Metabolomics in Regulatory Toxicology?

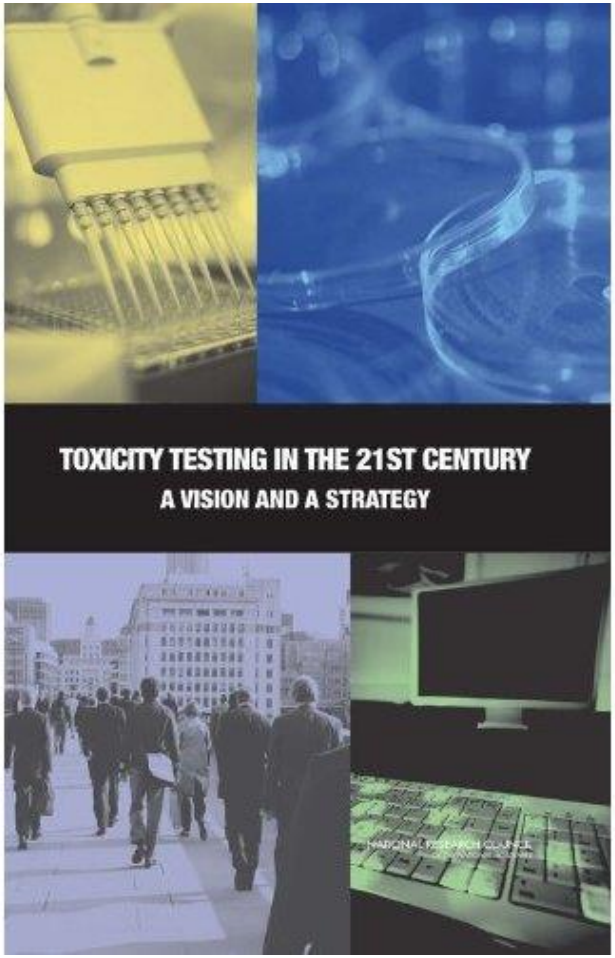
IGHRC/RSC London 13th October 2017

George Loizou

Setting The Scene

- More than 130,000 new synthetic chemical compounds have been developed over the last 60 years.
- The US EPA was established in 1970. HSE was established in 1975.
- Since then the safety and risk assessment of about 1000 chemicals have been “adequately” evaluated.
- Mostly based on rodent two-year bioassay, (developed in the early 1990s)
- Can take four to five years to complete at extraordinary cost
  - Several million dollars for a properly conducted good laboratory practices compliant bioassay in rats and mice.

Next Generation Risk Assessment



US National Research Council Report (2007)

Has stimulated activities described as working toward a ‘Paradigm Shift’ in toxicology

Tox21

Toxicology in the 21st Century (Tox21)

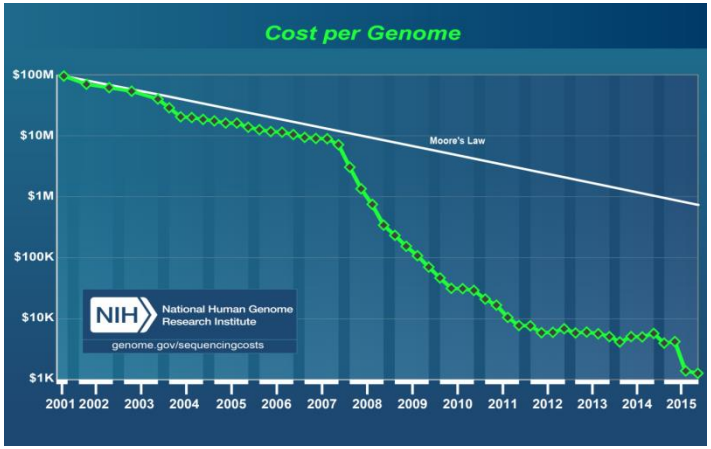
Collaboration of US federal agencies

EPA, NIH, NIEHS, FDA…

Develop more efficient and less time-consuming approaches to predict how chemicals may affect human health

The Human Genome Project

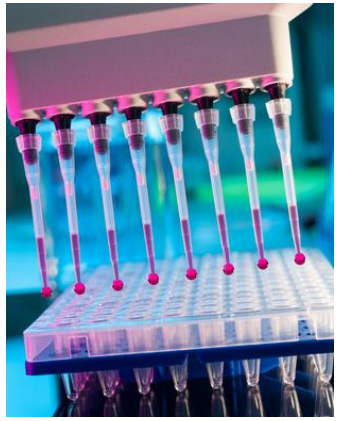
- In 1990 US Congress established the Human Genome Project
- Completed two years ahead of schedule in April 2003 and cost $2.7 billion



Cost is dropping greater than exponential rate!

Huge Economic benefits

- Every $1 invested by the U.S. government in the HGP generated $141 in spin-off economic activity
- Robotics technology to screen thousands of chemicals for potential toxicity
- *In vitro,* high-throughput, cell-based toxicity testing
- A paradigm shift in toxicology



Human Genome Sequencing

In 2016 your genome could be sequenced for under $1000 in a day….

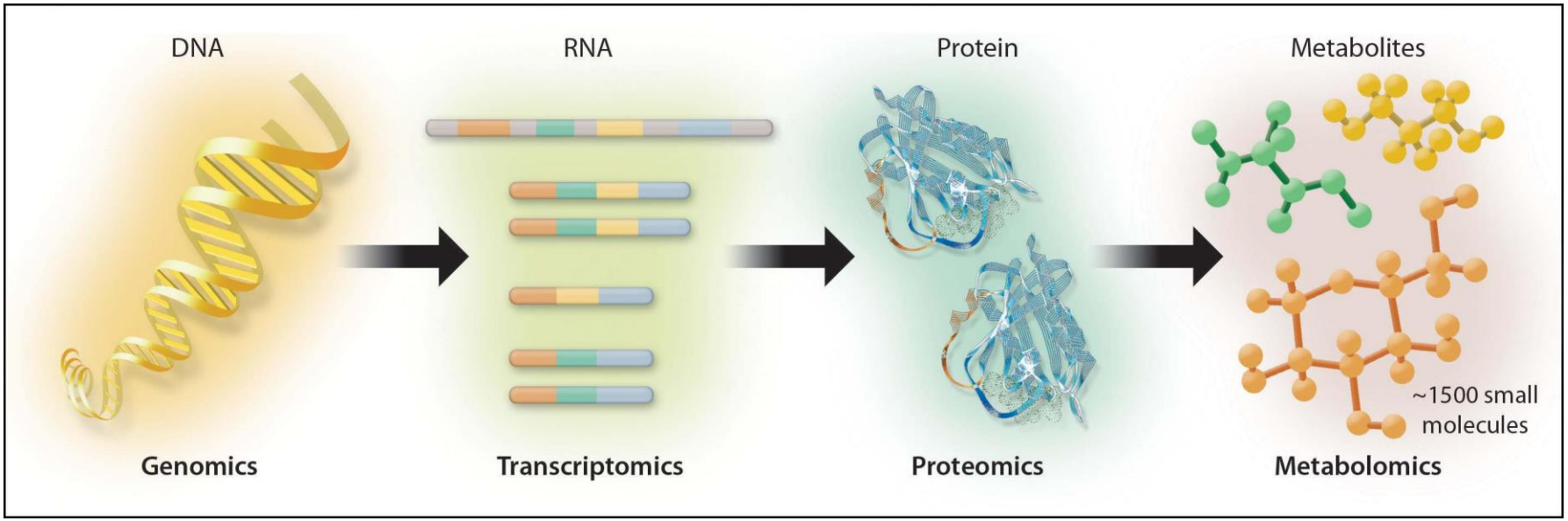
And today…in 12 to 15 minutes!

Genes versus Environment

Although there are genetic influences, 98% people die of environmental causes

The “Omics” Era

Genes (DNA sequences) switched on/off → 1st step in gene translation → Protein products (mainly enzymes) of genes → Arrays of metabolites (due to enzyme activity)



Huge Potential

- Specific molecular end points
- Early detection
- At lower exposure levels compared with histopathology, clinical chemistry or haematology
- Cell-wide detailed analysis of mechanisms of toxicity
- Without need for *a priori* knowledge on mode of action

Big Data

- Grown out of high throughput experimental technologies
- Terabyte-scale (10^{12}) datasets for systems-level measurements of cellular and molecular phenomena
- Generating approximately 1.8 zettabytes (10^{21}) of biological data every year, roughly doubling the world's information resource every two years!*

*enough to fill a stack of DVDs that would reach from Earth to Mars

Omics Databases

- More than 50,000 “genomics” papers per year
- Publicly available databases storing omics responses of human disease, surveys and clinical assays measuring human exposure and health outcomes
- Pose considerable challenges in data processing and extraction of biological meaning

Human Toxicity Data?

For environmental chemical safety assessment human toxicity data are rare

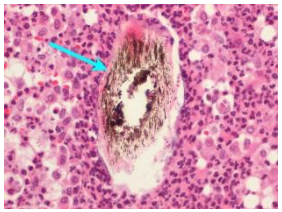
Mostly inappropriate (accidental poisoning)

Appropriate mechanistic, dose-response data cannot be generated for obvious ethical reasons

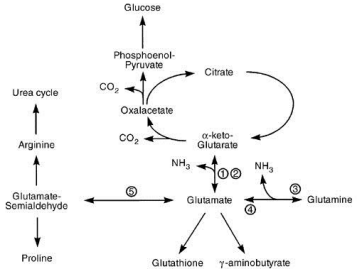
However, this could be changing!

Old Versus New

- Traditional toxicology
  - Described ‘apical’ endpoints
    - i.e., tissue damage and disease measured by histopathology, clinical chemistry, haematology
  - Reliant on animal studies



- New Toxicology
  - Detect biochemical disturbances that precede tissue damage and disease
  - At much lower exposure concentrations than apical endpoints
  - Can be measured *in vitro*



Metabotyping

Metabonomics

Metabolic responses through time as a result of perturbations of complex systems stimulated by disease, nutritional changes, drug therapy, genetic modulation, and environmental exposure to xenobiotics

Metabolomics

Endogenous small molecule composition of a given body fluid sample in terms of metabolite presence and concentration

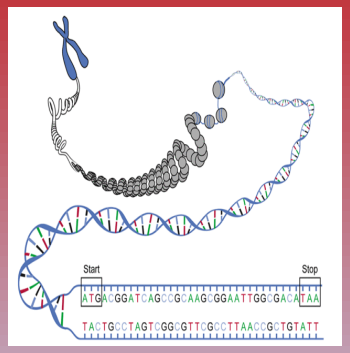
The Technology

Mass-Spectrometry or Liquid Chromatography coupled to Gas Spectrometry are able to resolve around, ~~1500~~, ~~2500+~~ metabolites, such as amino acids, fatty acids, nucleotides, and many other small molecules

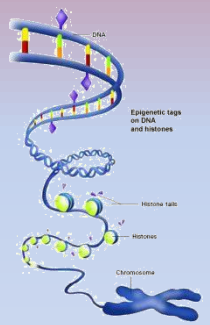
High-resolution metabolomics (HRM) using ultra-high resolution mass spectrometry with data extraction algorithms now enables measurement of greater than **10,000** chemicals in biological samples with quantitative reproducibility

(Walker, et al. (2016). *International journal of epidemiology* 45(5), 1517-1527)

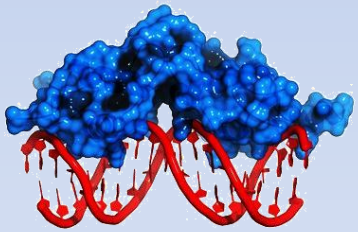
Genomics



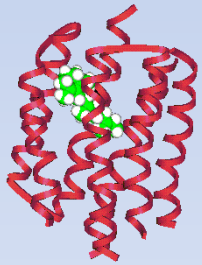
Epigenomics



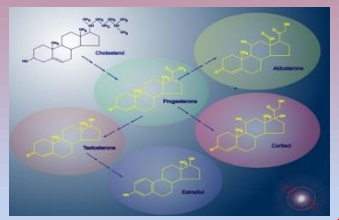
Transcriptomics



Proteomics



Metabolomics



Phenomics

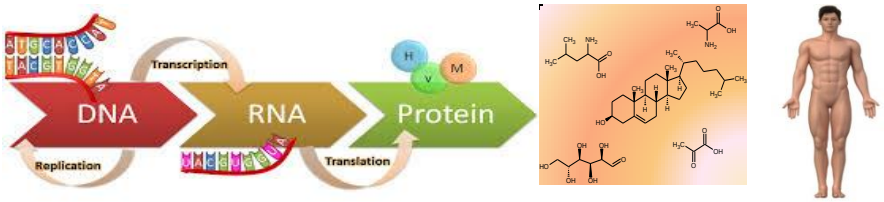
Lower

Higher

Level of Organisation

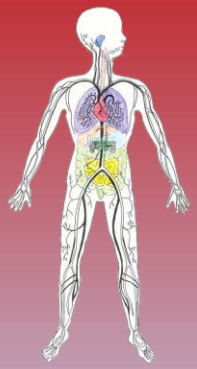
Why Metabolomics?

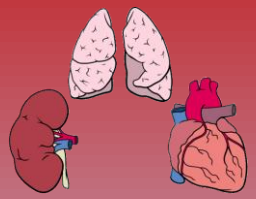
- Component of the Central Dogma

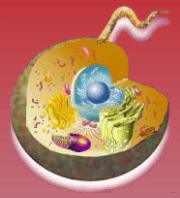


  - DNA encodes RNA, RNA encodes Protein, Enzymes metabolise…
- Reflect and magnify (several thousands of times) perturbations that occur at the genomic, transcriptomic and proteomic level
- Reflects changes at a higher level of organization, that is, closer to the phenotype
- Diagnostic readout of effect of environment/nutrition on phenotype
- Provide a more reliable indication of state of health of the individual

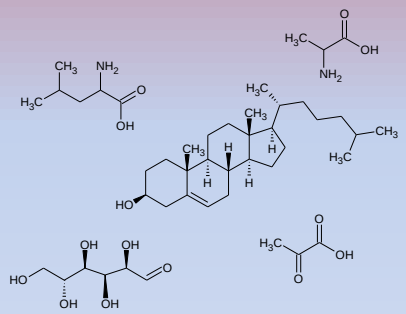
Body → Organs → Cells



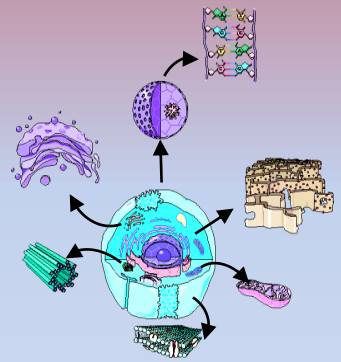




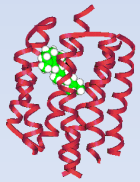
Microbiome



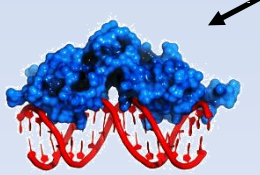
Metabolomics



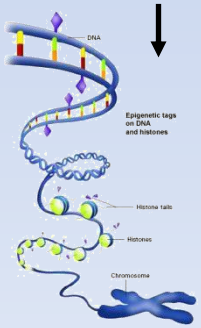
Sub-cellular organelles



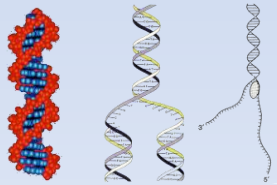
Proteomics



Transcriptomics



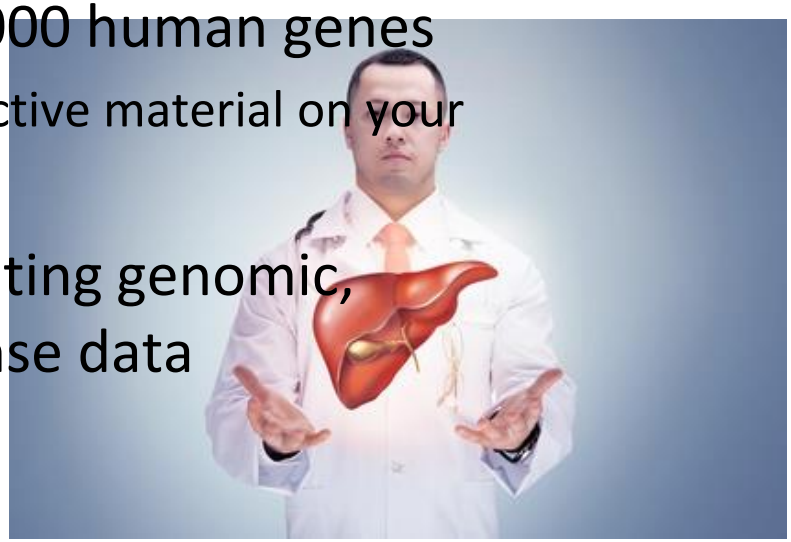
Epigenomics



Genomics

The Microbiome

- 100 trillion microorganisms (bacteria, fungi, viruses) in and on every human being
- Outnumber human cells by a ratio of 3-10:1
- 1–3% of body mass (0.75–2.25 kg in a 75 kg person)
- 3.3 million microbial genes to 23,000 human genes
  - Human genes just 0.7% genetically active material on your body
- Confounding factor when interpreting genomic, proteomic or metabolomic response data



Metabolic Signatures

- Patterns of metabolite changes predictive of the manifestation of toxicity and disease
- Serum, plasma, urine, mucosa, exhaled breath, saliva, hair, sweat
- Tissue and cultured cells
- Steady growth in signature identification
  - Pre-symptomatic, diagnostic, prognostic

Could provide human data that may be used in chemical safety assessment?

iKnife

Developed by Dr. Zoltan Takats of Imperial College London

Electronic Noses

MS-based pattern-recognition array sensors that capture volatile organic compounds in exhaled breath, providing disease-specific molecular signatures

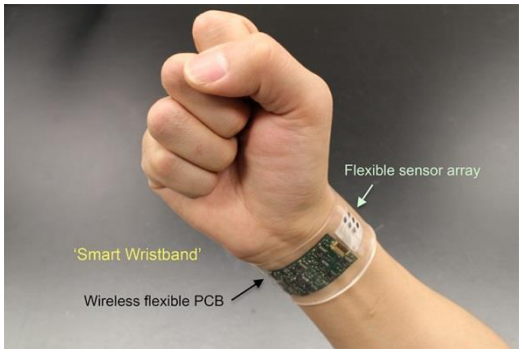
Promising results for early detection of lung, breast and prostate cancer and the distinction between asthma and COPD

Breath signatures produce an abnormal array of organic chemicals, thought to precede the transformation of normal to cancerous cells

http://www.owlstonenanotech.com/

Biosensors

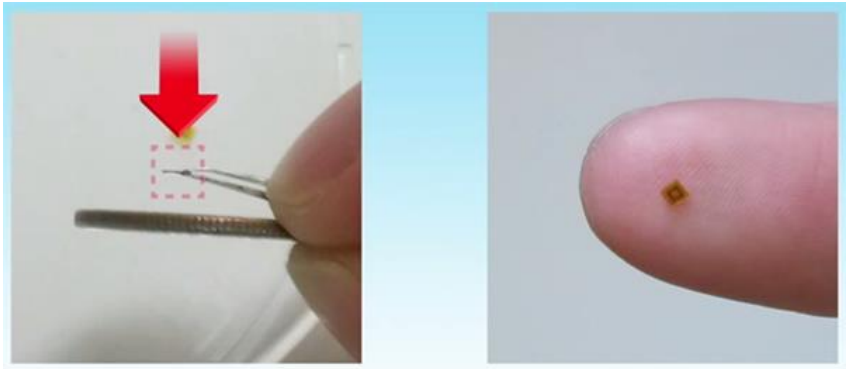
A small hand-held device that can prick your thumb, measure 2500 organ-specific proteins and send this information to a server for analysis and feedback the information on the state of your 50 organ systems (promising developments in this direction already exist, Hood et al (2013))



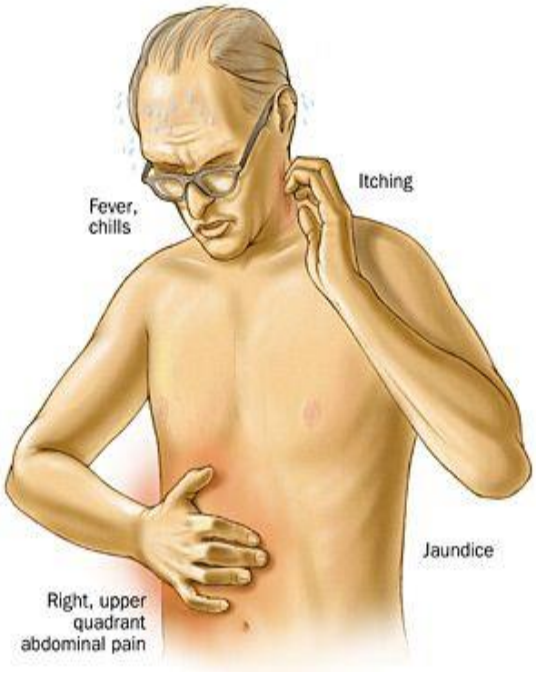
Sophisticated sweat sampling technology to provide sweat samples for frequent, non-invasive metabolic profiling

Implantable Real-Time Biosensors

- Blood glucose levels
- Blood and Intracranial pressures
- Blood biomarkers that precede heart attack
- All send information wirelessly to smartphone app



Medical Practice



Superstition-based to Symptoms-based to Molecular Medicine

Precision Medicine

- In 2015 President Barack Obama announced the Precision Medicine Initiative
- Instead of a one-size-fits-all solution designed to help the largest number of statistically average people
- Medical treatments tailored for an individual's unique genetic makeup, environment and lifestyle

P4 Medicine

1. Predictive
2. Preventive
3. Personalised
4. Participatory

Leroy Hood,Co-Founder of the Institute For Systems Biology (Seattle) receiving the National Medal of Science

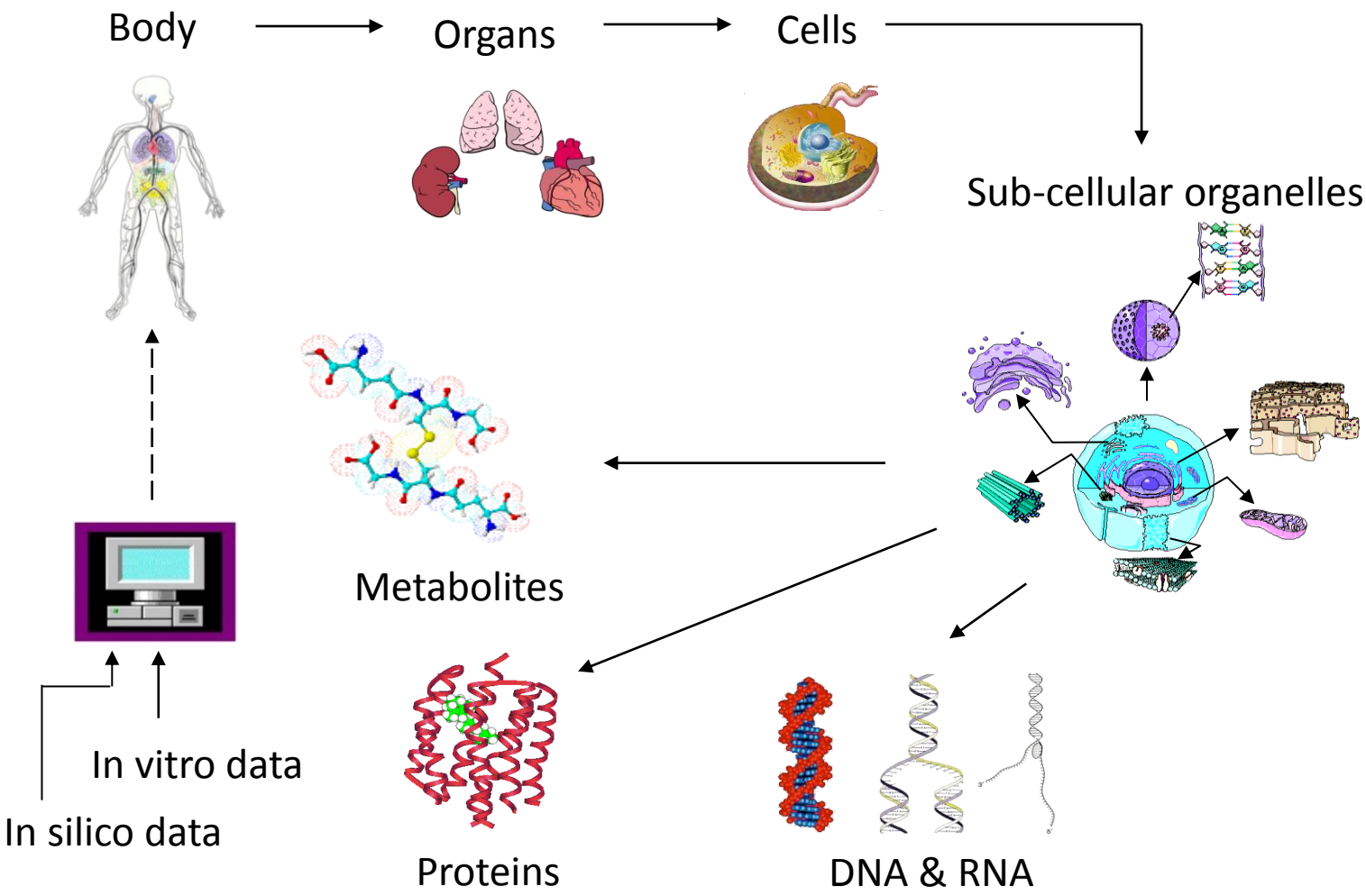
The Greatest Convergence in Our History!

"The coalescence of the rapidly maturing digital, nonmedical world of mobile (wireless) devices, cloud computing and social networking with the emerging digital medical world of genomics, biosensors and advancing imaging" Topol, E. J. (2012). *The Creative Destruction of Medicine: How the Digital Revolution Will Create Better Health Care. Basic Books, New York.*

The digital transformation of healthcare to a wellness paradigm (Smarr, 2012)

The Next 10-15 years will usher in the era of P4 Medicine (Hood et al, 2013)

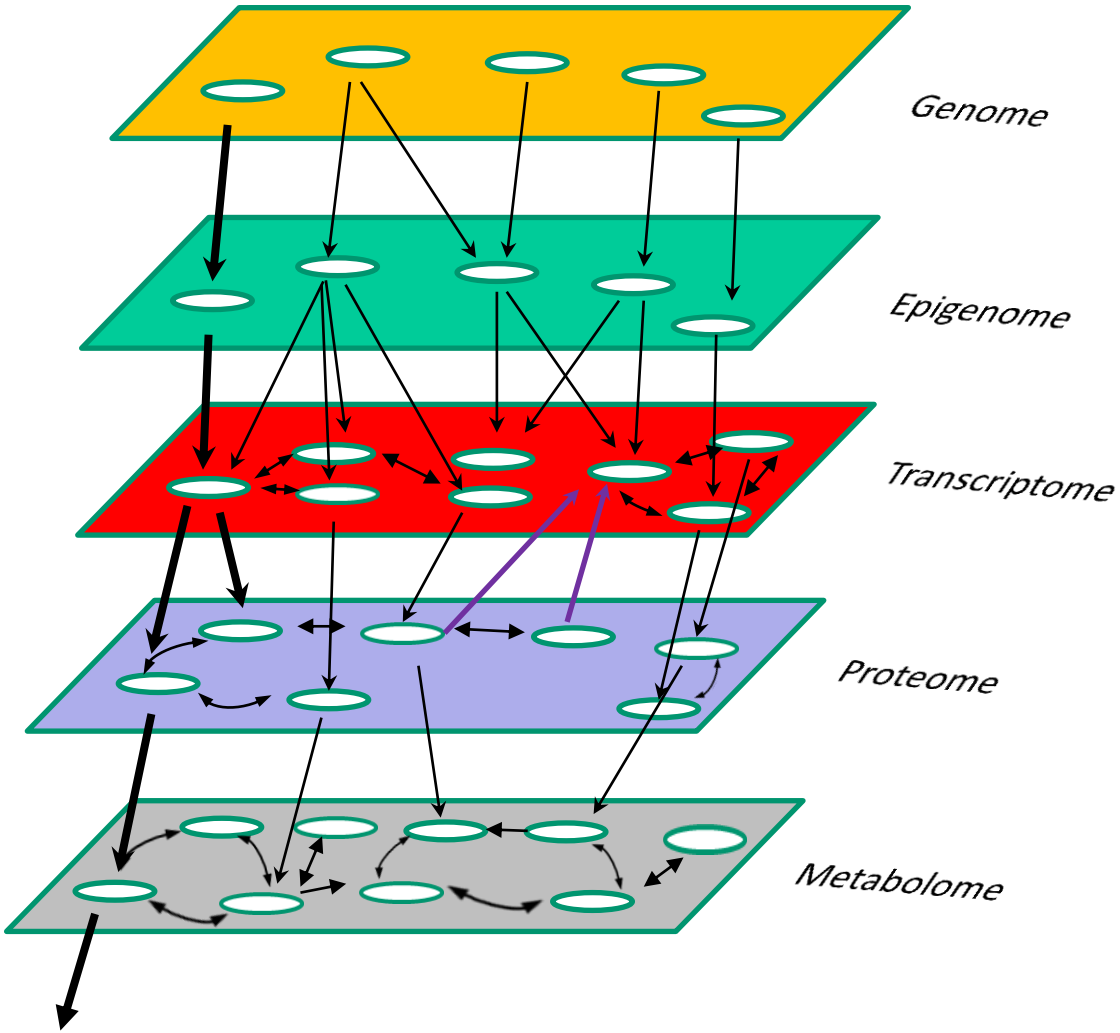
The Reductionist Approach



Systems Biology

- Altered molecular and cellular components that result from exposure to chemical and non-chemical stressors, are studied and *integrated* across multiple levels of biological organization
- From genes to gene expression products, to alterations in biochemical pathways and networks and the propagation of effects from cells to tissues to organs and the whole body

Multi-omic Analysis



Outcome: More complete view of biological activity

Pathway and Network Analysis

Database

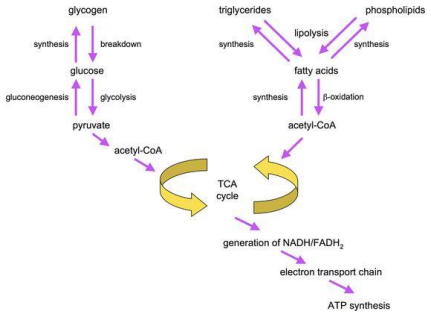
e.g.,
Consensus
Path DB
human

Protein interactions
Signalling interactions
Metabolic interactions
Gene regulations
Genetic interactions
Toxicant target
interactions
Biochemical Pathways

Integration
of
interaction
networks

Pathway of
Toxicity

Metabolite
signature



Pathways of Toxicity

- Metabolite signatures used to identify a Pathway of Toxicity which connects the molecular initiating event (MIE) of a toxicant with an adverse outcome
- Omics databases e.g., Human Metabolome Database and ConsensusPathDB-human
- Bioinformatics and statistics

Perturbed Networks

Disease a consequence of disease-perturbed networks in an organ

Initial disease perturbations may be genetic (e.g., mutations) and/or due to environmental exposure (e.g., infectious organisms, environmental chemicals)

Perturbations propagate from one or a few perturbed networks to many as the disease progresses

Pathophysiology of toxicity and disease due to altered dynamics of information flow through networks

P4 Medicine

Genomes and attendant medical, molecular, cellular and environmental data will be a routine part of each patient's medical record

Biosensors and wireless consumer devices will allow digital self-tracking via smartphone apps

Measurement will be longitudinal and throughout our lives, immediately identifying any transitions from health to disease

Alerts will be issued early on and preventive measures suggested such as changes in diet, exercise habits or avoid exposure

Anonymised Databases

Human *in vivo* metabolic signatures reflecting perturbed networks arising from disease and environmental chemical exposures

Accessed by qualified researchers and health practitioners

Will afford unparalleled opportunity to mine these data for the predictive medicine of the future and the risk assessment of environmental stressors

Genomic and Phenomic Databases in Development

- 100,000 Genomes project 'Clinical Interpretation Partnerships' (Genomics England)
- UK Biobank – world's largest imaging study. 10,000 participants already scanned. Combined with existing wealth of data
- Human Longevity Inc. (28,000 to date. 1 million integrated health records with genome, molecular and clinical data by 2020)
- eMERGE (Electronic Medical Records and Genomics) (National Human Genome Research Institute (NHGRI))
- The Clinical Genome Resource (US National Human Genome Research Institute, disease-related variants)

Genomic and Phenomic Databases

- Complete history and physical examination
- Whole genome sequencing
- 2400 blood metabolites
- Oral, urinary and faecal microbiome sequencing
- Structural brain MRI for volumetric assessments
- Whole body MRI for visceral and subcutaneous adipose tissue, skeletal muscle mass and intrahepatic triglycerides
- Bone density
- Ocular, visual and hearing acuity
- Over 40 blood biomarkers
- Urinalysis
- Resting energy expenditure
- Maximal oxygen consumption (VO_2Max)
- Echocardiography
- Electrocardiography
- Carotid Intimal Media Thickness
- Full pulmonary spirometry

Life at The Speed of Light with Craig Venter
https://youtu.be/pp2BZND7xLc

Implications for Regulatory Toxicology:
A New Dawn for Chemical Safety Assessment?

- Human *in vivo* metabolic signatures identified for precision medicine
- Stored in a reference data base e.g., MSEA, a library of ≈ 1000 predefined metabolite sets covering various metabolic pathways, disease states, biofluids and tissue locations[1]

[1]Xia et al (2010).. *Nucleic acids research* ***38(Web Server issue), W71-7, 10.1093/nar/gkq329.***

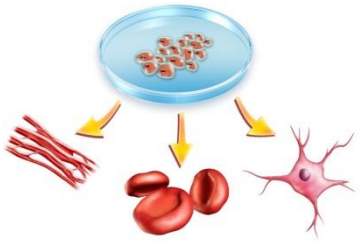
MSEA = Metabolite Set Enrichment Analysis

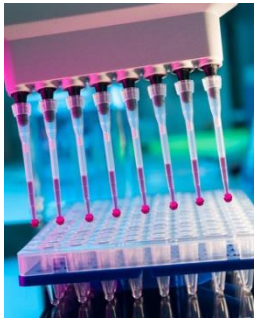
Implications for HSE:
Metabolomics based regulatory submissions?

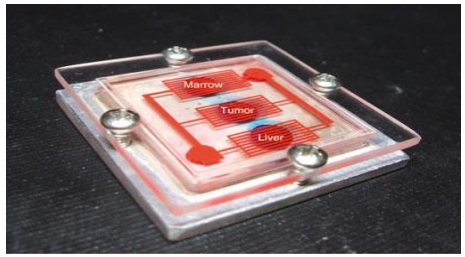
In vitro metabolic signatures and perturbed networks measured in 3D cell assays, organs-on-chip etc.,

Validated by comparison with *in vivo* metabolic signatures in a reference data base e.g., MSEA

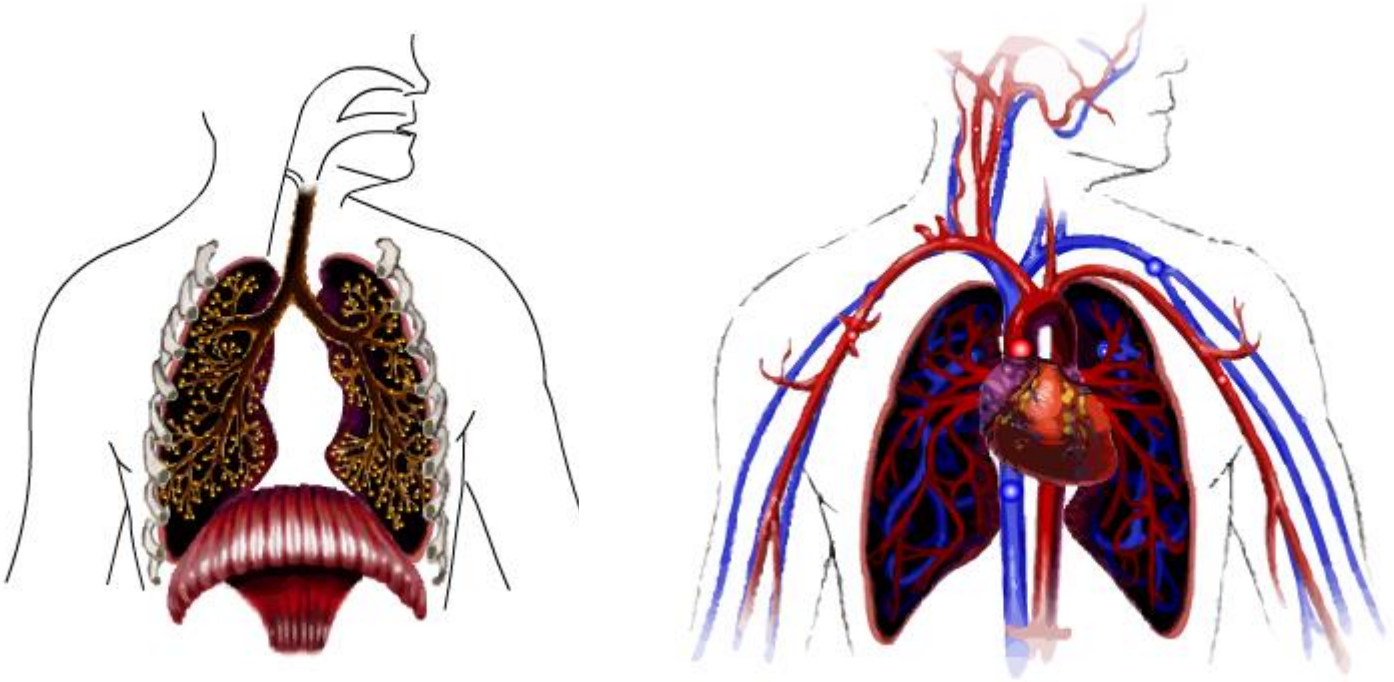
External exposure concentration-response relationships predicted from *in vitro* "signature" concentration-response relationships





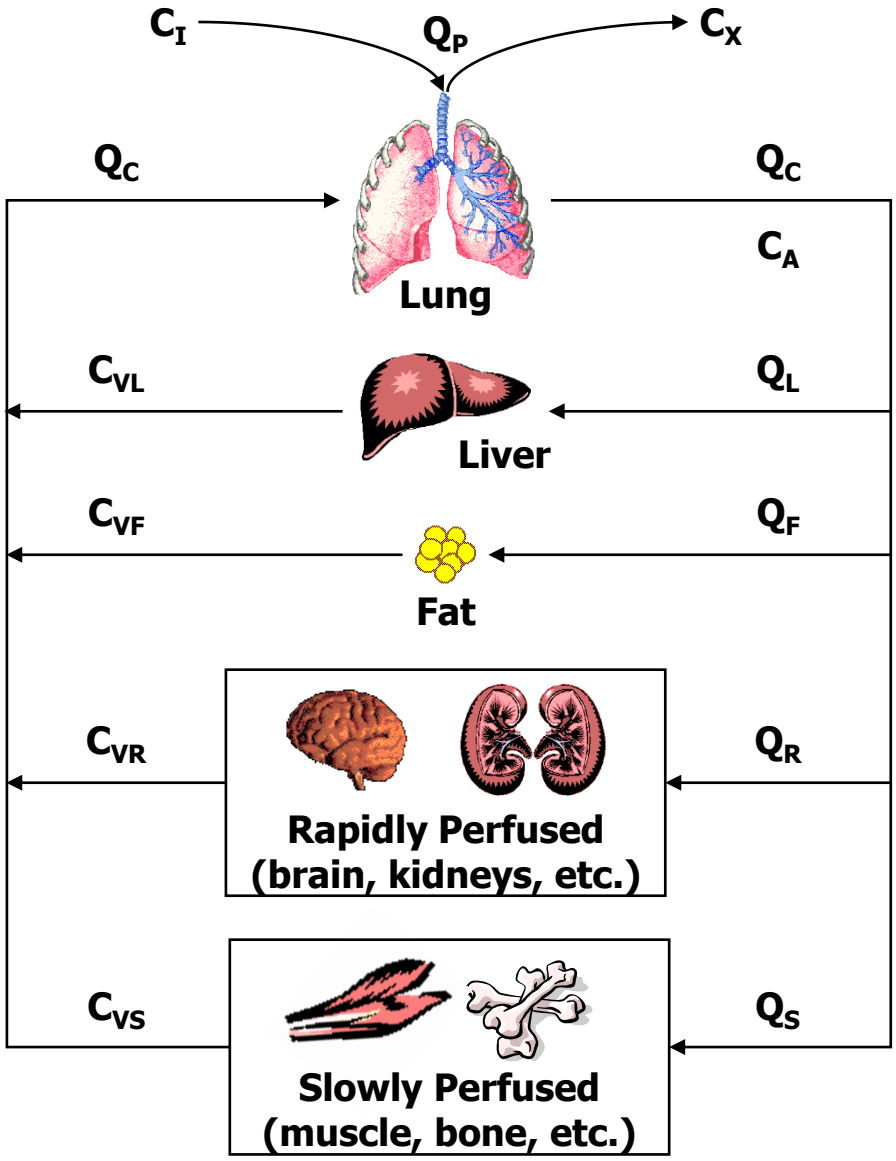


What is a PBPK Model?

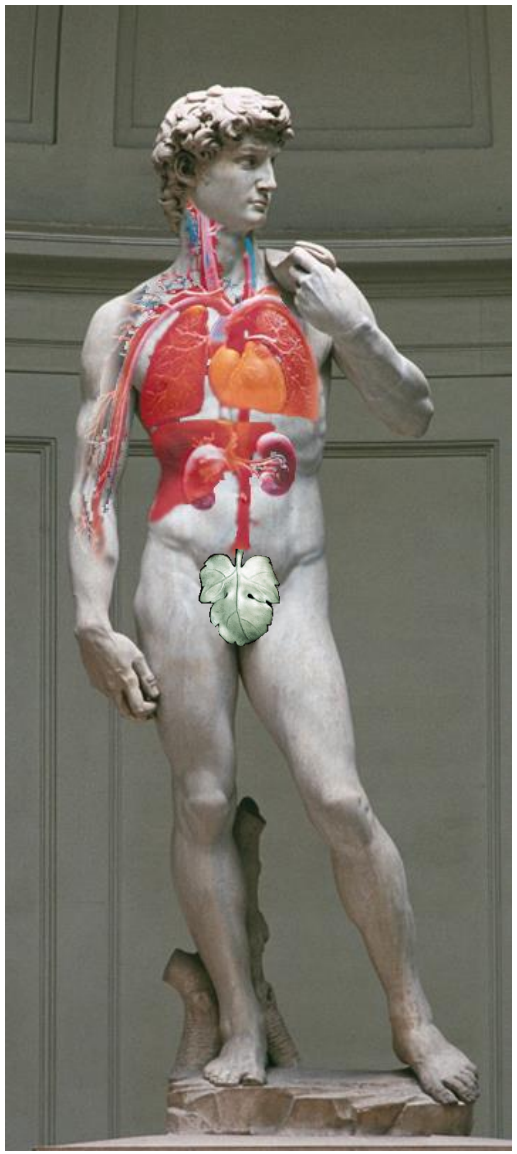


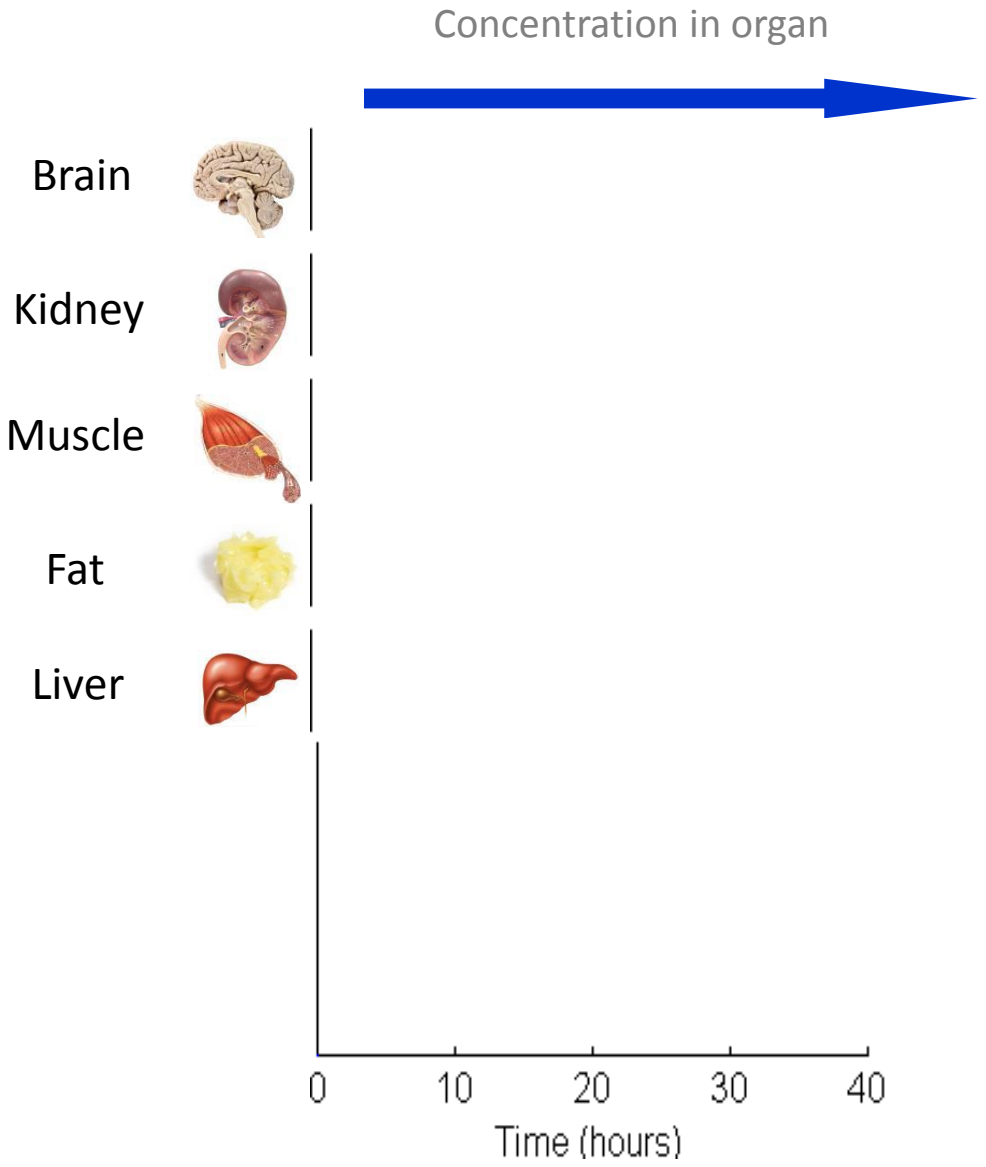
A biologically realistic, simplified model

Physiologically Based Pharmacokinetic Model



Distribution to the organs





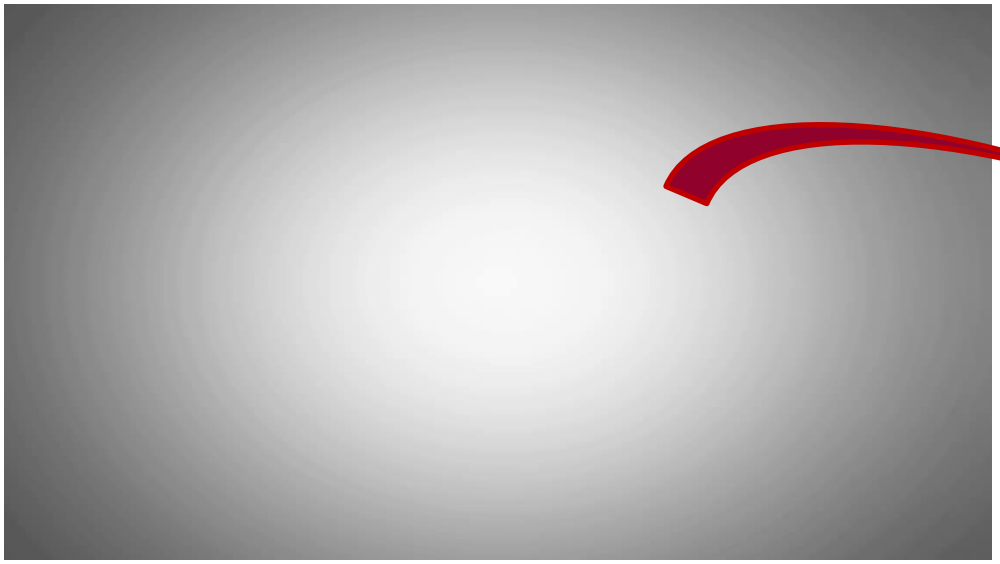
Strengths of PBPK modelling

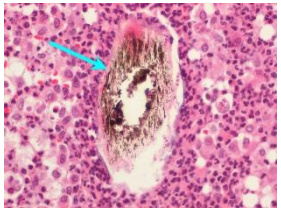
- Their mechanistic nature explains the very basis of observed data
  - Uniquely suited to hypothesis testing

- Tissue concentrations of drugs and chemical accurately predicted (tissue dosimetry).

- Tissue dosimetry described as ‘linchpin’ of chemical risk assessment

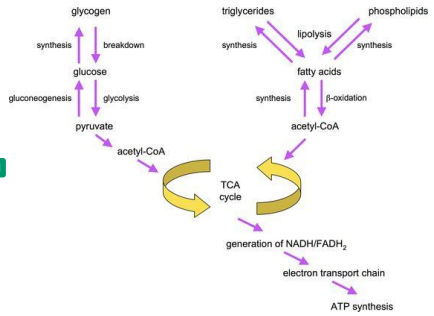
Pathways of Toxicity- Molecular Initiating Events



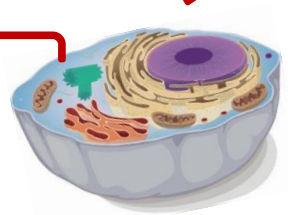


Toxicity/disease

Metabolite signatures



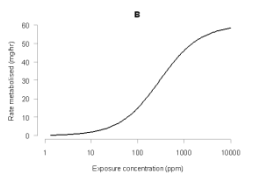
Perturbed Biochemical pathways



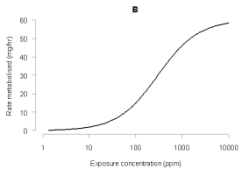
Tissue Dosimetry

Reverse Dosimetry – In vitro Data

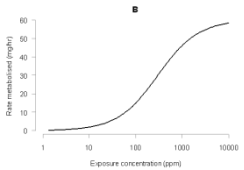
Exposure Reconstruction

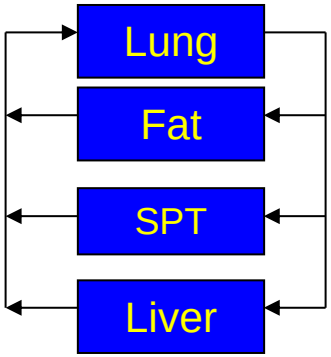


No Model



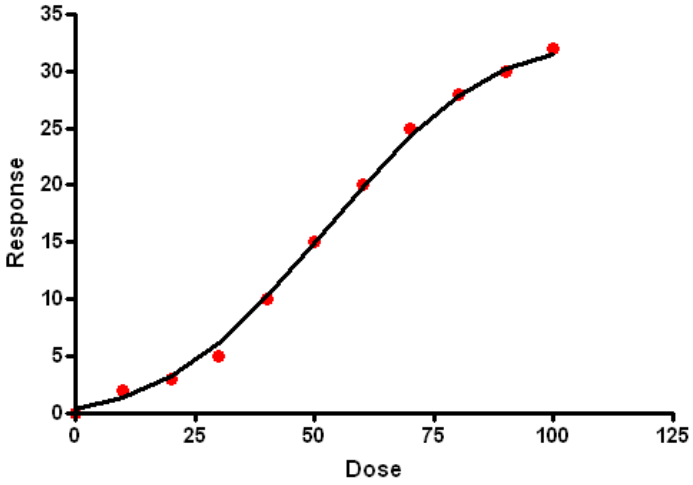
Empirical Model



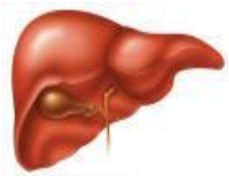


Physiologically-Based Pharmacokinetic Model

In vitro Concentration-Response



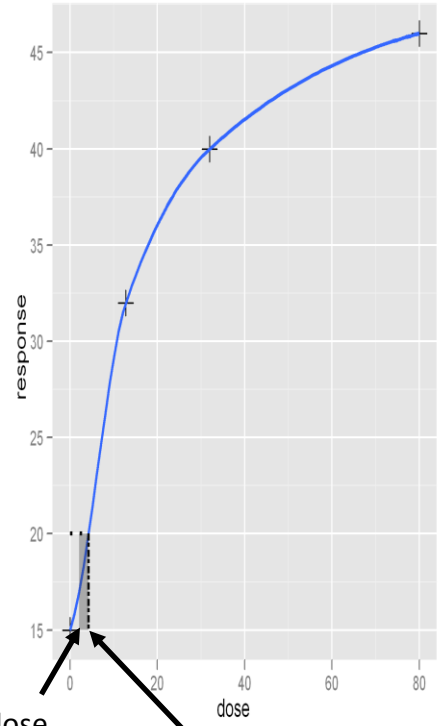
In vitro cell line concentration response data are surrogates for venous effluent concentration from *in vivo* organ or tissues

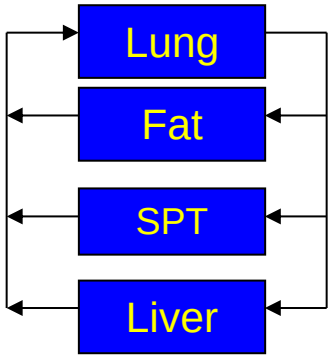
Arterial input →  → Venous effluent

Liver

Point of departure: in vitro to in vivo extrapolation

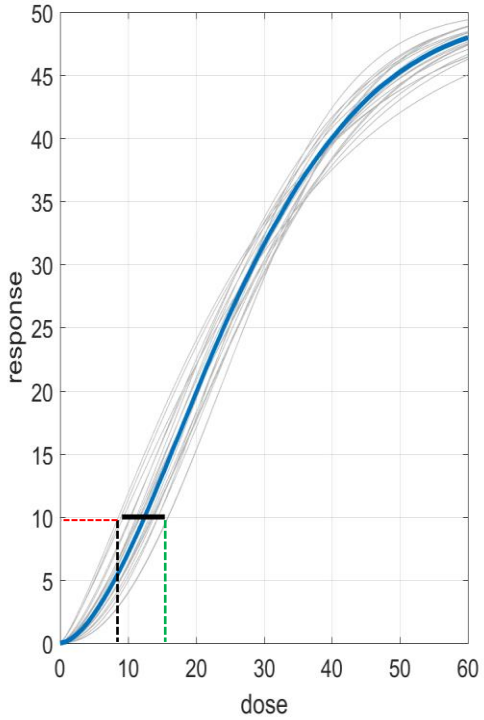
In Vitro Point of Departure





In vivo dose reconstruction using Physiologically Based Pharmacokinetic modelling

In Vivo Point of Departure



Black line denotes interval of doses from 2.5th to 97.5th at 10% response level

Chemical Safety Assessment?

How long will it be before a more accurate estimate of risk to protect public health is available, than an estimate based on current practice, which is based on a few animal test results in rodents and a bunch of poorly supported (scientifically that is) uncertainty factors?

Within 10-15 years?

Machine Learning

- **Machine learning** is a type of artificial intelligence (AI) that provides computers with the ability to learn without being explicitly programmed.
- **Machine learning** focuses on the development of computer programs that can teach themselves to grow and change when exposed to new data.

Genotype-Phenotype Correlations

Genotype: PKD1, c.9884A>G (p.Asn3295Ser), Autosomal Dominant, VUS-notable
Phenotypes:
Body MRI Finding: Polycystic Kidney Disease (PCKD)
DEXA: Osteopenia
Metabolomics: Renal function normal range, but 26th percentile; elevated thyroxine and low steroid hormones, may be causing osteopenia and could be related to PCKD
Interpretation: Definite changes in surveillance and management based on these data, evaluate for thyroid cysts related to PCKD

Genotype: TNNT2, c.862C>T (p.Arg288Cys), Autosomal Dominant, Likely Pathogenic, associated with Familial Hypertrophic Cardiomyopathy
Phenotypes:
4D Echocardiography: Left Ventricular Hypertropy (LVH) with mild valvular disease
Brain MRI: Meningioma
Interpretation: Definite changes in surveillance and management based on these data

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