International Science Forum on Computational Toxicology

MAY 21-23, 2007
US EPA Main Facility
Research Triangle Park, NC

Description and Objectives of the Platform Sessions

Plenary Sessions

I. Virtual Tissues - the Next Big Step for Computational Biology. Implications for Toxicology and Risk Assessment: Relatively simple computational models of biological systems, best exemplified by PBPK models, have provided a new level of rigor for the analysis of the pharmacokinetic data and for our understanding of how pharmacokinetics influences dose-response. The "omics" revolution in the laboratory, and parallel developments in computer software and hardware, have moved the idea of virtual tissues (VT) from the realm of science fiction to forming the basis for a new, if ambitious, field of research. The potential payoffs from development of VT in toxicology are significant. VT development will build on current successes with PBPK modeling and take the development of quantitative descriptions of biological mechanisms to a new level of complexity. VT will have much greater capabilities than PBPK models for providing insights into dose-response and time-course behaviors and will promote inclusion of larger amounts of integrated biological data into risk assessment.

II. Use of Computational Tools for Ecological Assessments: Molecules to Ecosystem: Ecological risk assessments need to focus on responses across multiple biological levels of organization. An understanding of processes at molecular, biochemical and cellular levels of organization enables insights into mechanisms underlying biological changes and, as such, provides a basis for extrapolation across species and chemicals. However, to make decision about possible risk it is necessary to link alterations at these lower levels of organization to adverse effects in individuals and populations. An ultimate goal would be to understand how changes in populations of plants or animals affect specific ecosystems. This session will explore how computational approaches can be used as the basis for understanding and predicting effects of chemical stressors across the biological continuum of molecules to ecosystems.

III. Understanding Gene-Environment Interactions for Improved Risk Characterization: It is well known that different species and individuals within species react different ways to identical exposures to environmental chemicals. This

is in part driven by genetic variation in systems ranging from adsorption, transport, metabolism and receptor interactions. Understanding the mechanisms behind variable response can help determine vulnerable individuals and species. This can in turn drive testing protocols and (potentially) regulatory thinking. Additionally, performing toxicogenomic experiments in genetically heterogeneous populations can help to determine the connectivities in complex biochemical networks. Application of emerging tools in molecular biology is facilitating investigation of genetic contributions to consequences of environmental exposures. The objective of this session is to consider available tools and approaches for studying gene-environment interactions with specific focus on improving environmental risk characterization.

Concurrent Sessions

IV. Computational Models

IV A. Modeling Signaling as a Determinant of Systems Behavior: The goal of this session is to describe how biological signaling processes lead to overall systems behaviors (e.g., doseresponse relationships). Modeling simpler and more complex systems provides insights into how different regulatory modules function. The modeling can provide insights into therelationships between the behaviors of parts of the system and the overall behavior when the parts are combined. Once the behavior of the biological system has been described, questions can be raised about perturbations of the system. Such perturbations can include altered physiological states (e.g., stress), disease states, and exposures to pharmaceutical or environmental compounds.

IV B. Computational Modeling of HPG Axis: Over the past decade there has been a focused international effort to identify possible adverse effects of endocrine active compounds (EAC) on humans and wildlife. Effects on development, reproduction, aging, and hormone-sensitive cancers mediated through alterations in the hypothalamus-pituitary-gonadal (HPG) axis have been of particular concern. The development and application of computational models of the HPG axis can improve our understanding of the complex linkages between chemical exposures, biological dose, and effects of EAC to help predict the dose-response behavior and identify predictive biomarkers indicative of adverse effects. This session will focus on the development of computational models of the HPG axis at all levels of biological organization (i.e. intracellular, tissue, multiorgan systems), and the use of EAC to perturb the HPG axis to understand function and/or dynamic.

IV C. Predicting the Environmental Fateand Transport of Chemical Contaminants: Exposure assessment requires knowledge of the environmental concentration and speciation of the chemical contaminant(s) of interest. This session will focus on: (1) the presentation of the computational tools currently available for predicting/simulating the fate and transport of chemicals in the environment; and (2) the identification of the most significant sources of uncertainty concerning our ability

to predict chemical fate and transport.

IV D. Dose Response and Uncertainty in Risk Assessment: This session will examine some issues surrounding probabilistic dose response assessments. While the Agency has some experience using probabilistic methods in risk assessment, virtually all of it is in using probabilistic methods to characterize uncertainty and variability in exposure. The goals of this session are to: (1) explore some methods for better characterizing the uncertainties inherent in estimating dose-response from toxicity data; (2) look at issues involved in extrapolating from animal to human dose-response: (3) look at approaches to dose-response assessment that probabilistically characterize uncertainty.

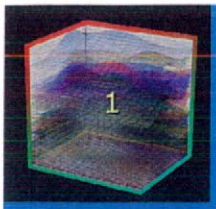
V. Hazard Prediction Tools

V A. Toxico-informatics: This session will cover the integration of chemical and toxicity data, toxicity data models, chemoinformatics, and the harnessing of legacy toxicity data to advancing predictive technologies.

V B. Computational Molecular Modeling Applied to Understanding and Predicting Chemical Toxicity: This session will cover research modeling the modes and mechanisms for chemical toxicity from the viewpoint of the physico-chemical interactions between molecules in biological systems. This will deal primarily with a causal approach to this problem. It will also include advanced methods that increase the speed of these computationally rigorous applications so they may be used for screening.

V C. Application of Drug Discovery Technologies in Environmental Chemical Prioritization: Use of modern drug discovery technologies, including high-throughput biochemical and cellular assays, provides a new opportunity to survey environmental chemicals for potential for hazard. This session will focus on methods traditionally used in the drug discovery process for characterizing the bioactivity of small molecules with regard to target specificity and toxicity and how they can be applied to the field of environmental toxicology.

V D. Using Genomics to Predict Potential Toxicity: Genomics provides detailed molecular data about the underlying biochemical mechanisms of disease or toxicity, and could represent sensitive measures for detecting effects of environmental exposures. Thus genomics can provide useful data along the source-to-outcome continuum, when appropriate bioinformatic and computational methods are available for integrating molecular, chemical and toxicological information.



Prediction system for Chemical Safety Using PERCELLOME TOXICOGENOMICS

Kanno Jun, Aisaki Ken-ichi, Igarashi Katsuhide, Nakatsu Noriyuki, Kitajima Satoshi, Kodama Yukio

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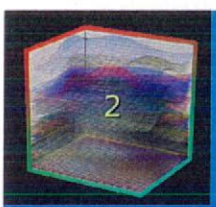
Biological Safety Research Center,
National Institute of Health Sciences,
Tokyo, Japan



Percellome Projects

Aim:

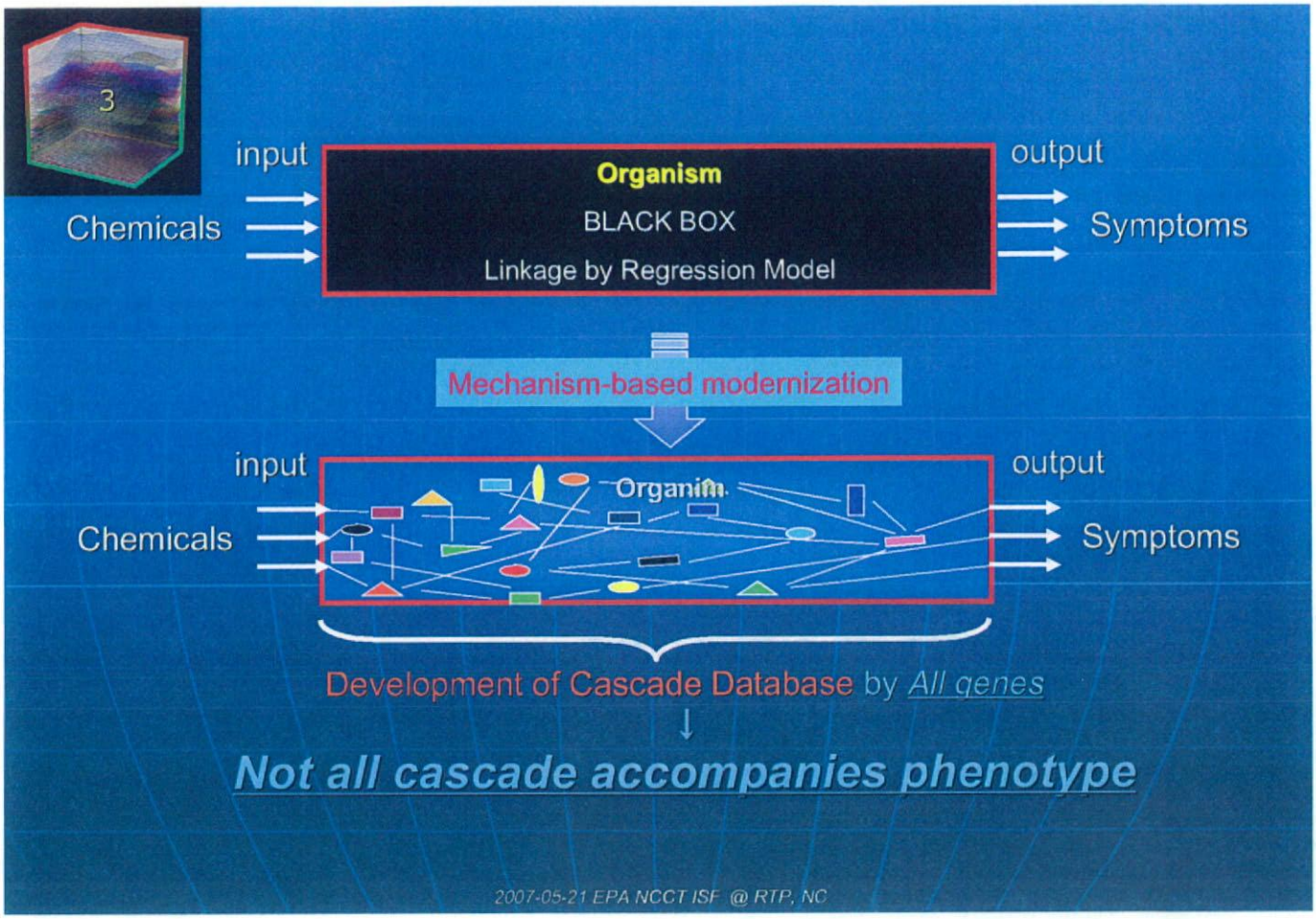
Comprehensive Gene Cascade Database by **Phenotype-Independent Approach**
(we cannot do phenotypic anchoring for all genes).

Ultimate Goal:

Virtual mouse, virtual human *in silico*

Tentative Goal:

High-Resolution, Mechanism-based Toxicology to reinforce Traditional Toxicology
(faster, cheaper, more accurate)


3
input
Chemicals
Organism
BLACK BOX
Linkage by Regression Model
output
Symptoms
Mechanism-based modernization
input
Chemicals
Organism
output
Symptoms
Development of Cascade Database by All genes
Not all cascade accompanies phenotype
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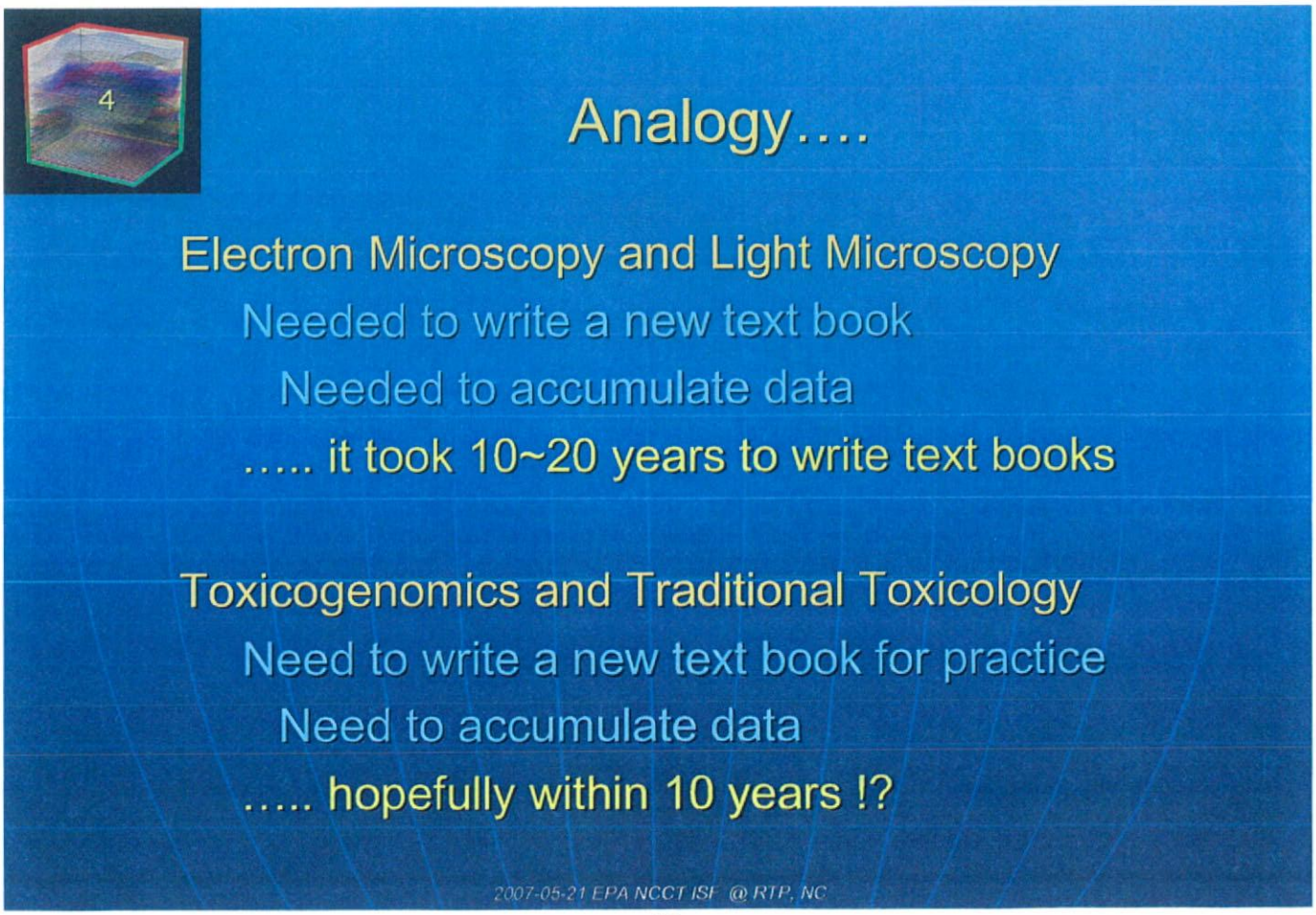
4
Analogy….
Electron Microscopy and Light Microscopy
Needed to write a new text book
Needed to accumulate data
….. it took 10~20 years to write text books
Toxicogenomics and Traditional Toxicology
Need to write a new text book for practice
Need to accumulate data
….. hopefully within 10 years !?
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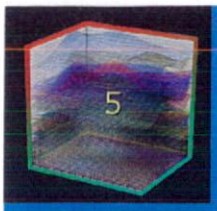
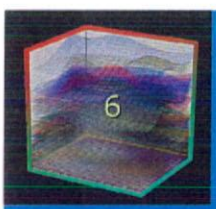


MHLW* Toxicogenomics Projects

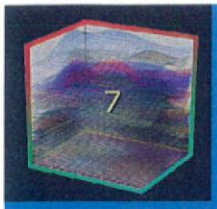
*Ministry of Health Labour and Welfare

- Toxicogenomics Project (2002~2006, 5y)
  NIHS +17 Pharmaceuticals
  - rat (oral; liver, kidney) 150 chem, single/ repeat exposure
  - + In vitro (rat primary hepatocyte, human primary hepatocyte)
  - Now @ Nat'l Institute of Biomedial Innovation (Osaka) 2005~2006
  - 2nd round project at Osaka (2007~2011)
- Percellome Project (Mouse)
  Chemical Safety Database
  - Div. Cellular and Molecular Toxicology/ BSRC/ NIHS
    - TTG1: 2003~2005 (single exposure), oral. liver, kidney 90 chem
    - ITG: 2005~2007 Inhalation Toxicogenomics
    - TTG2: 2006~2008 (repeated exposure, multi-organ etc.), oral
    - FTG: 2004~ Fetus (developmental)
    - NTG: 2006~ Behavior -> Brain TG (HC, BS, Cx, Cl)
    - (Food TG): 2007~ Functional Health Food (CoQ10, α-lipo)



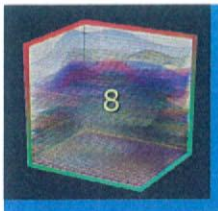
Why mouse?!

- Gene Knockout Organism data will be important for writing a "toxicogenomics text book" (validation)
  - p53 KO
  - ER α/β KO, KI
  - hSXR KI



Percellome Method

Obtain transcriptome data in
copy number of mRNA per one cell (average)
in order to
compare the accumulated transcriptome data.



BMC Genomics

BioMed Central

Methodology article

Open Access

"Per cell" normalization method for mRNA measurement by quantitative PCR and microarrays

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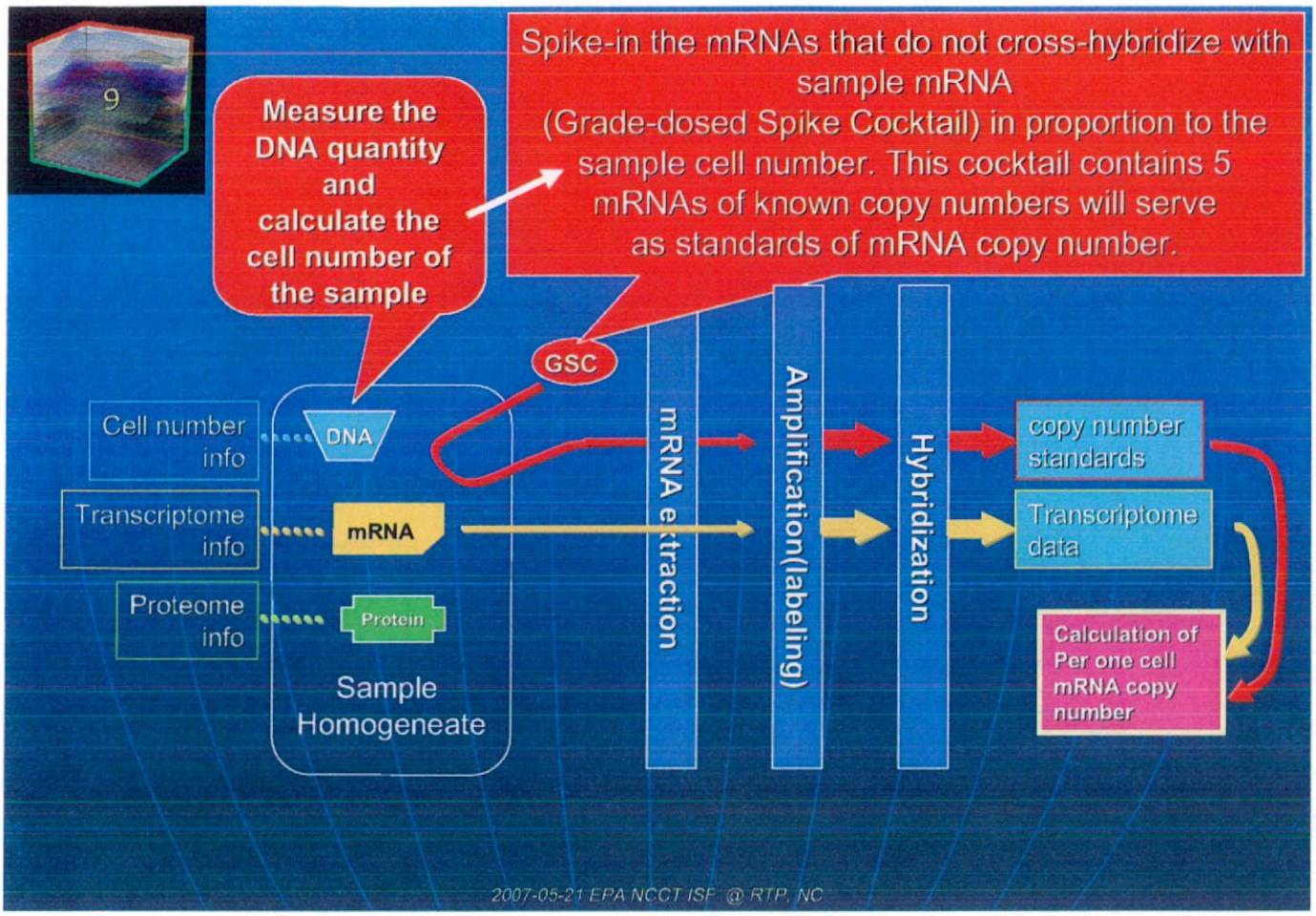
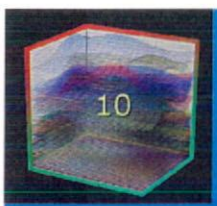
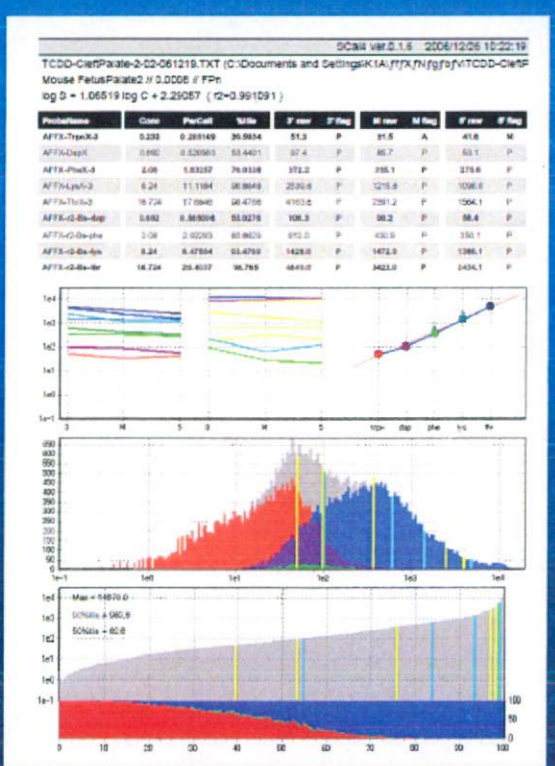
on line journal: BMC Genomics. 2006 Mar 29;7(1):64
PMID: 16571132





Percellome Normalization, calculation and QC (for microarray)

SCal4

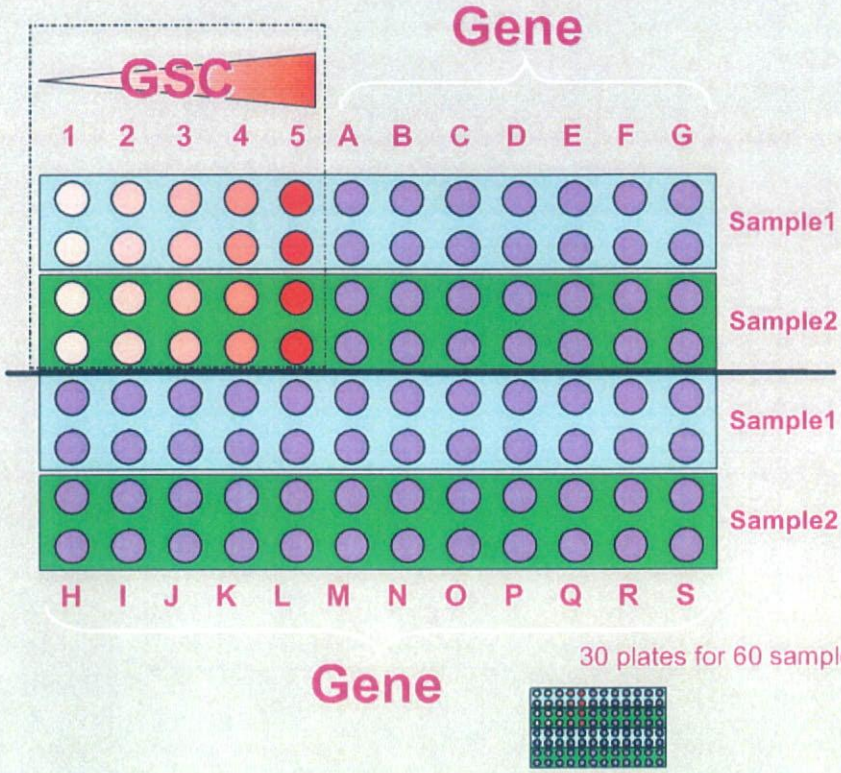


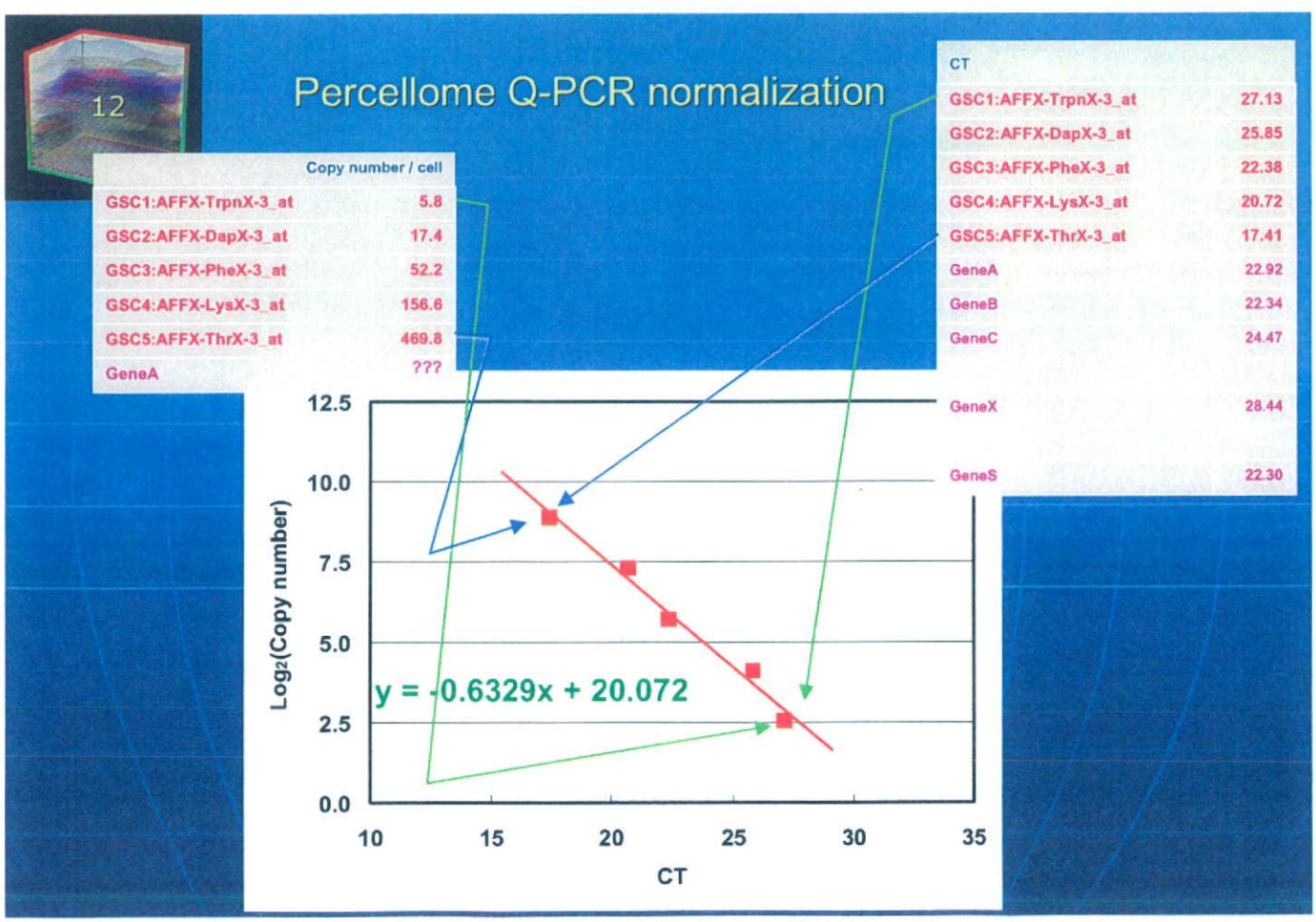
Affymetrix GeneChip
(Mouse 430 v2
Rat, Human, Xenopus)

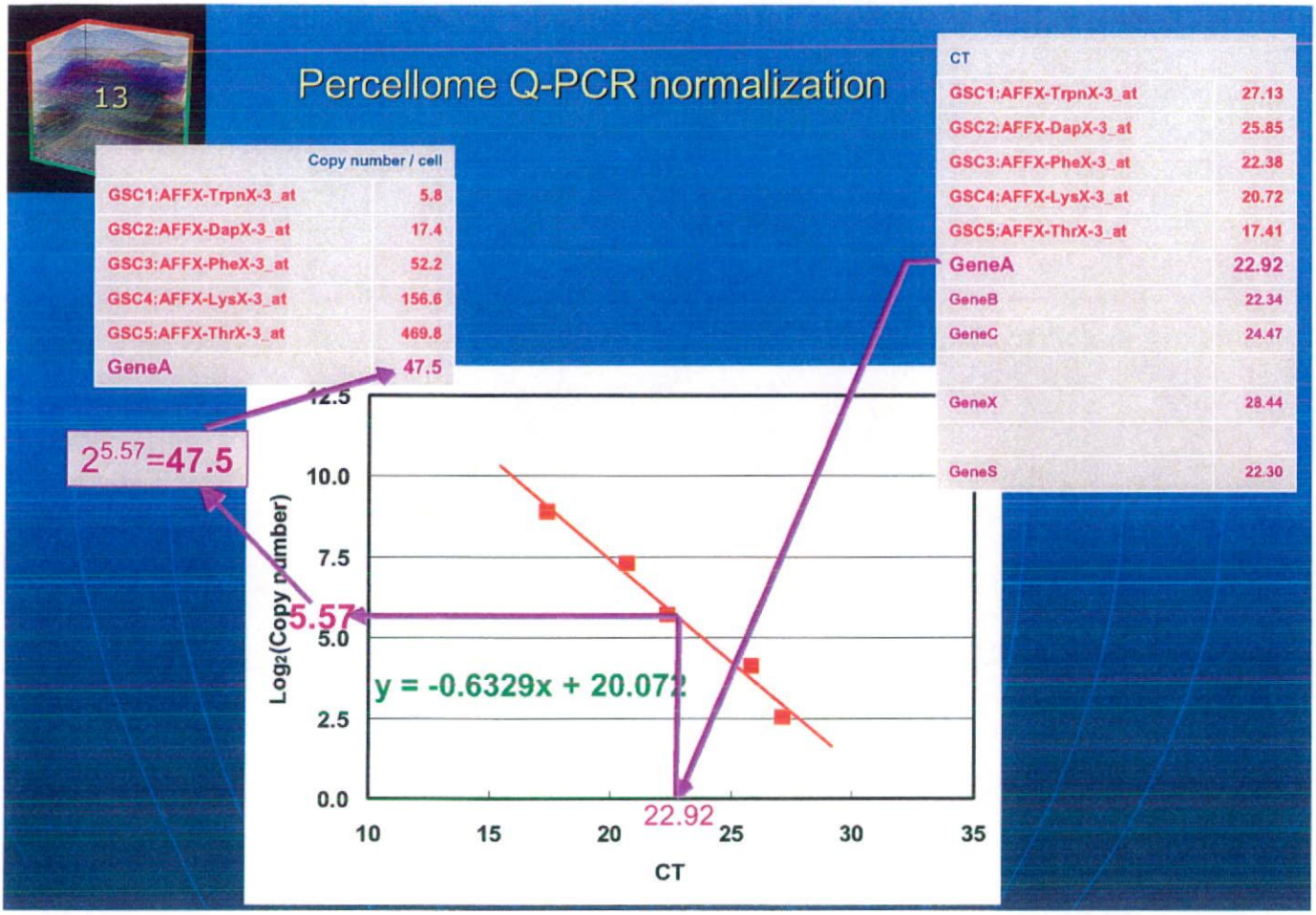
Exon Array
(human and mouse)
now under trial

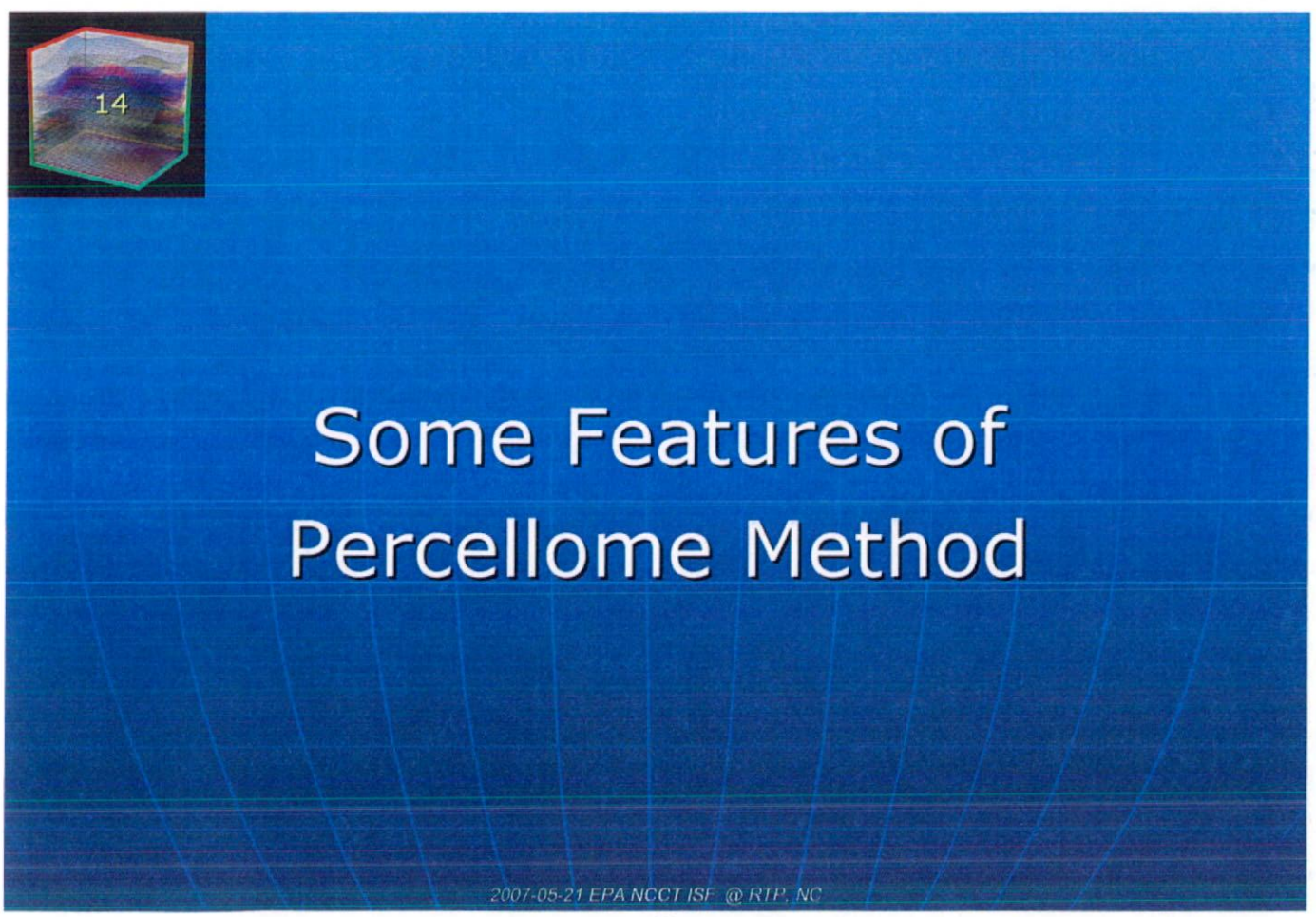
Percellome Quantitative-PCR

(by ABI PRISM 7900HT / SYBR Green)






13
Percellome Q-PCR normalization
Copy number / cell
GSC1:AFFX-TrpnX-3_at 5.8
GSC2:AFFX-DapX-3_at 17.4
GSC3:AFFX-PheX-3_at 52.2
GSC4:AFFX-LysX-3_at 156.6
GSC5:AFFX-ThrX-3_at 469.8
GeneA 47.5
$2^{5.57}=47.5$
CT
GSC1:AFFX-TrpnX-3_at 27.13
GSC2:AFFX-DapX-3_at 25.85
GSC3:AFFX-PheX-3_at 22.38
GSC4:AFFX-LysX-3_at 20.72
GSC5:AFFX-ThrX-3_at 17.41
GeneA 22.92
GeneB 22.34
GeneC 24.47
GeneX 28.44
GeneS 22.30
Log2(Copy number)
12.5
10.0
7.5
5.0
2.5
0.0
5.57
y = -0.6329x + 20.072
22.92
10
15
20
25
30
35
CT


14
Some Features of Percellome Method
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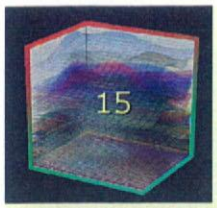
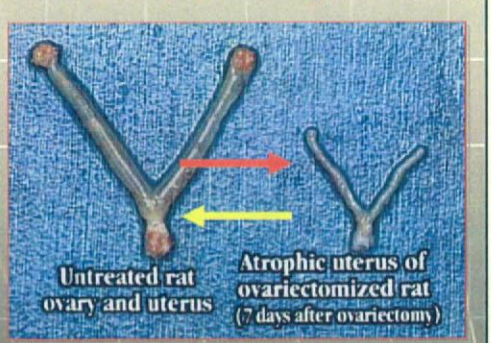
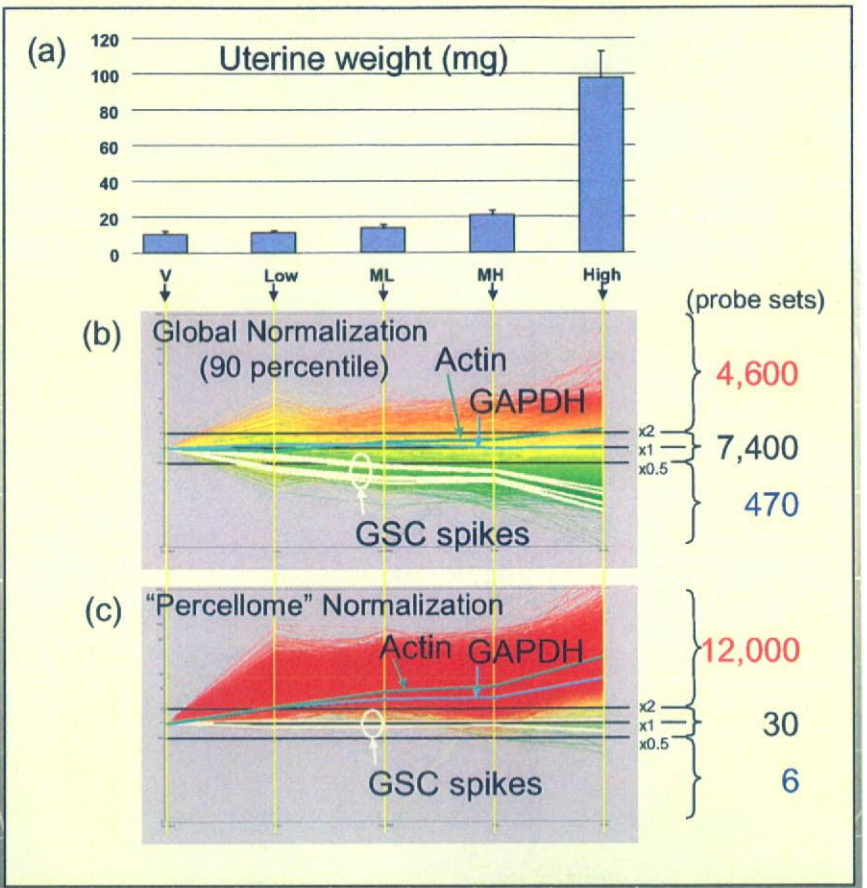


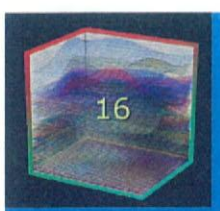
Profile-independent normalization

Ovariectomy



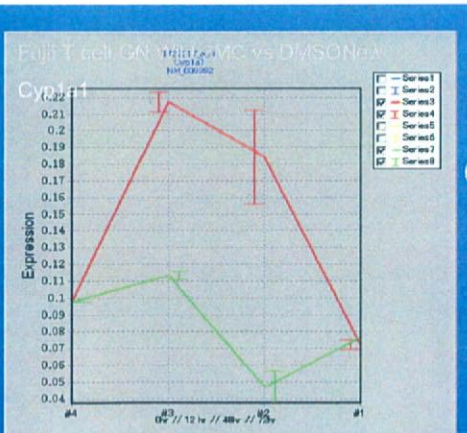
Chemical (estrogenic) effect



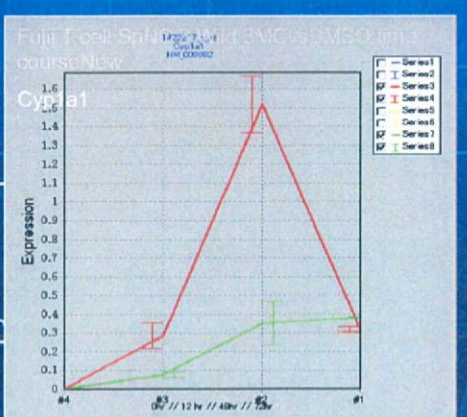


In vitro Experiment

Induction of Cyp1a1 in cultured cell treated by an AhR ligand, time course study

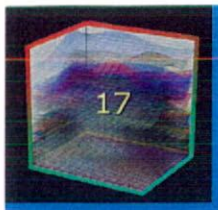


Global Normalization



Percellome

copy number per cell

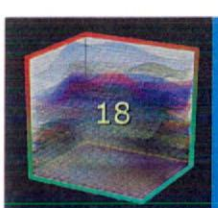


Percellome Toxicogenomics Database

Single oral gavage:

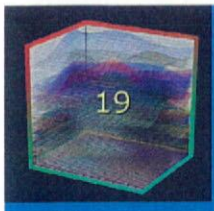
Dose selection:

- No morphologic change in 24 hrs
- No overt symptoms in 24 hrs

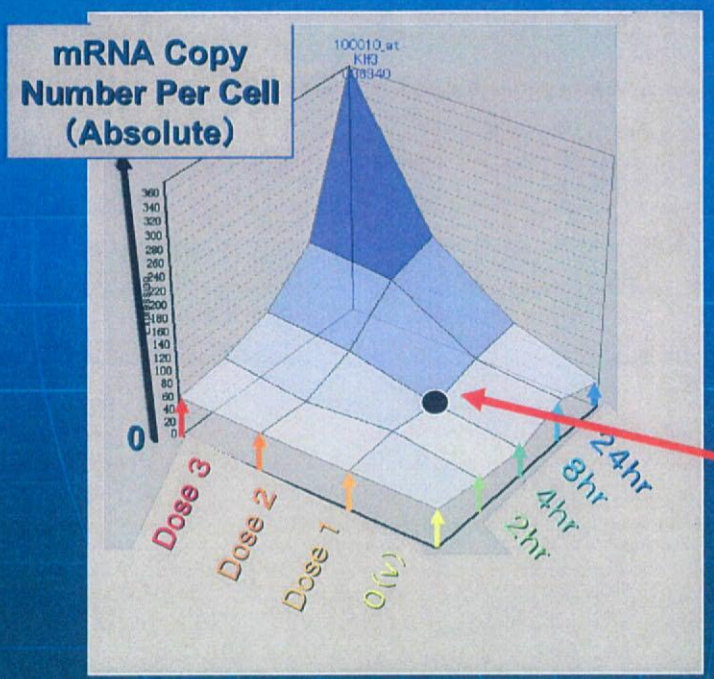


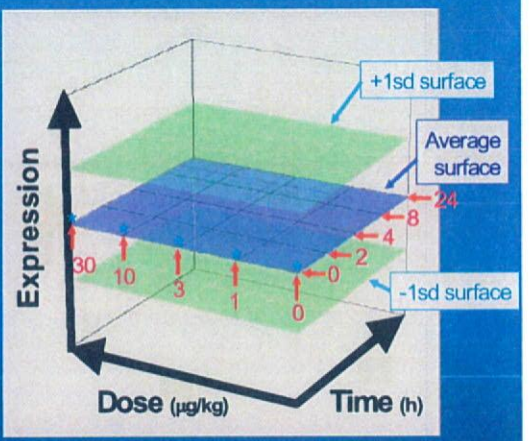
Data base Chemicals

Medicine	Isoniazid	Chemicals related to Chemical Substances Control Law in Japan	2,4-dinitrophenol
	Cisplatin		4-amino-2,6-dichlorophenol
	(Transplatin)		Pentachlorophenol
	Acetaminophen		2-Aminomethylpyridine
	Aspirin		2-Vinylpyridine
	Ibuprofen		1,2,3-Triazole
	Dexamethasone		1,2,4-Triazole
	Omeprazole		3-Amino-1H-1,2,4-triazole
	Phenobarbital		N-Methylaniline
	Valproic Acid		2-Chloro-4,6-dimethylaniline
	Thalidomide		1,2-Dichloro-3-nitrobenzene
	Sodium arsenite (NaAs2HO4)		4-Ethylnitrobenzene
	Diethylstilbestrol		
	Tamoxifen	Industrial chemicals	Toluene
	Paclitaxel (Taxol)		Bromobenzene
	Phenytoin		Carbon tetrachloride
	Rifampicin		Methanol
	PCN		DMSO
	AraC		Tributyltin
			Bisphenol A
Agricultural chemicals	Paraquat		MEHP
	Methoprene		DEHP
	Pyriproxyfen		Fullerene
	Tebufenozide		Indigo
	Acephate		
	Carbaryl	DNA demethylating drugs	Azacytidine
	Warfarin		
	Permethrin		
	Deet	inhalational toxic chemicals	Formaldehyde
			Acetaldehyde
Food-derived chemicals	Citric acid		
	Hydroxycitric Acid	Agonists on nuclear receptor	Ethynyl estradiol
	Forskolin		Testosterone propionate
	Caffeine		Clofibrate
	Monocrotaline		Troglitazone
	Ethanol		Levothyroxine
	Coenzyme Q10		All trans retinoic acid
	Genistein		9-cis retinoic acid
	Genistin		Methoprene acid
	Daizein		TCDD
			TCDF
Mutagen	Diethylnitrosamine		3-methylcholanthrene
	N-ethyl-N-nitrosourea		



Millefeuille data (MF surface data)





One point:
Triplicate
(three GeneChips)
Mean ±SD

4x4x3=48 animals

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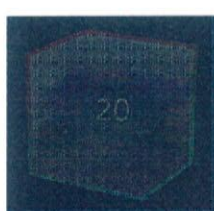
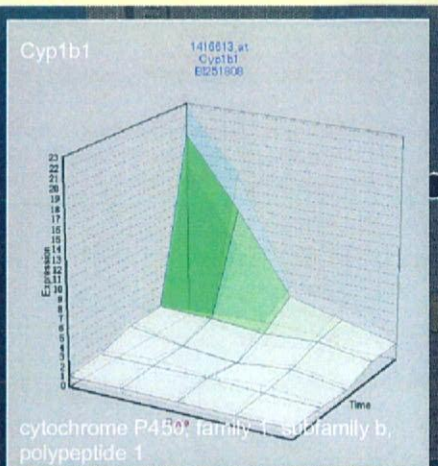
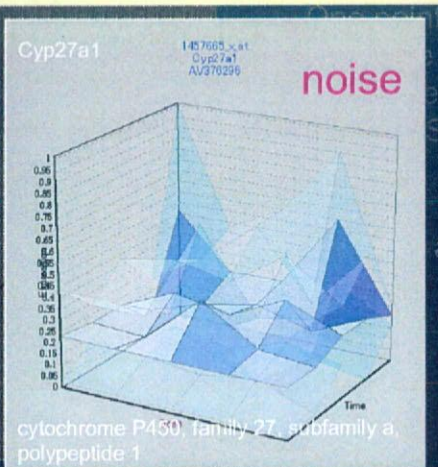
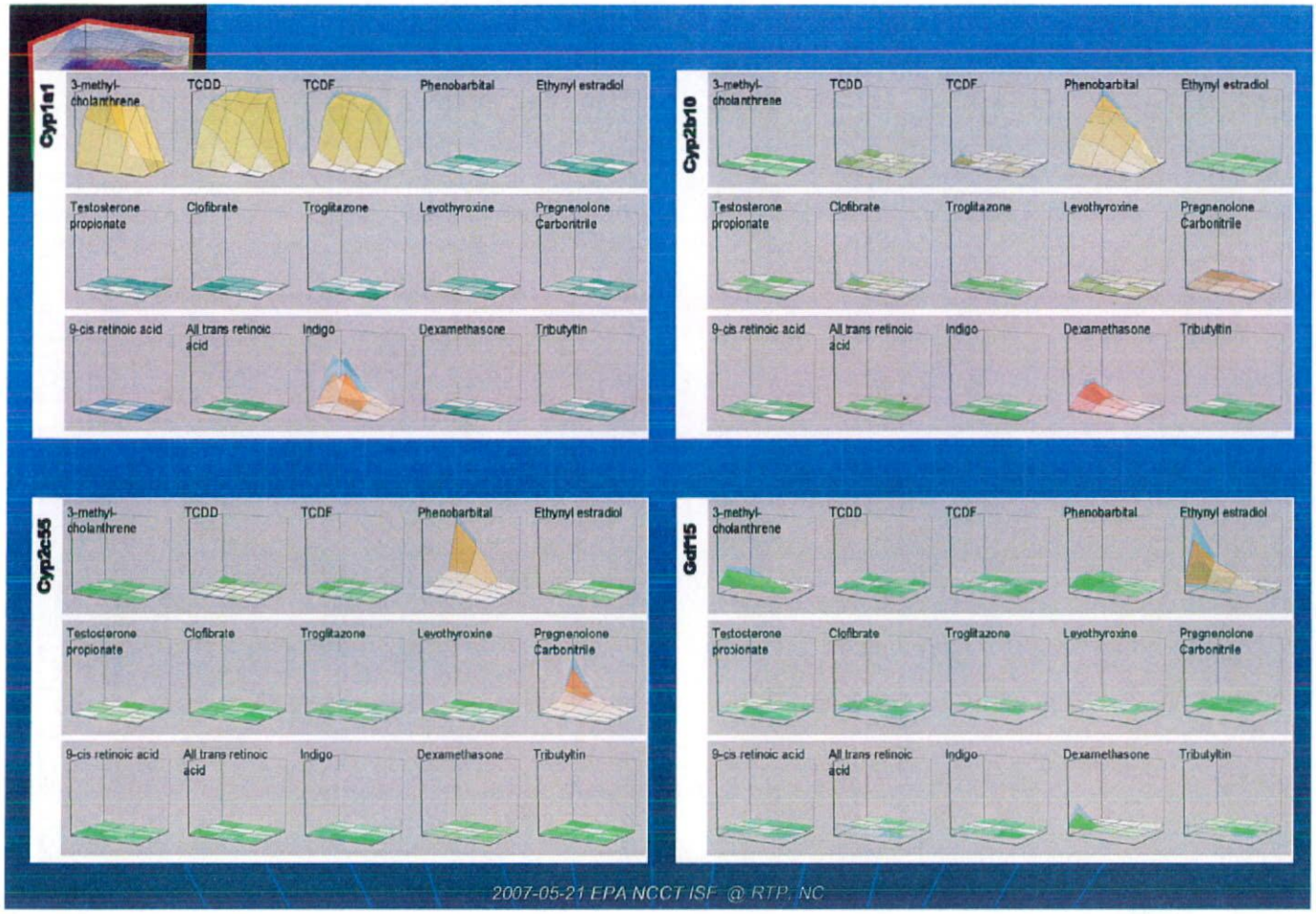


Millefeuille data (MF surface data)

Biologist-friendly!
Easy to check the data by eyes






Cyp1a1
Cyp2b10
Cyp2c55
Gstl5
3-methyl-cholanthrene
TCDD
TCDF
Phenobarbital
Ethynyl estradiol
Testosterone propionate
Clofibrate
Troglitazone
Levothyroxine
Pregnenolone Carbonitrile
9-cis retinoic acid
All trans retinoic acid
Indigo
Dexamethasone
Tributyltin
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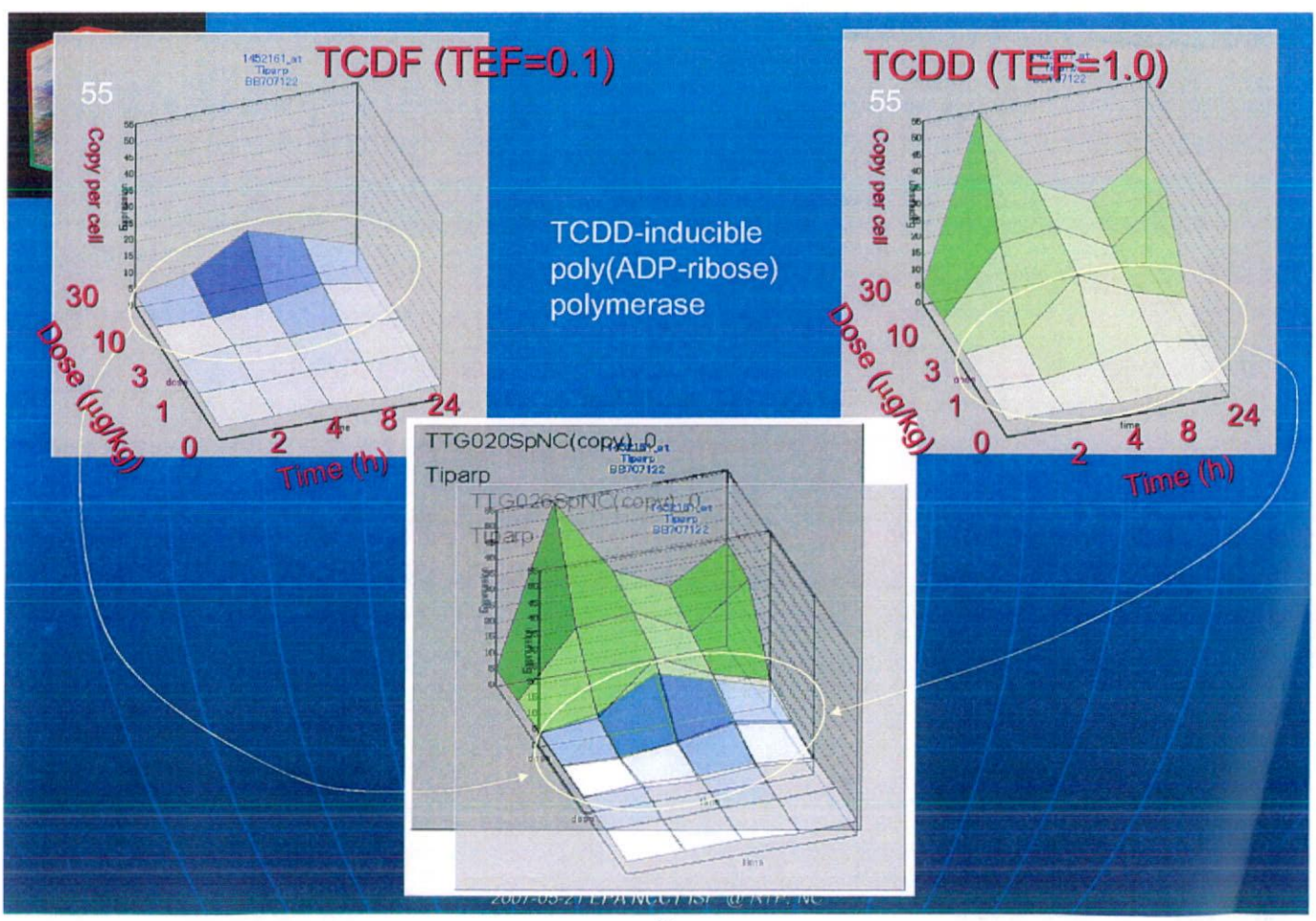
TCDF (TEF=0.1)
TCDD (TEF=1.0)
Copy per cell
55
30
10
3
1
0
Dose (μg/kg)
2
4
8
24
Time (h)
TCDD-inducible
poly(ADP-ribose)
polymerase
TTG020SpNC(copy)_0
Tiparp
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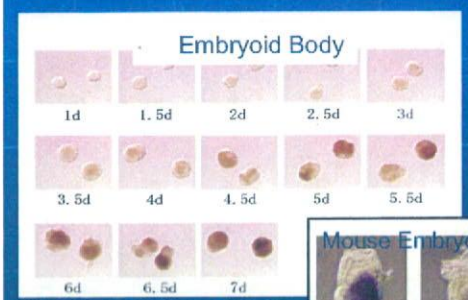
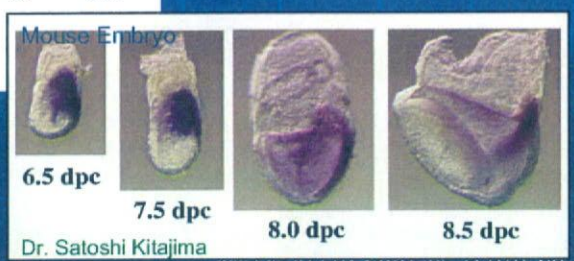
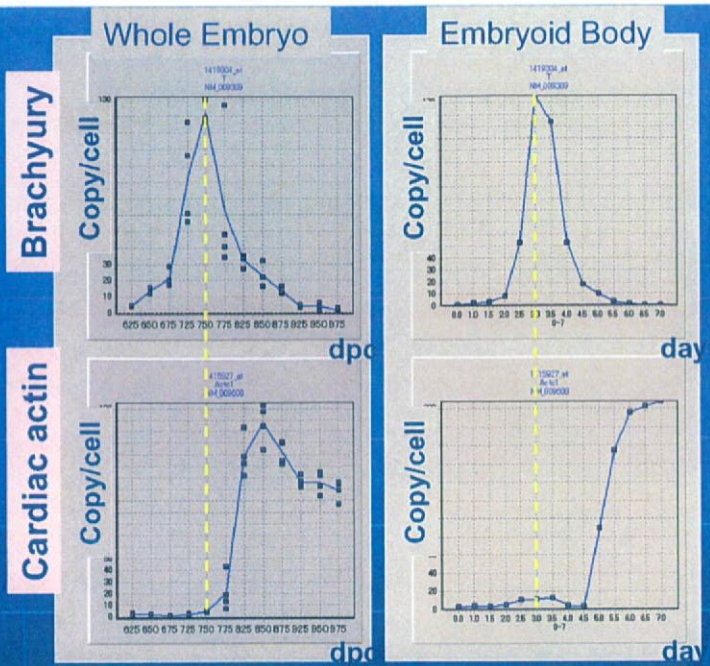
Fetus (Developmental) toxicogenomics

(Percellome)







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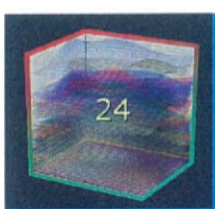
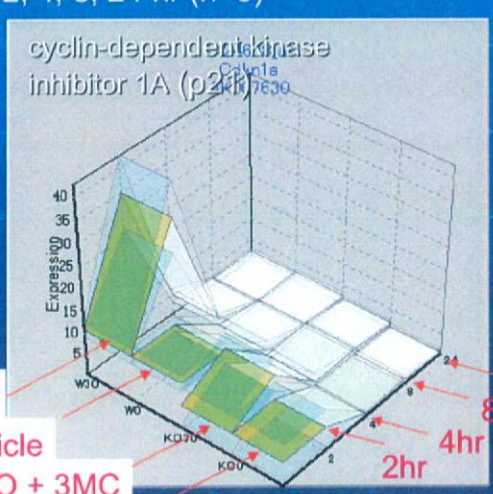


KO mouse study

p53 +/- mouse
wild type (C57BL/6)
3-methylcholanthrane (3-MC) 30gm/kg
Single gavage
time: 2, 4, 8, 24 hr (n=3)



Wild + 3MC
Wild + vehicle
p53KO + 3MC
p53KO + vehicle

Search for p53-dependent genes

Search for p53-independent genes

Probe chemical = 3-MC (AhR ligand as well as mutagen)

p21 (cdkn1a) is induced

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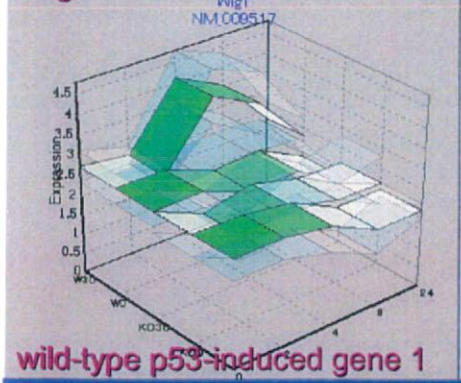
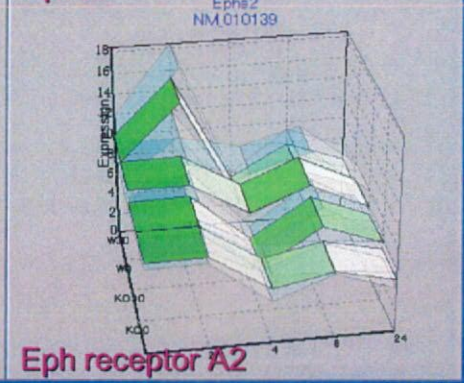
Wig1



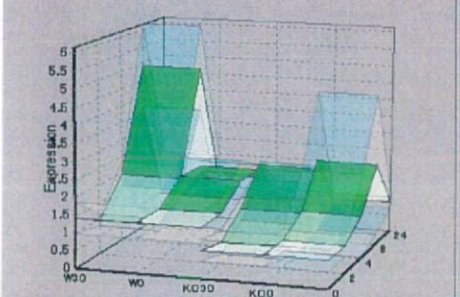
wild-type p53-induced gene 1

Epha2



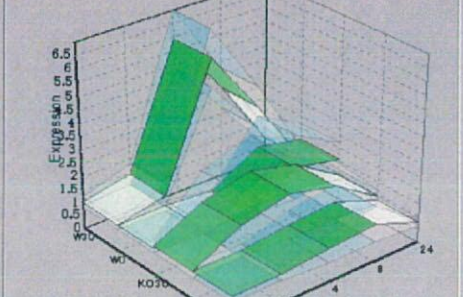
Eph receptor A2

Pdzm3



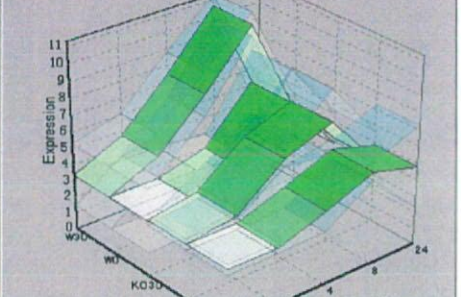
PDZ domain containing RING finger 3

Zfp746

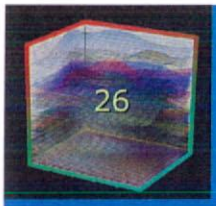


zinc finger protein 746

Clstn3

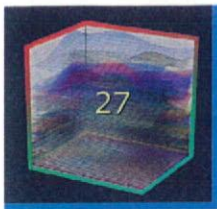


calsyntenin 3

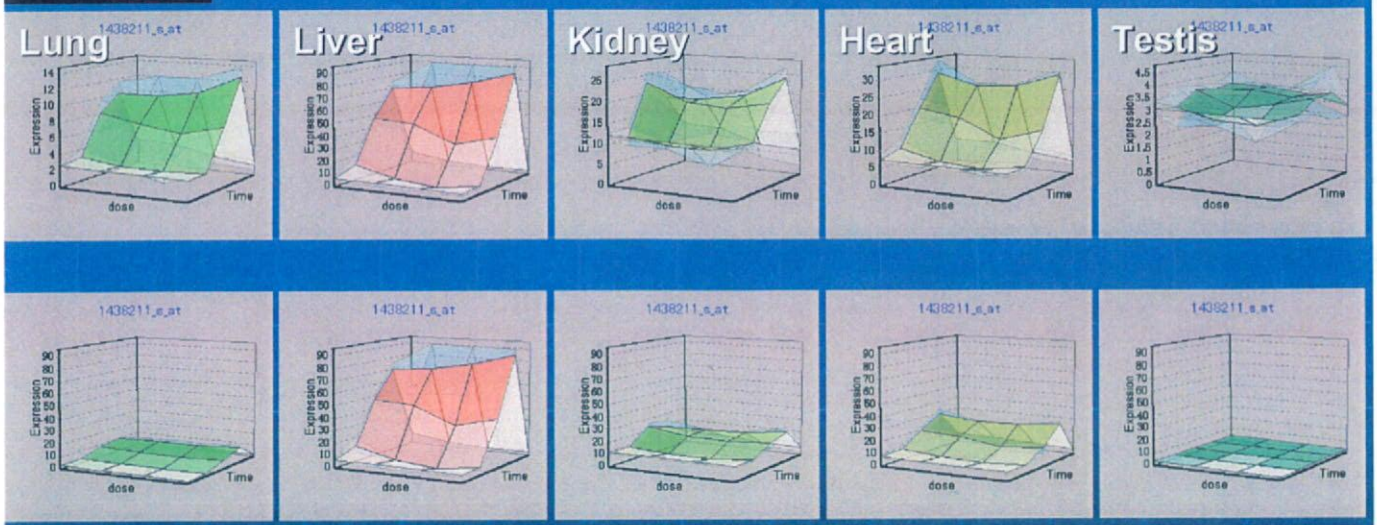


Multiorgan analysis

- Pattern -1
  - Lung
  - Liver
  - Kidney
  - Heart
  - Testis
- Pattern -2
  - Brain
    - Cerebral cortex
    - Hippocampus
    - Brain stem
    - Cerebellum
  - Liver

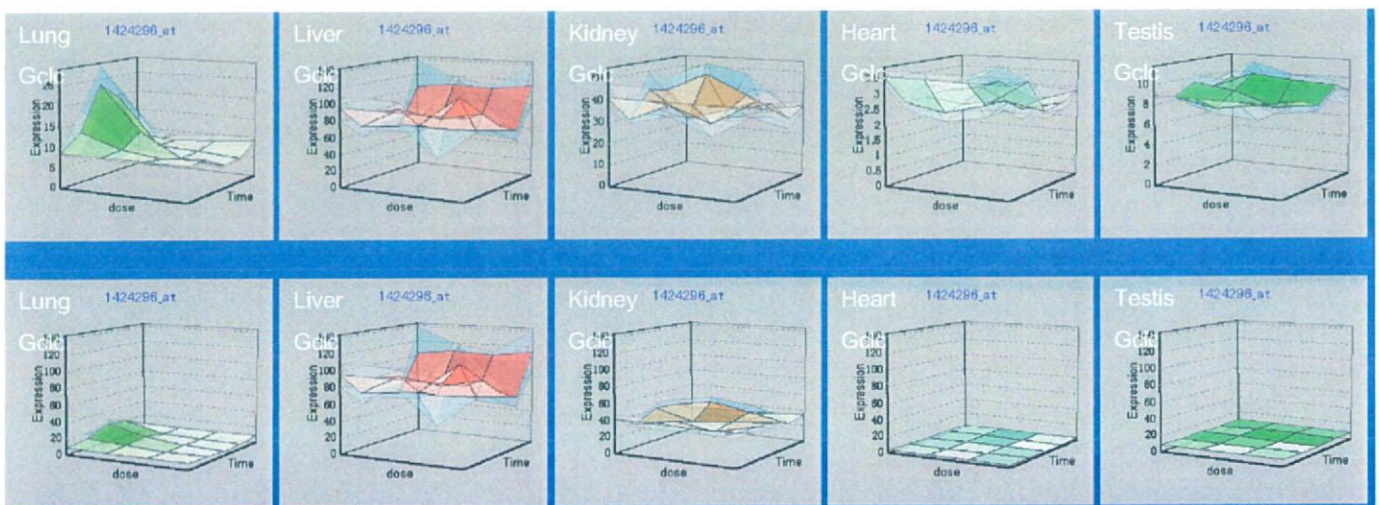


Circadian rhythm



Dbp

D site albumin promoter binding protein

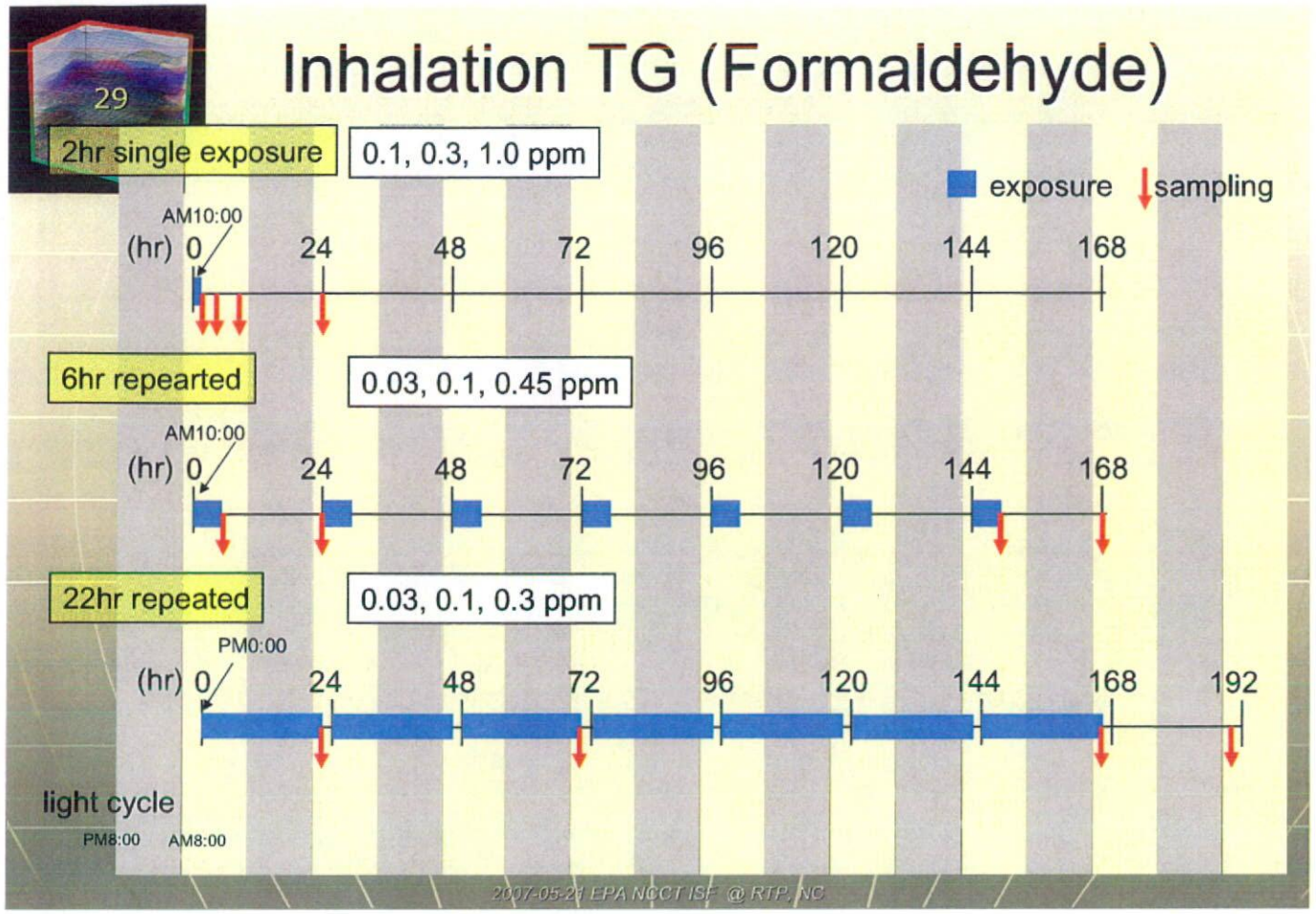


Pattern -1 CCl_4

Single oral gavage
2, 7, 20 mg/kg
time: 2, 4, 8, 24

Gclc

glutamate-cysteine ligase, catalytic subunit


29
Inhalation TG (Formaldehyde)
2hr single exposure
0.1, 0.3, 1.0 ppm
exposure
sampling
AM10:00
(hr) 0 24 48 72 96 120 144 168
6hr repearted
0.03, 0.1, 0.45 ppm
AM10:00
(hr) 0 24 48 72 96 120 144 168
22hr repeated
0.03, 0.1, 0.3 ppm
PM0:00
(hr) 0 24 48 72 96 120 144 168 192
light cycle
PM8:00 AM8:00
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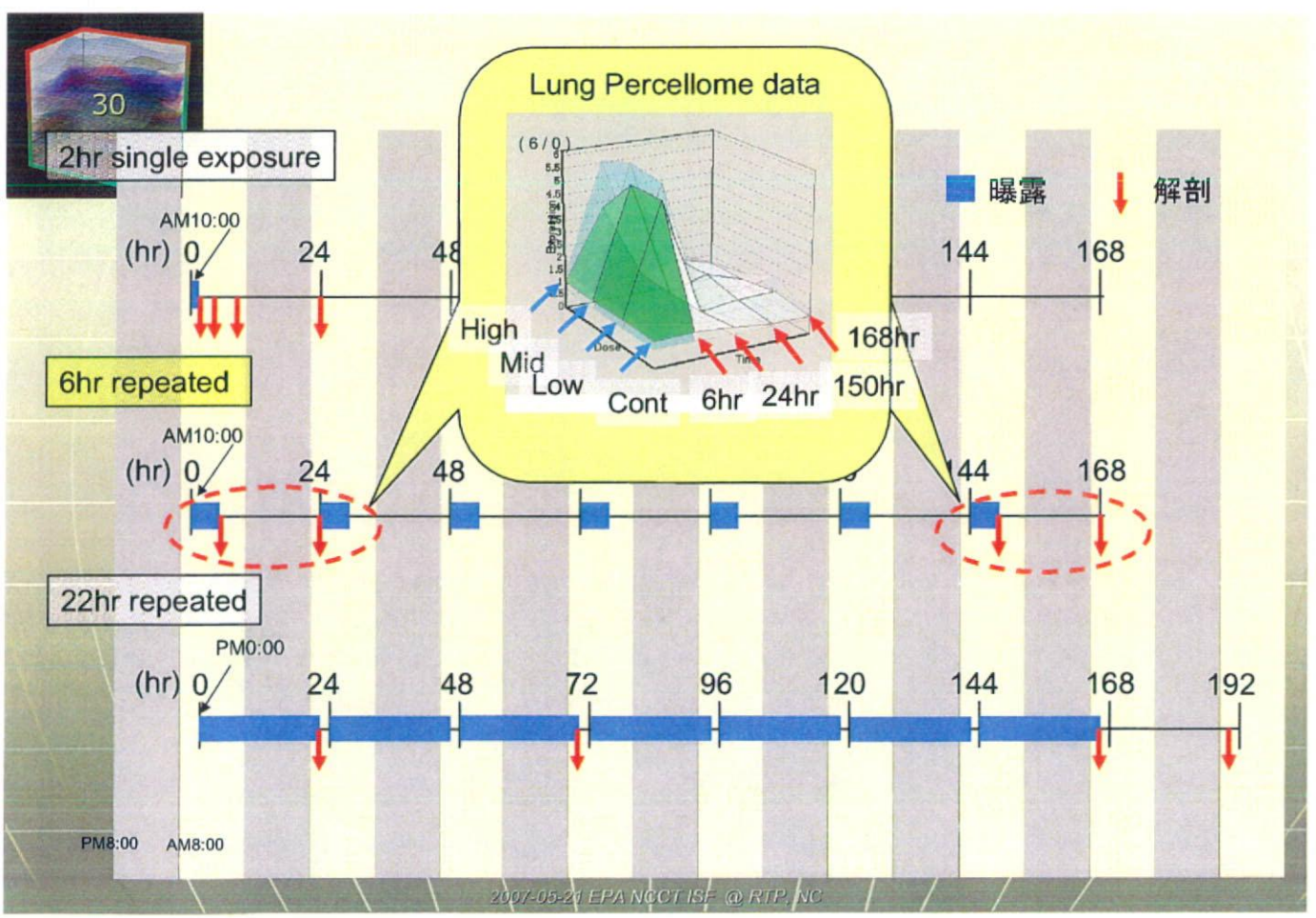
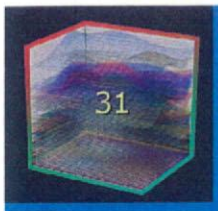
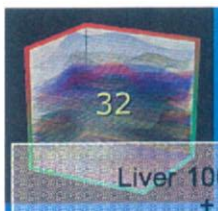
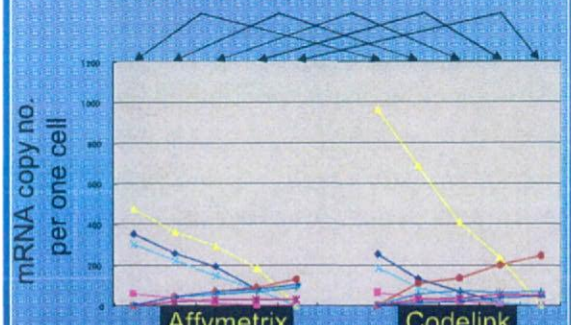

30
Lung Percellome data
2hr single exposure
曝露
解剖
AM10:00
(hr) 0 24 48 144 168
High
Mid
Low
Cont
6hr
24hr
150hr
168hr
6hr repeated
AM10:00
(hr) 0 24 48 144 168
22hr repeated
PM0:00
(hr) 0 24 48 72 96 120 144 168 192
PM8:00 AM8:00
2007-05-21 EPA NCCT ISF @ RTP, NC



Data-bridging among Affy, Agilent and Q-PCR



Liver 100% 75% 50% 25% 0%
+
Brain 0% 25% 50% 75% 100%



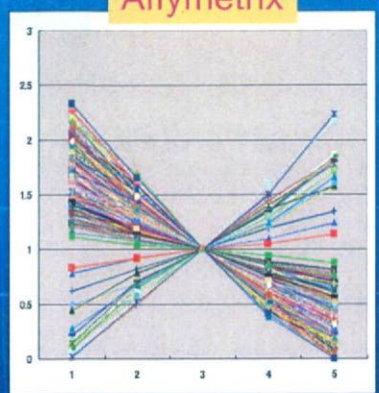
$y = f(x)$ $y' = g(x)$

$y = f(g^{-1}(y'))$

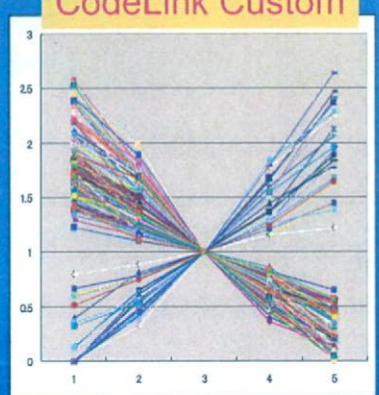
$y = F(y')$

To 250
High linearity
probesets
(R^2 closest to 1)

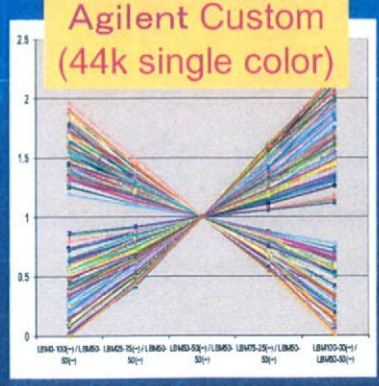
Affymetrix

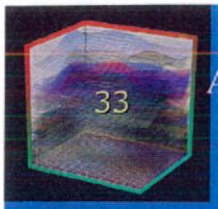


CodeLink Custom

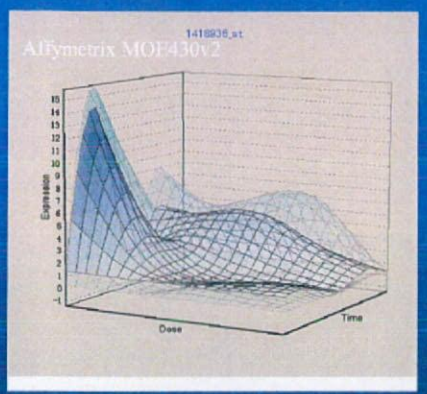


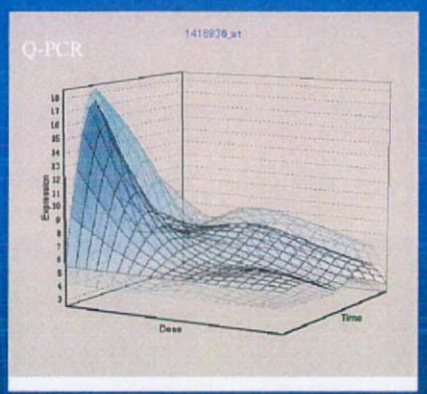
Agilent Custom
(44k single color)

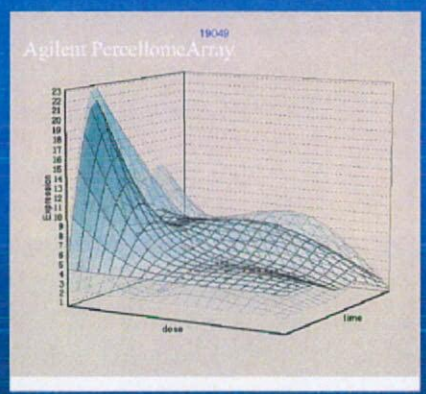


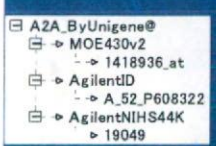


Affy Q-PCR Agilent









Maff

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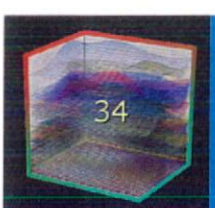

Strategy of data analysis (single gavage studies for early responses)

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