Current state-of-the-art of genomics in toxicology

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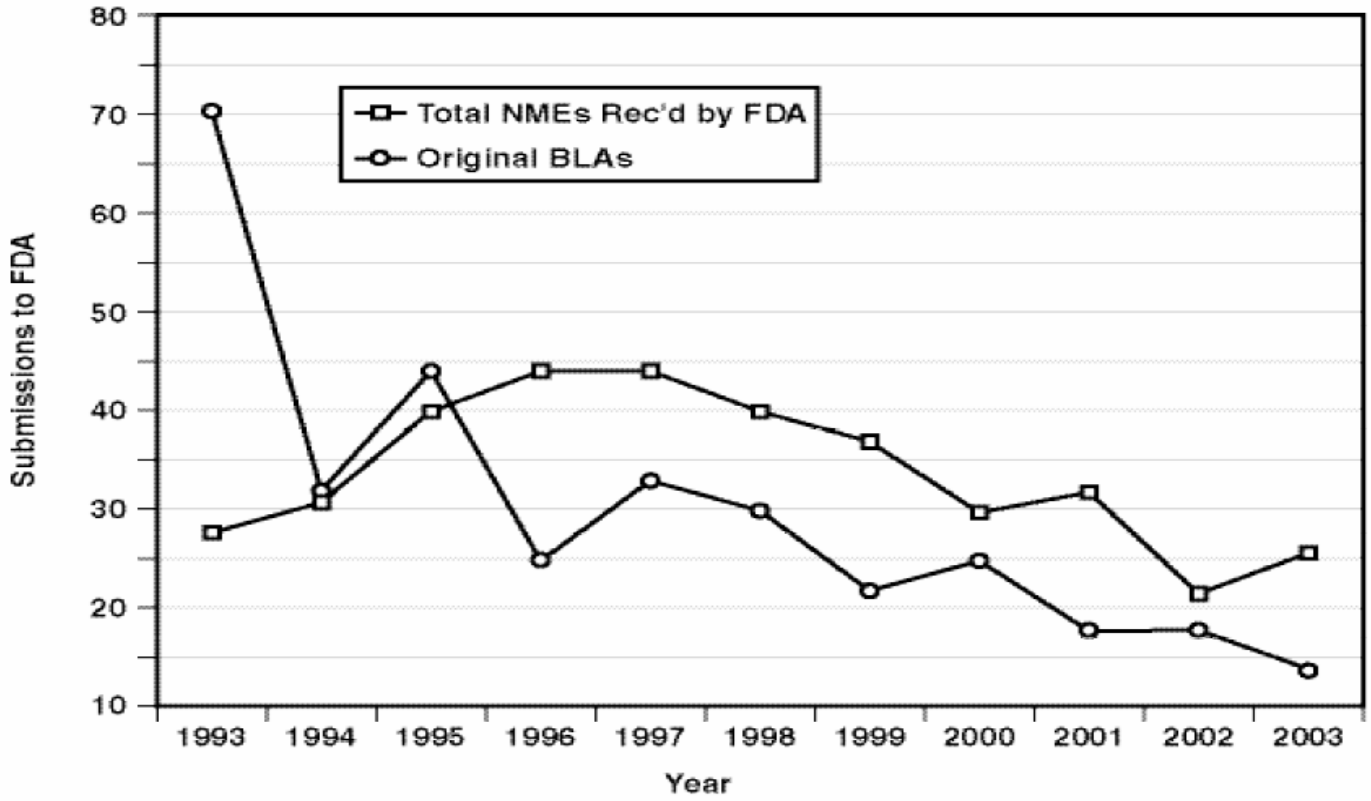
Slowdown in new drug and biologicals submissions to regulatory agencies worldwide starting in 2000

Figure 2: 10-Year Trends in Major Drug and Biological Product Submissions to FDA



The figure shows the number of submissions of new molecular entities (NMEs) — drugs with a novel chemical structure — and the number of biologics license application (BLA) submissions to FDA over a 10-year period. Similar trends have been observed at regulatory agencies worldwide.

Challenge and Opportunity on the Critical path to New Medicinal Products, U.S. FDA, March 2003.

Stagnation on the critical path to new medical products

In FDA's view, the applied sciences needed for medical product development have not kept pace with the tremendous advances in the basic sciences.

The new science is not being used to guide the technology development process in the same way that it is accelerating the technology discovery process.

→ *A new product development toolkit...is urgently needed to improve predictability and efficiency along the critical path*

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Challenge and Opportunity on the Critical path to New Medicinal Products, U.S. FDA, March 2003.

REACH: Registration, Evaluation and Authorization of Chemicals

1-10 tonnes per annum **~19,000 chemicals**	**10-100 tonnes per annum** **~5,000 chemicals**
-in vitro skin irritation -in vitro eye irritation -in vitro bacterial gene mutation -in vivo skin sensitization -In vivo Acute toxicity – oral -Aquatic toxicity – acute Daphnia and algae	-in vitro cytogenicity/gene mutation -in vivo skin irritation -in vivo eye irritation -in vivo acute toxicity – dermal, inhalation -in vivo 28 day repeated dose toxicity -in vivo reproductive toxicity - screening -in vivo toxicokinetics -in vivo short-term fish toxicity
100-1,000 tonnes per annum **~2,400 chemicals**	**>1,000 tonnes per annum** **~2,700 chemicals**
-in vivo repeated dose: 90 day sub-chronic toxicity -Reproductive toxicity: in vivo prenatal development & 2 gen reproductive tox -in vivo long-term daphnia and fish toxicity -Bioconcentration/accumulation -Terrestrial toxicity (if relevant)	-in vivo somatic cell test (if relevant) -in vivo 2-generation reproductive toxicity -in vivo carcinogenicity -in vivo terrestrial toxicity (if relevant) -In vivo sediment toxicity (if relevant) -In vivo long-term reproductive toxicity in birds – if relevant

Testing requirements of existing chemicals, per tonnage

EC, 1907/2006

Estimated laboratory animal usage: 16 million

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Senate resolution 3-1843:
Concerning scientific alternatives for animal testing in biomedical research

- The senate asks the Federal Government to:
  - perform a scientific study to evaluate the reliability of "Science Based Toxicology" (SBT) as an alternative for Animal testing in biomedical research
  - perform a feasibility study for the establishment of a "Belgian Centre for Toxicogenomics"
  - submit the same request to the European Council to ensure that more specialized centres are involved on an European level

- Accepted on November 29, 2006 with 11 votes, 1 abstention.

- Discussion in Senate on 28 Februari, 2007

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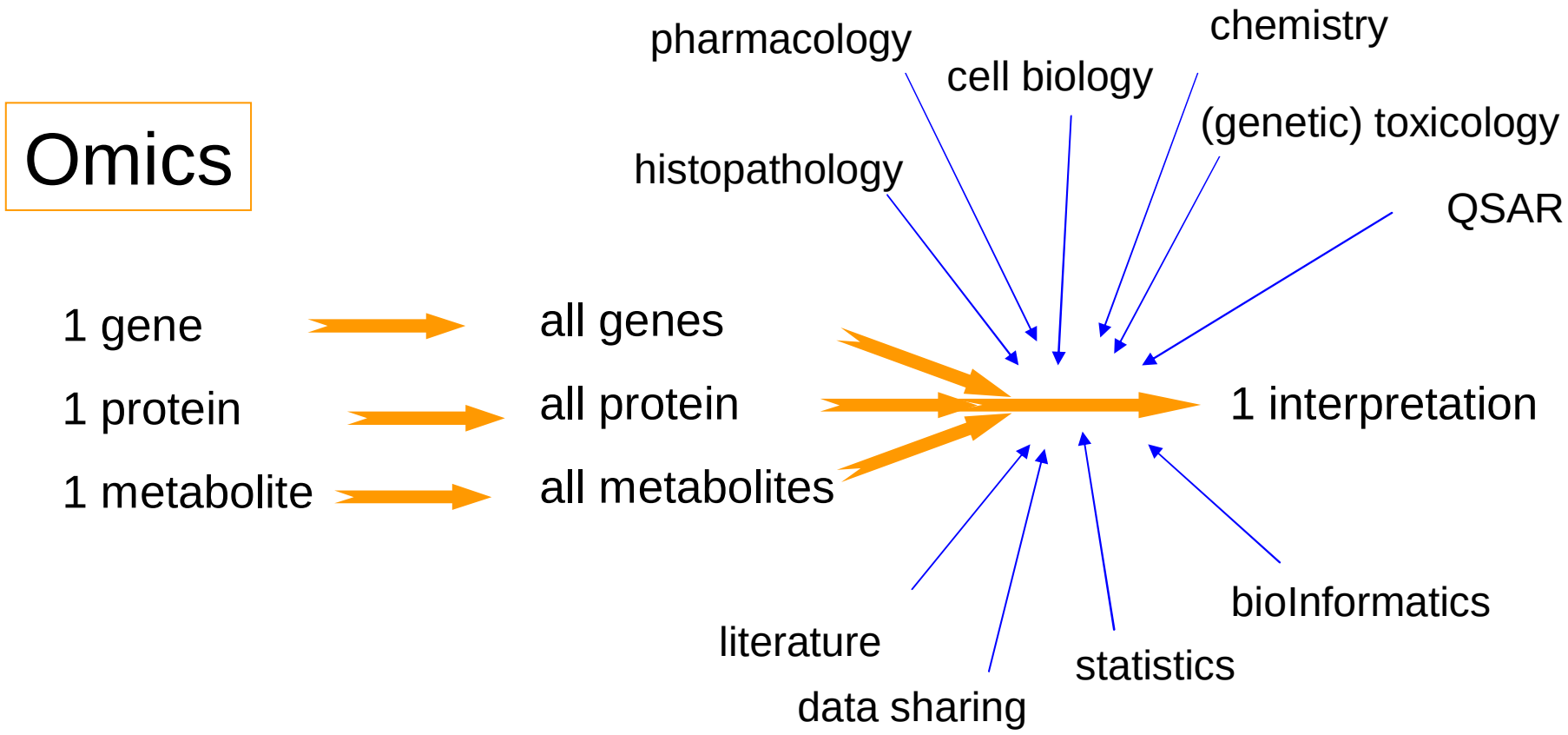
New Paradigm in Toxicology: TOXICOgenOMICS



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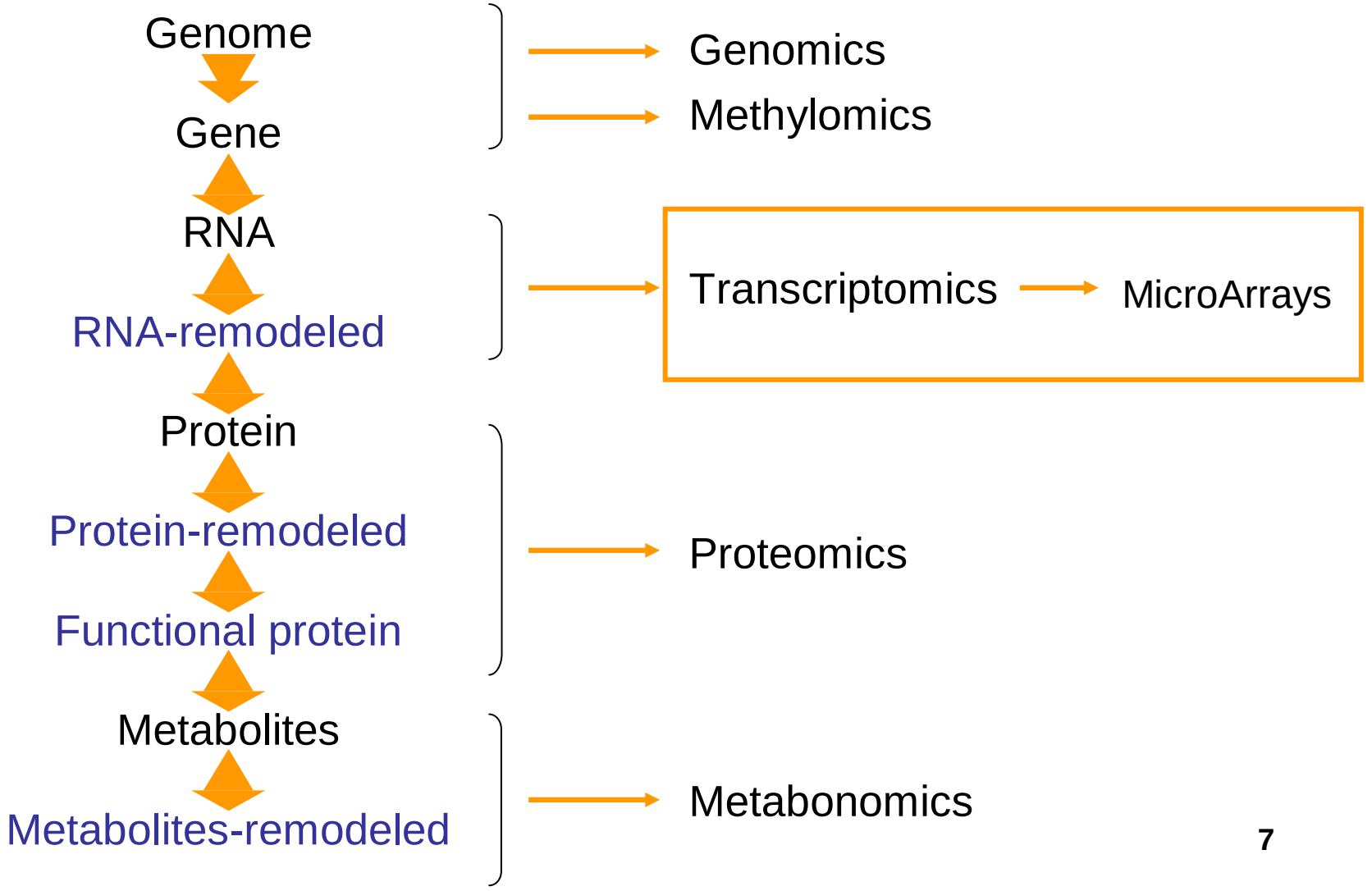
The Omics world



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Toxicogenomics: current state of the art

Academic questions

- Can it classify toxicants?
- Is it predictive?
- Can it explain mechanisms/mode of action?

Compared to current toxicology

- Is it more sensitive?
  - Conc/dose
  - Time
- Will it reduce testing?
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- Is it affordable or cost saving?
- What is the added value?

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Toxicogenomics: current state of the art

Gordon Conference Toxicogenomics, 2007

- Can agent specific patterns be detected?
  - McMillian et al., 2004; Biochem Pharm; 68
- Can patterns be detected associated with dose and exposure?
  - Auman et al., 2007, Environ Health Perspect; 115

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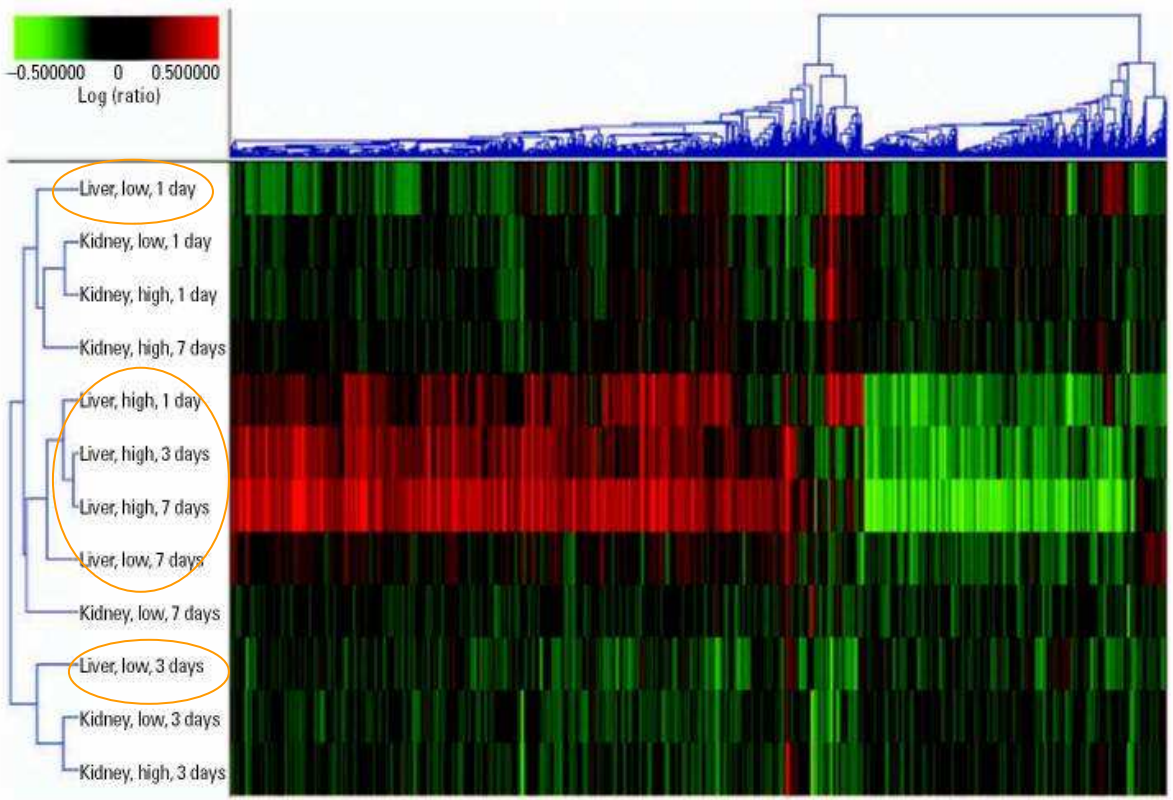
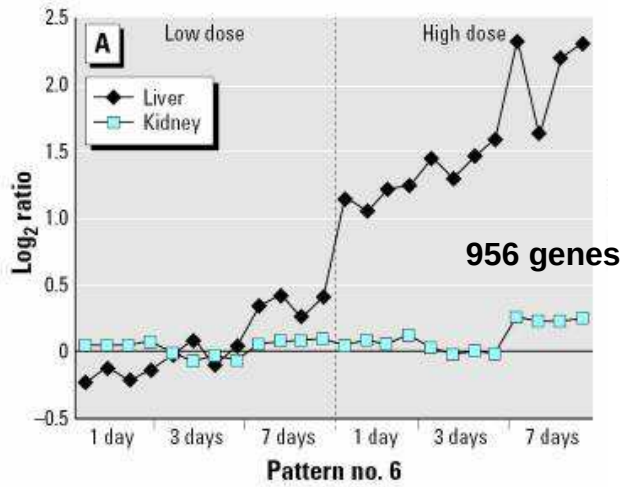
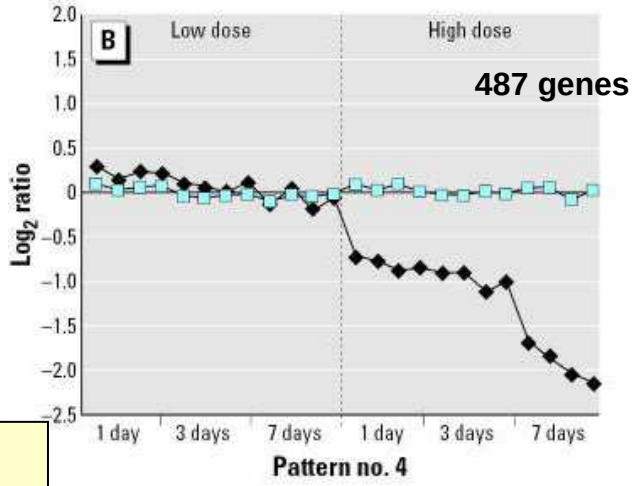
Identification of genes implicated in methapyrilene induced hepatoxicity by comparing differential expression in target and nontarget tissue

Auman et al., 2007, Environ Health Perspect; 115







Liver and kidney expression profiles in Rats

- Low and high dose - 1, 3, 7 days of dosing
- Liver 7 days-low dose: minimal hepatocyte necrosis
- Kidney: no histopatholgy findings

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- Can biomarkers of early effects be identified?
  - Heinloth et al., 2004, Toxicol Sci; 80
- Is genomics more sensitive then histopathological analysis?
  - Heinloth et al., 2007, Toxicol Pathol; 35

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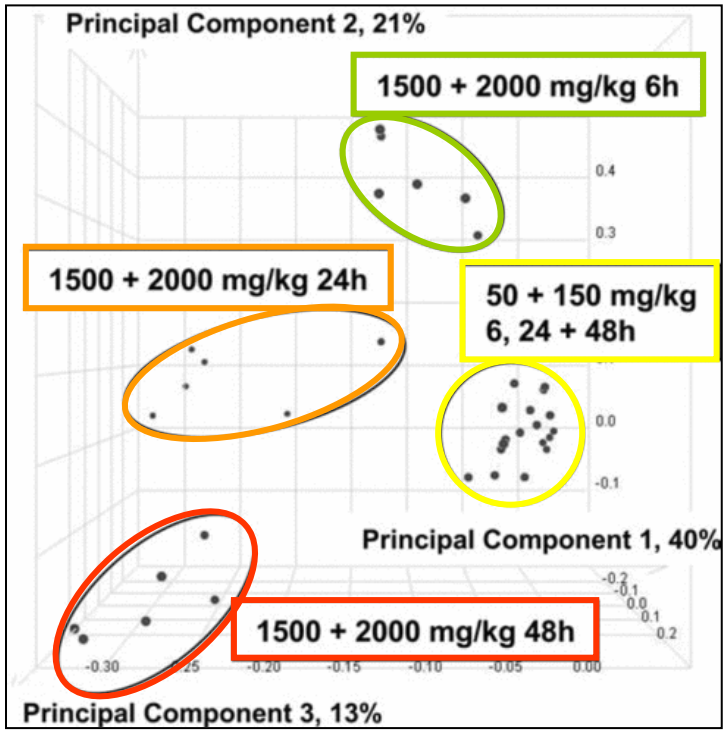
Gene expression analysis offers unique advantages to histopathology in liver biopsy evalutations

Heinloth et al., 2007, Toxicol Pathol; 35

Table 1 Quantification of necrosis in biopsy-like samples. *1500 mg/kg*

Animal #	Sample #	Time (hours)	% Necrosis	Mean % Necrosis	Std. Dev. % Necrosis
3018	1	24	0	3.33	5.77
3018	2	24	0		
3018	3	24	10		
3019	1	24	0	17.66	22.50
3019	2	24	10		
3019	3	24	43		
3022	1	24	40	21.66	20.21
3022	2	24	0		
3022	3	24	25		
3006	1	48	25	13.67	12.66
3006	2	48	16		
3006	3	48	0		
3007	1	48	29	24	8.66
3007	2	48	29		
3007	3	48	14		
3008	1	48	7	3.66	3.51
3008	2	48	0		
3008	3	48	4		



Paracetamol expression profiles in Rat liver

- Single doses 50, 150, 1500, 2000 mg/kg
- 6, 24 or 48 hr post exposure
- No clinical chemistry parameters altered at 50, 150, 1500 (6h) mg/kg
- Hepatotoxicity from 1500 mg/kg, 24h

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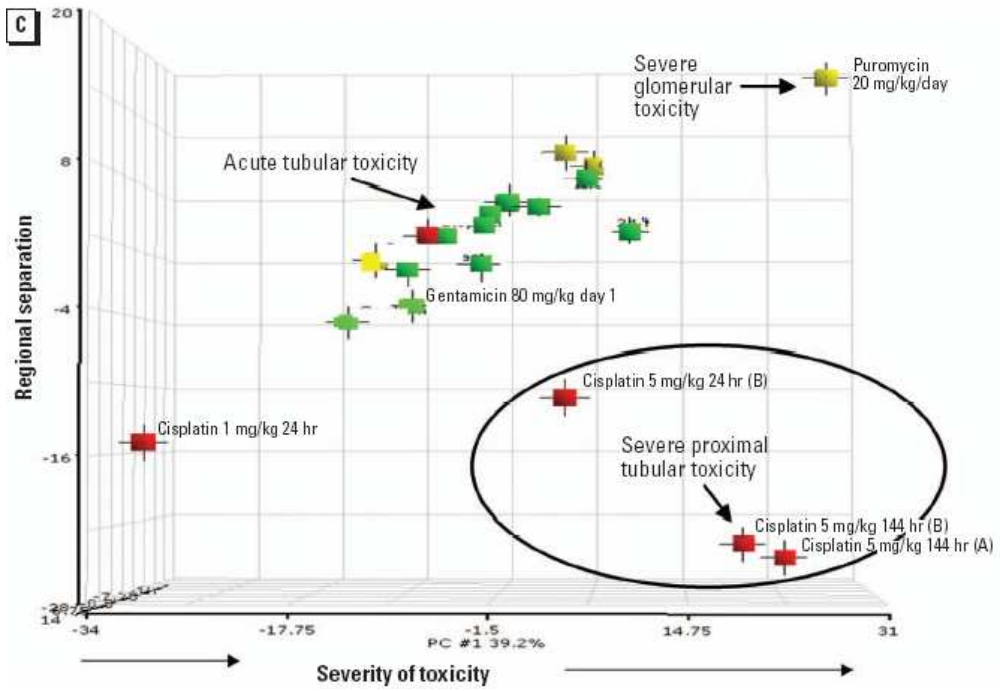
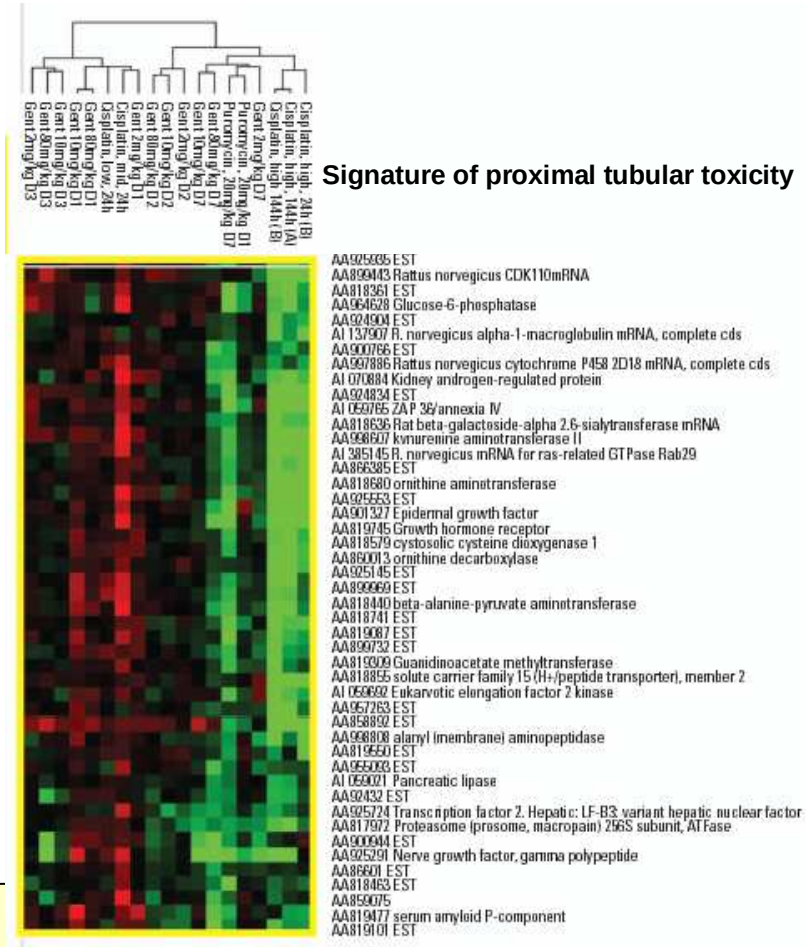
  - Amin et al., 2004; Environ Health Perspect; 112

Identification of putative gene-based markers of renal toxicity

Amin et al., 2004; Environ Health Perspect; 112





Expression profiles in Rat kidney

- Cisplatinin: proximal tubular necrosis; glucosuria
- Gentamicin: severe renal injury; increased BUN and creatine
- Puromycin: dilation Renal tubules - focal glomeruloscleorisis; proteinuria

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- Can genomics be used to bridge in-vitro / in-vivo?
  - Boess et al., 2007, Toxicol In Vitro
  - Werle-Schneider et al., 2006; Int J Tox; 25
- Can genomics be used to bridge organisms?
  - Mattingly et al., 2006, Toxicol Sci; 92

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Gene expression profiles in rat liver slices exposed to hepatocarcinogenic enzyme inducer, peroxisome proliferators and 17a-ethinylestradiol

Werle-Schneider et al., 2006; Int J Tox; 25

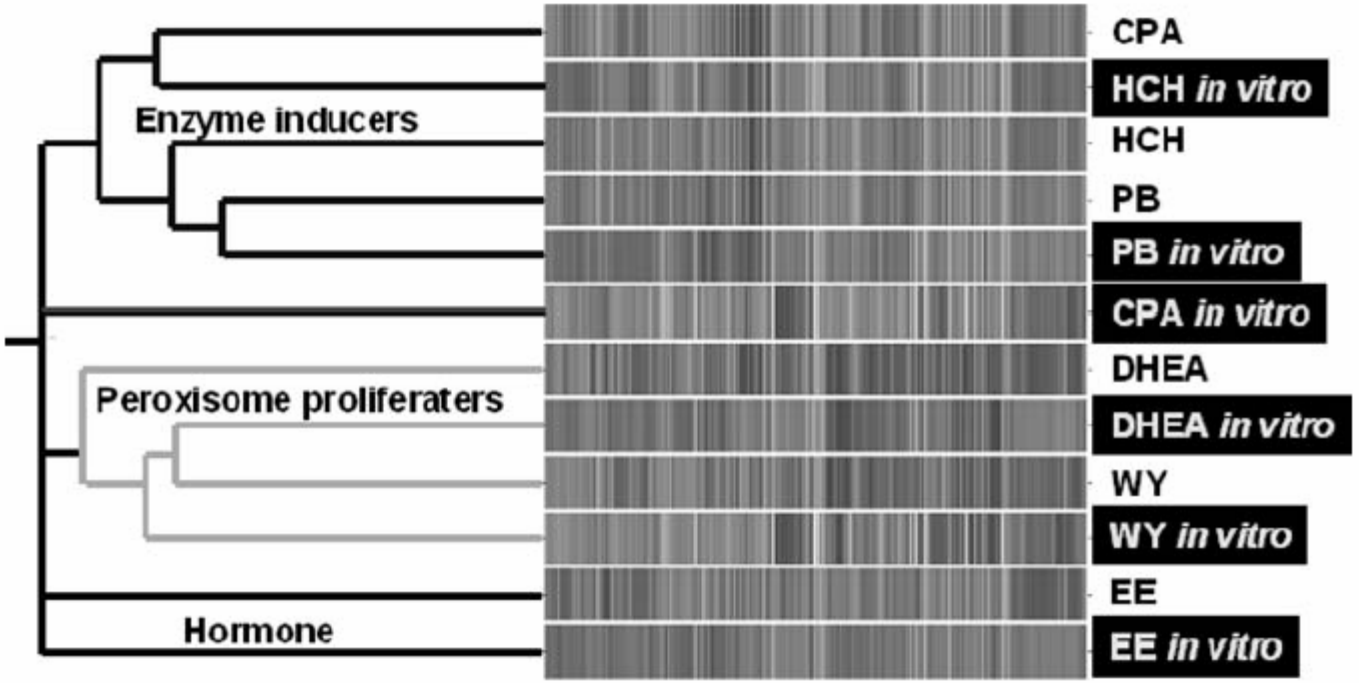

FIGURE 2

Expression profiles in Rat liver slices treated In Vitro compared to
In Vivo treatment: Scheel et al., 2003

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} More time/studies needed

- What is the added value? ✓

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Applications of genomics in preclinical drug safety evaluation

Lord et al., 2005; Pharm Toxicol; 98

Gene expression patterns can provide supportive evidence for mechanisms when analyzed in context with other findings/endpoints

Transcriptional profiling approaches have a lot to offer in drug development and safety assessment but they should not necessarily be expected to be definitive or standalone

Making distinction between predictive data versus definitive data is proving to be problematic in the regulatory setting of drug risk assessment

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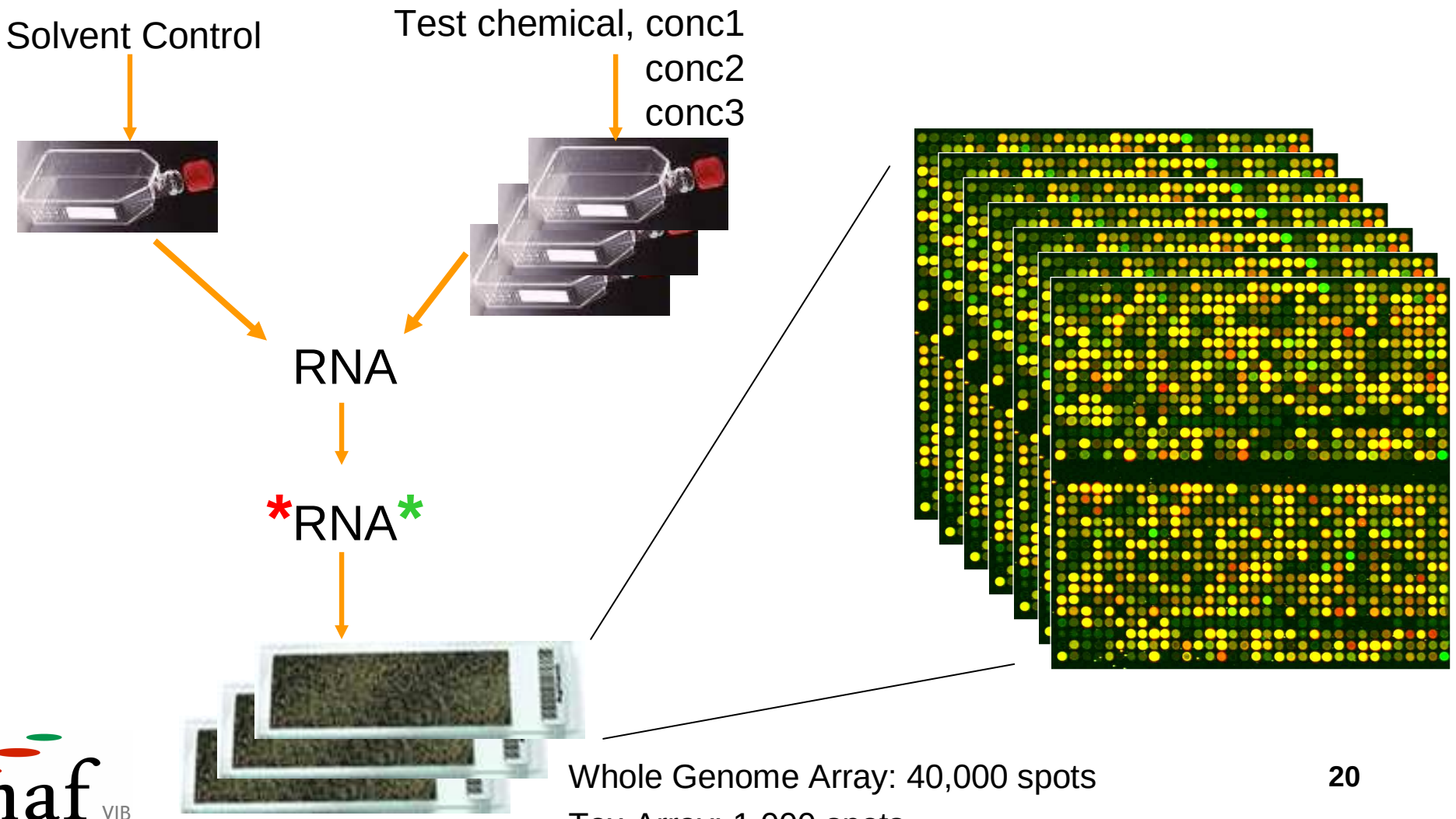
DNA MicroArrays for High Throughput Expression Profiling



Whole Genome Array: 40,000 spots

Tox-Array: 1,000 spots

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