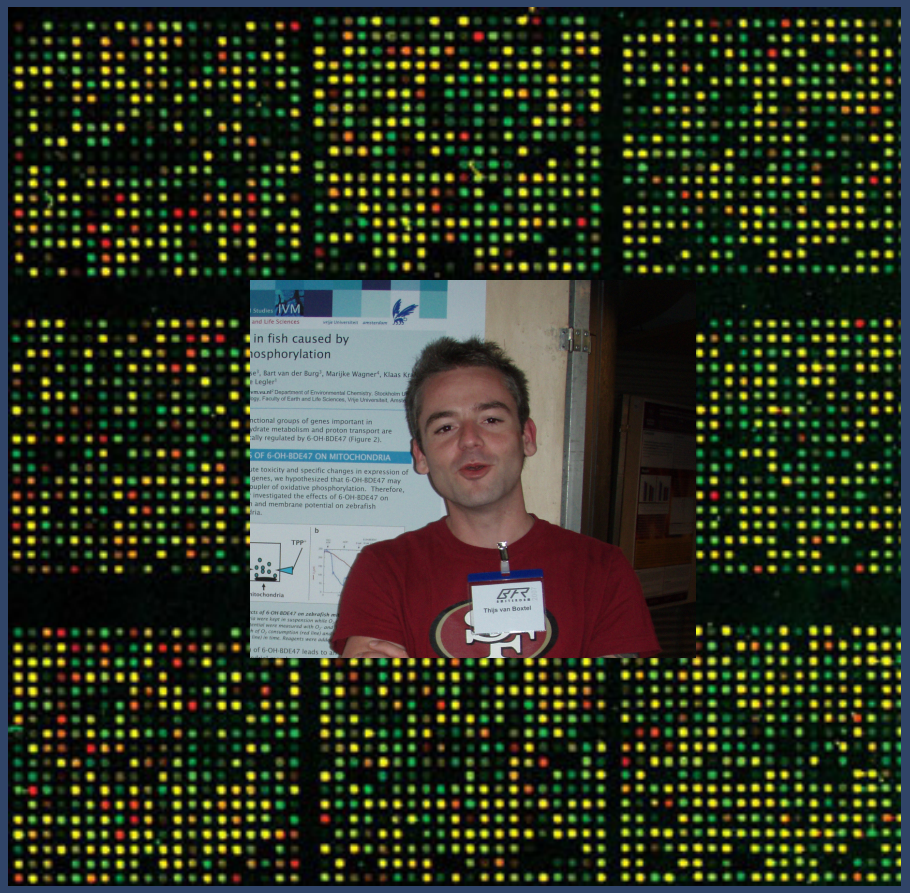
-experimental design, analysis, interpretation, costs

-identify signatures in mixtures and environmental samples?

-lack of commercial arrays for non-model species

-linking effects at gene expression level to physiological and population effects

# Acknowledgements




Thijs van Boxtel

- Netherlands Organization for Scientific Research
- Ecogenomics project
- EU FIRE project