



Centrum pro výzkum  
toxických látek  
v prostředí

# BIOMARKERS AND TOXICITY MECHANISMS

## 10 – BIOMARKERS

### Introduction

Luděk Bláha, PŘF MU, RECETOX  
[www.recetox.cz](http://www.recetox.cz)

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

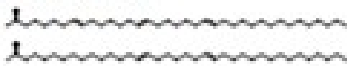


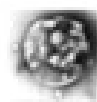








- markers in biological systems with a sufficiently long half-life which allow location *where* in the biological system change occur and *to quantify* the change.

### Various definitions and applications of „biomarkers“

- Ecology / Geology
- Human health and diseases
- **Toxicology** (special focus in this class)



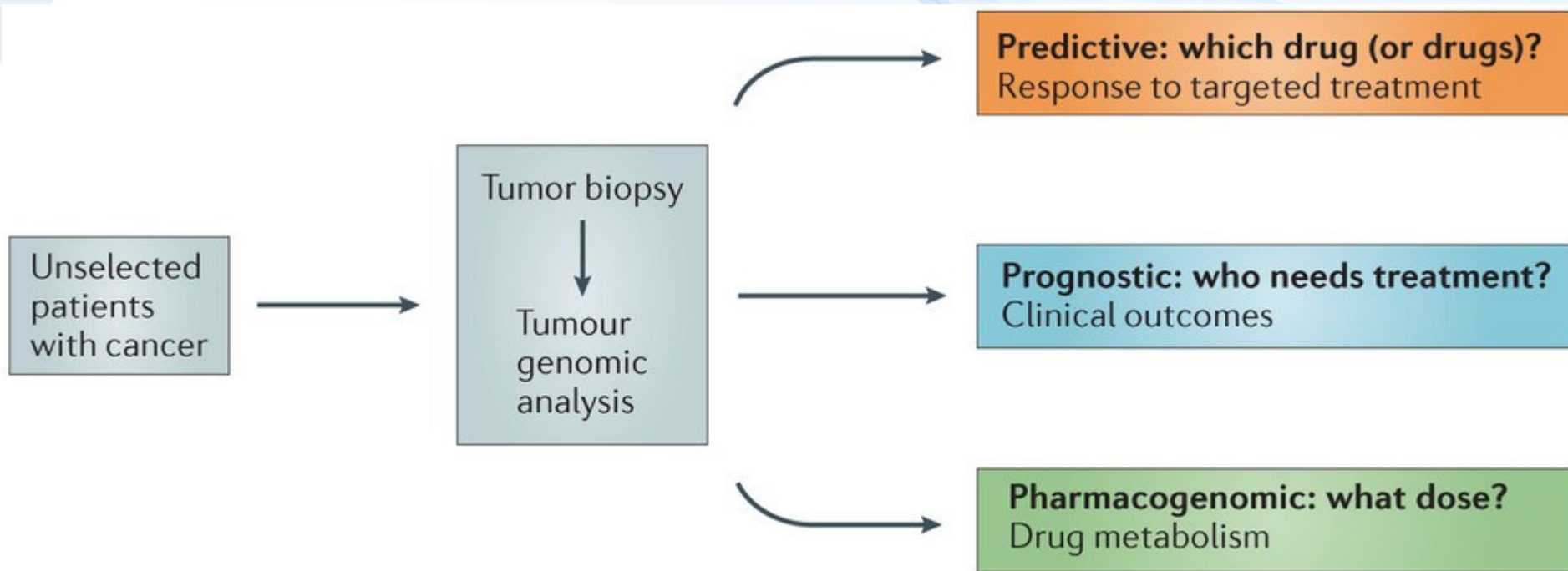
# Biomarkers in ECOLOGY / GEOLOGY

Molecular Biomarker	Known or postulated source	Application
<b>Alkenones</b> 	<b>Haptophyte Algae</b> 	$U^{K_{37}}$ → Sea surface and lake temperatures $\delta^{13}C$ → Paleo- $pCO_2$ $\delta D$ → Hydrography, salinity
<b>Isoprenoidal GDGTs</b> 	<b>Thaumarchaeota</b> 	$TEX_{86}$ → Sea surface and lake temperatures MI → Anaerobic oxidation of methane
<b>Long chain Diols</b> 	<b>Eustigmatophytes</b> 	DIX → Sea surface temperatures
<b>Branched GDGTs</b> 	<b>Anaerobic soil and peat bacteria</b> 	BIT → Relative inputs of terrestrial material MBT → Terrestrial Temperature (MAT) CBT → pH
<b>Plant Waxes</b> 	<b>Higher Land Plants</b> 	Land plant organic matter inputs. $\delta^{13}C$ → Changes in carbon cycle/ reservoirs $\delta D$ → P/E, hydrography, paleotopography
<b>Hopanes</b> 	<b>Soil bacteria</b> 	$\delta^{13}C$ → Changes in methanogen populations



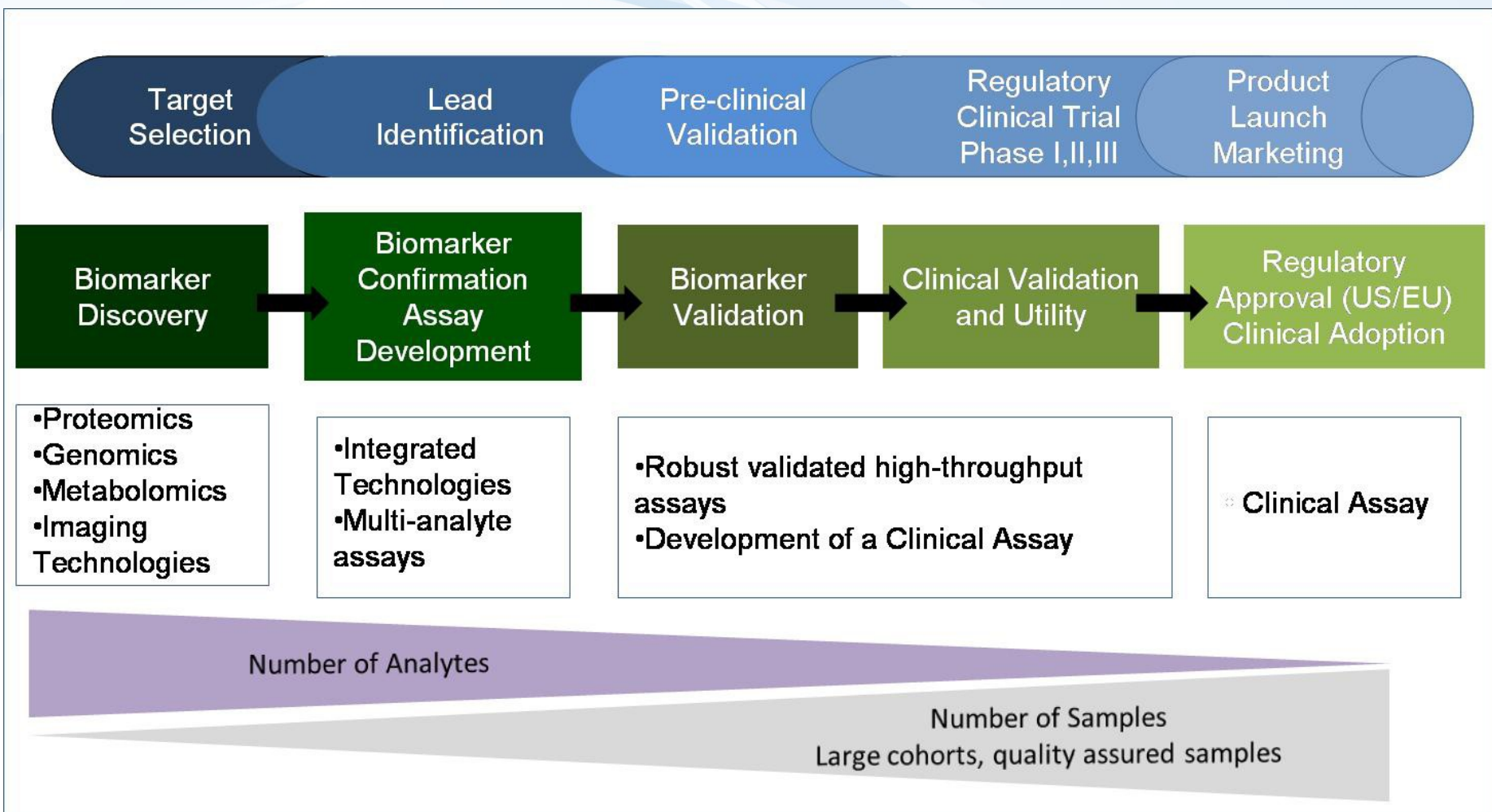
# Biomarkers in HUMAN HEALTH

Potential applications  
of biomarkers:



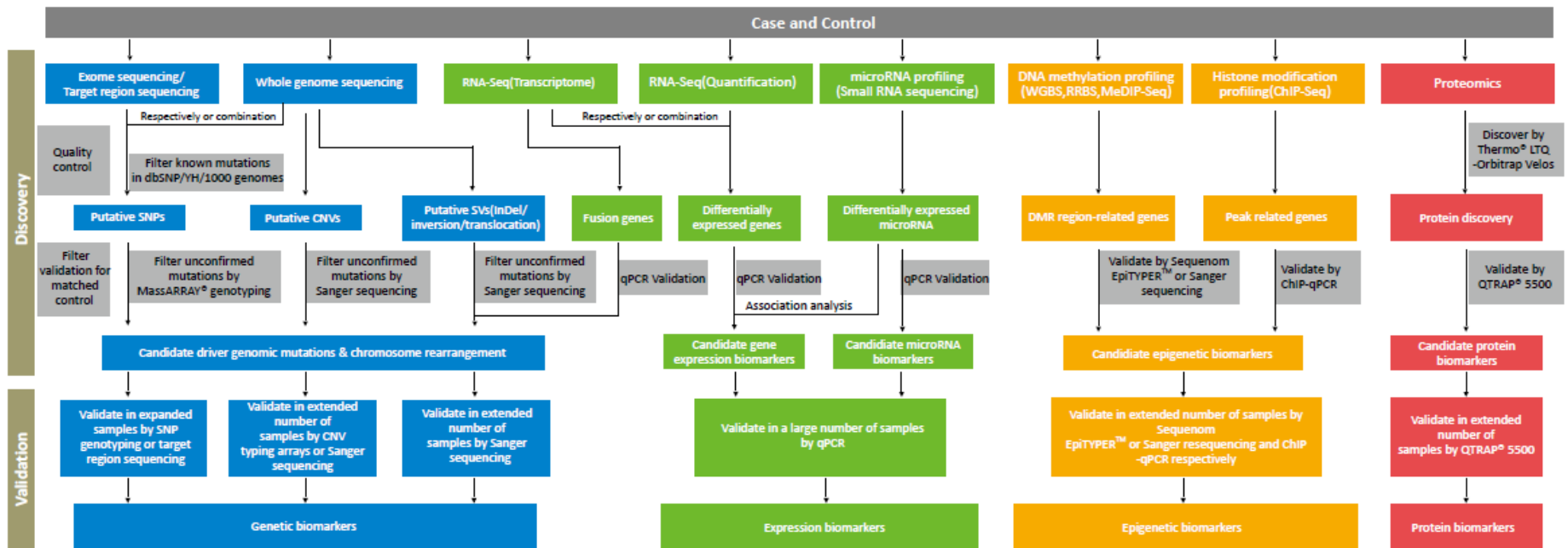
Nature Reviews | **Drug Discovery**

# Biomarkers in HUMAN HEALTH ... a lot of work



# Biomarkers in HUMAN HEALTH ... a lot of work

## Overview of Multi-omic Approaches Applied in Biomarker Discovery



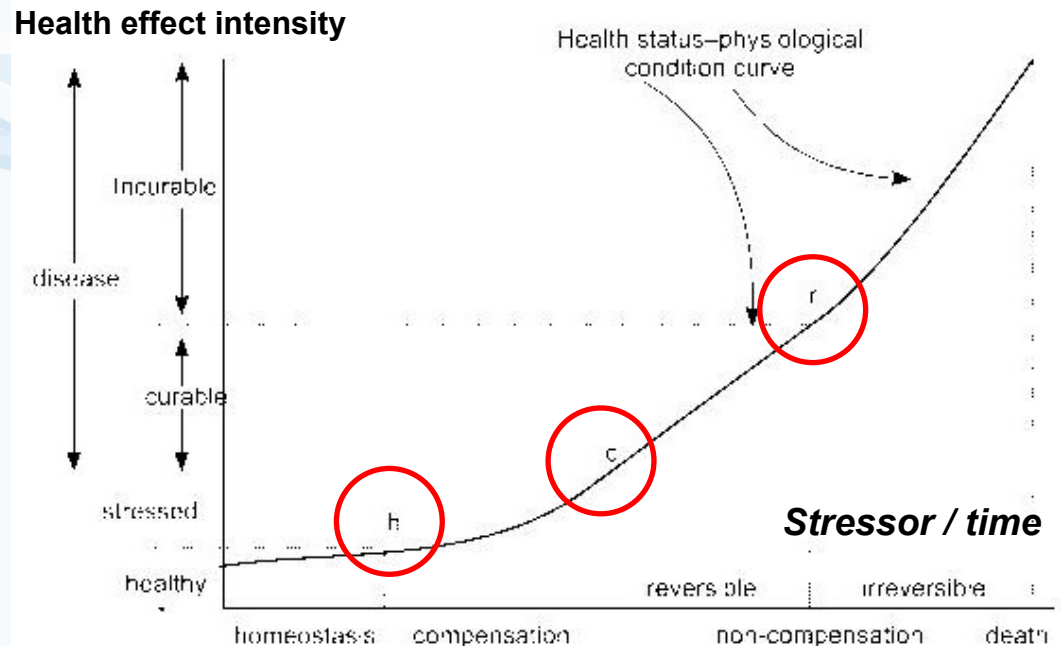
- **Identification of markers of long-term risks**
  - Human: health, toxicology and carcinogenesis
  - Ecotoxicology: early markers of toxic effects
- **BIOMARKER**
  - Change which occurs as response to "stressors" (xenobiotics, disease, temperature...) **extending the adaptive response beyond the normal range**
- **In vivo biomarkers:**
  - changes measured in stressed organisms ("classical biomarkers")
- **In vitro biomarkers**
  - in vitro testing characterizing potencies of xenobiotic to induce specific biological activity (or toxicity mechanism)
    - = biological potencies (markers of potential hazards)





# Biomarkers & Exposure

h: homeostatic conditions  
 c: reversible stage  
 r: irreversible effects of pollutants

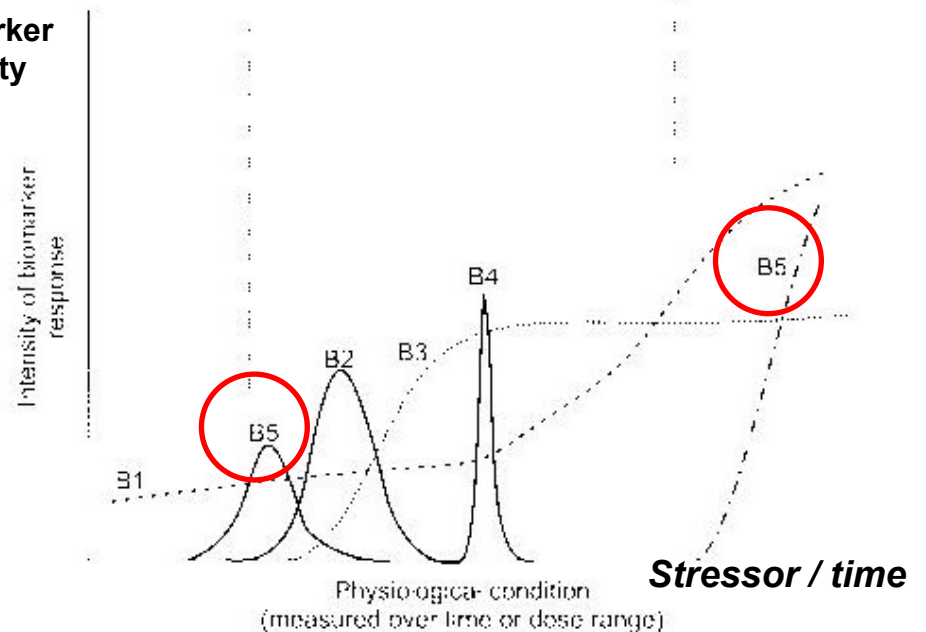


## Various biomarker profiles

- temporal changes—B2; B4
- repeated occurrence (**B5**)
- continuous increase (B1)
- increase with maximum (B3)

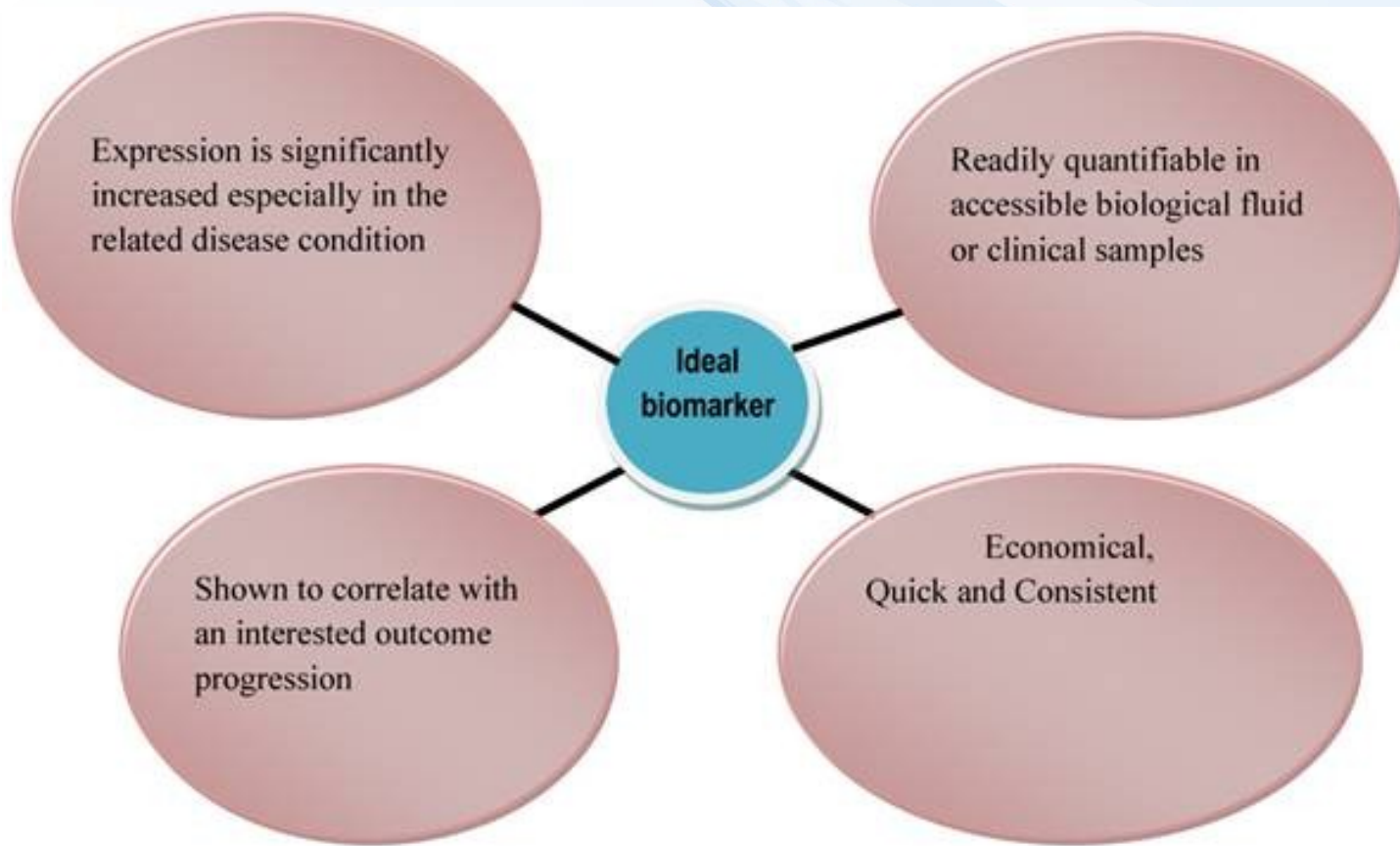
**: B1 + B3 are candidate biomarkers !**

## Biomarker intensity

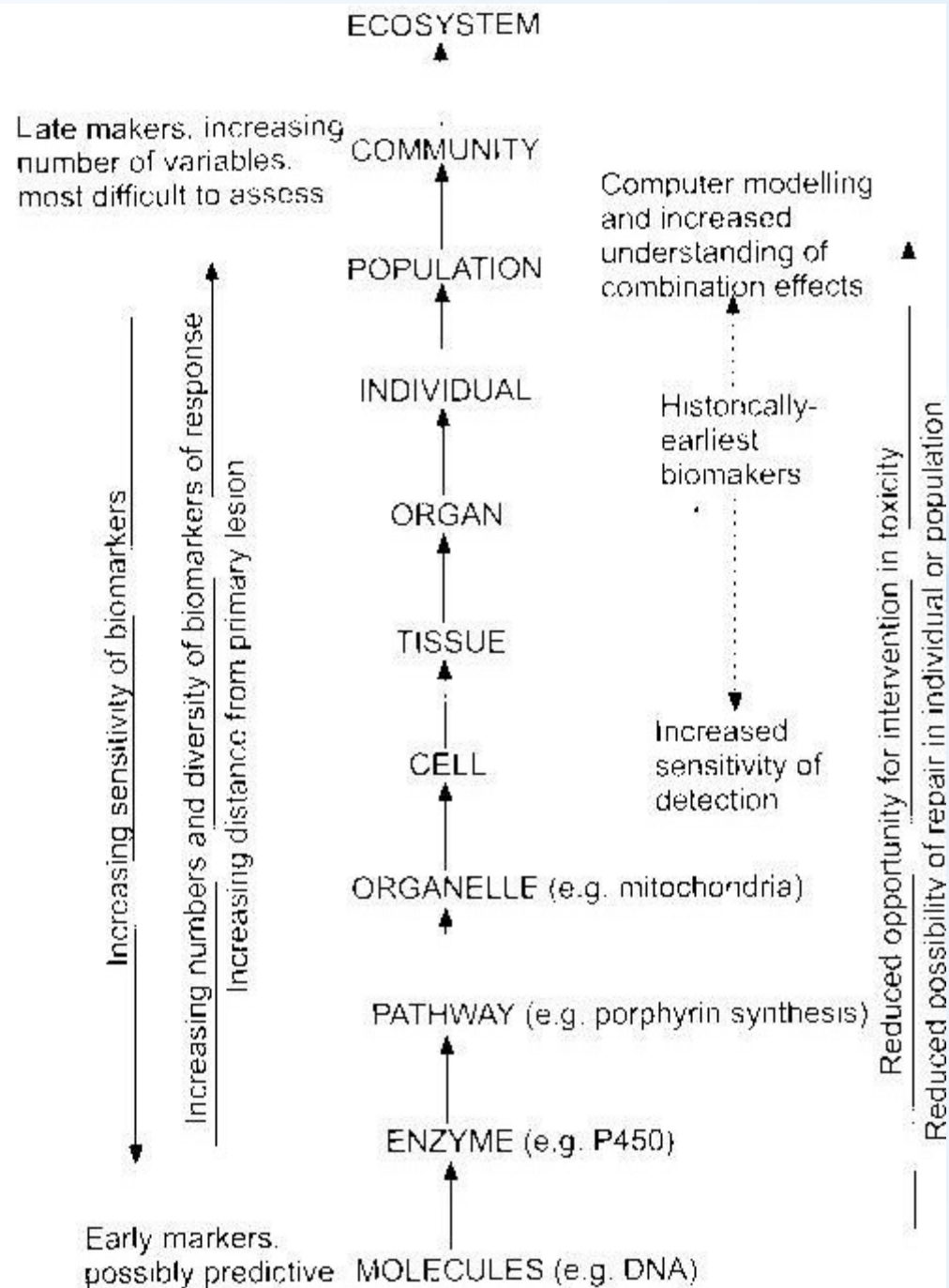




# Ideal biomarker



# Biomarkers at different levels of biological organisation



# Biomarkers - classification

## Categorization by US National Academy of Sciences

- Biomarkers of exposure
- Biomarkers of response or effect
- Biomarkers of susceptibility

## Continuum exists among biomarkers

example: adducts of toxicant to DNA

? *biomarker of exposure* / ? *response*

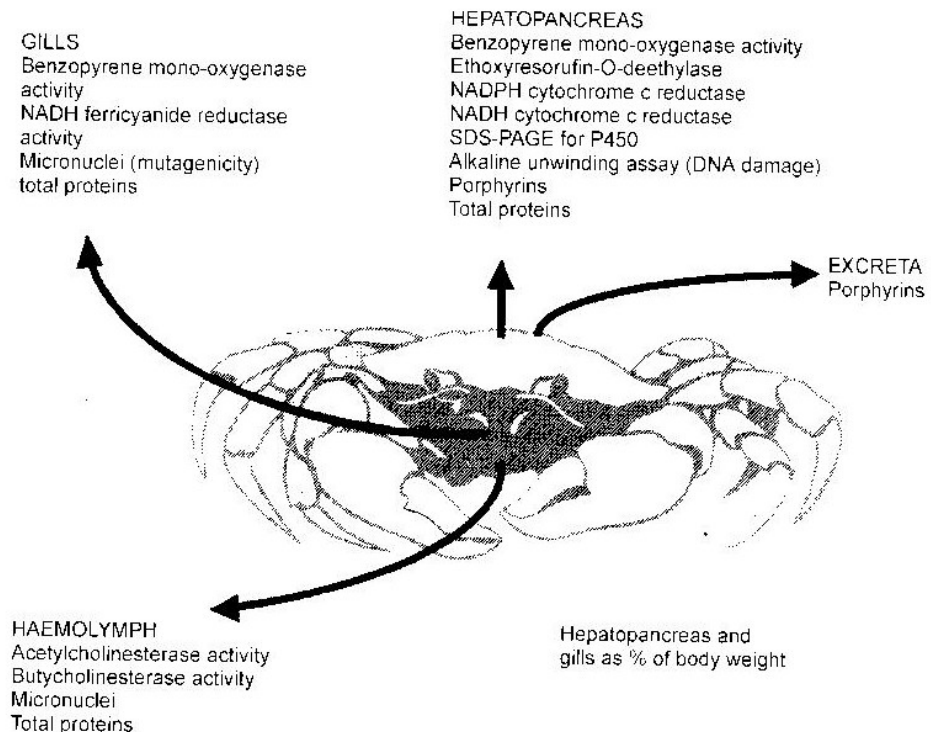


# Various biomarker types

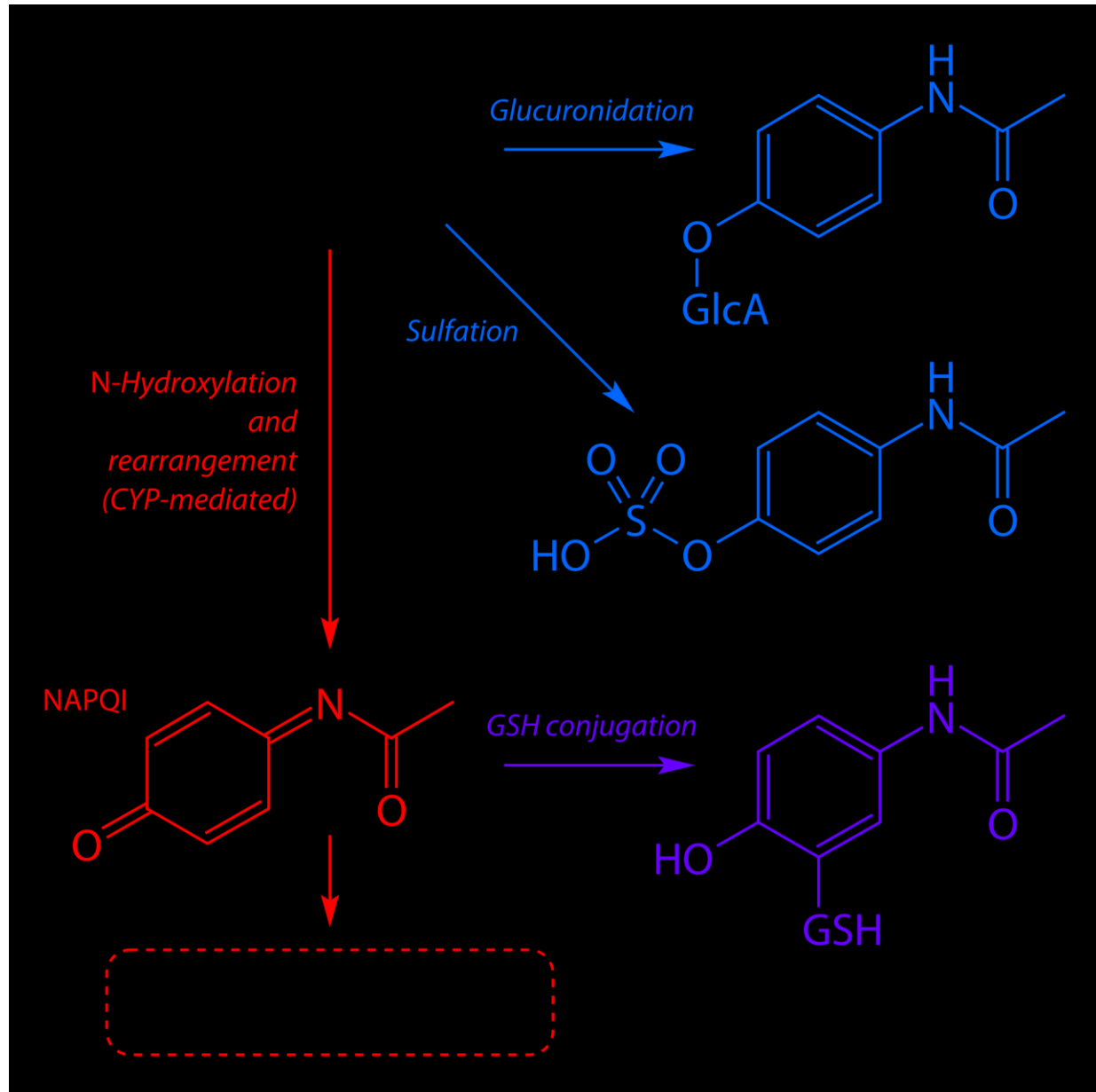
- **Specific (selective) in vivo biomarkers**
  - Biomarkers selectively reflecting specific types (mechanisms) of toxicity
    - E.g. inhibition of AcCholE :  
exposure = organophosphates; effect = neurotoxicity
  - + provides specific information
  - multiple biomarkers must be measured in parallel
  
- **Non-specific (non-selective) in vivo biomarkers**
  - Biomarkers of general stress
    - E.g. induction of Heat Shock Proteins (hsp)
  - + general information about stress
  - sensitive to many "stressors" (temperature, salinity ...)

# Sampling biological materials for biomarker analyses

- **Non-destructive (non-invasive)**
  - blood / haemolymph collection & analyses
  - skin, feather, hair ...
  - (life of the organism not affected)
- **Destructive (invasive)**
  - whole animal
  - 3R principles: maximum use of the material
  - multiple biomarker evaluation



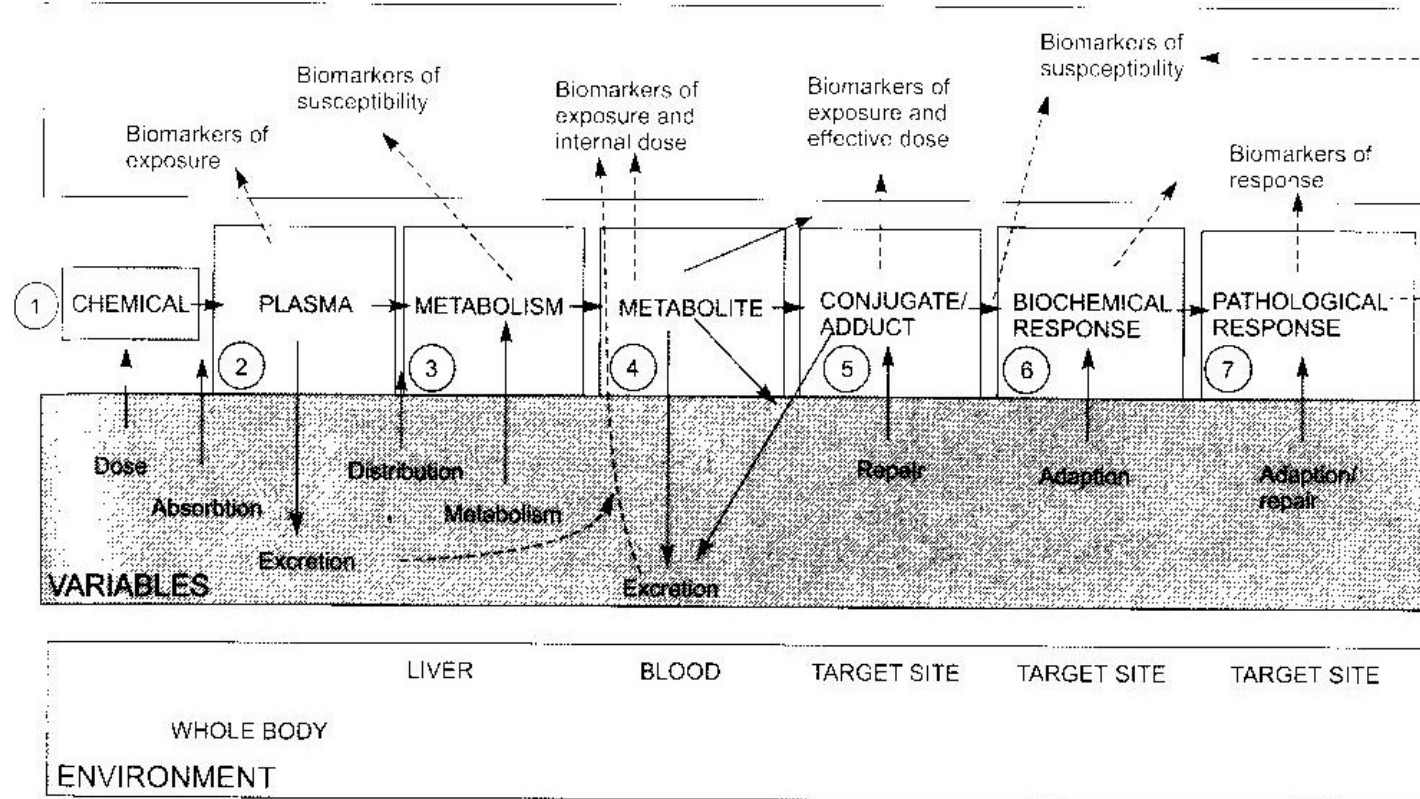
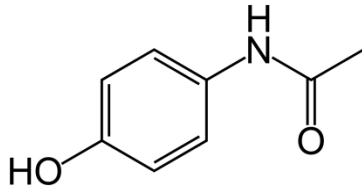
EXAMPLE  
- Paracetamol





## BIOMARKERS

EXAMPLE  
- Paracetamol



- (1) paracetamol
- (2) parent compound measurement - **biomarker of exposure**
- (3) activation to reactive metabolite (N-ac-p-benzoquinone, NAPQI) by CYP  
→ reaction with GSH / measurement – levels of CYPs; **levels of GSH – susceptibility**
- (4) GSH-NAPQI conjugate – **exposure, susceptibility**
- (5) NAPQI-protein adducts → toxicity: **exposure, effective dose**
- (6) adaptations: GSH depletion, inhibition of protein synthesis – **biomarkers of response**
- (7) protein alkylation → degeneration of hepatocytes: necrosis  
→ increase concentrations of bilirubin in plasma + inflammation - **response / effect**



# Toxicity biomarkers – examples

**Table 1** Examples of different biomarkers illustrated with specific examples and examples of the stressor which may result in the biomarker changes

Type of biomarker	Biomarker	Specific example	Stressor
Exposure	DNA adducts	Styrene oxide- <i>O</i> <sup>6</sup> guanine	Styrene exposure
	Protein adduct	N <sup>7</sup> -Guanyl-aflatoxin B <sub>1</sub>	Dietary aflatoxin
	DNA fragments	7,8-Dihydro-8-oxoguanine	Reactive oxygen species
Exposure and effect (response)	Protein adducts	Carboxyhaemoglobin	CO inhalation
	Enzyme inhibition	Acetylcholinesterase inhibition	Organophosphates
	Urinary metabolites	Mercapturic acids	Buta-1,3 diene, allyl chloride
Effect (response)	Serum/plasma enzymes	AST (aspartate aminotransferase)	Xenobiotics causing necrosis
		LDH (lactate dehydrogenase)	Xenobiotics causing necrosis
		ALT (alanine aminotransferase)	Hepatotoxic compounds
		ALP (alkaline phosphatase)	Bile duct toxins
		CK or CPK (creatine kinase)	Heart/muscle toxins
		Urea (changes)	Hepatotoxic and nephrotoxic compounds
		Protein (reduced, e.g. albumin)	Hepatotoxic compounds
		Bilirubin	Liver injury
		Prothrombin	Warfarin (rodenticide)
		Glucose, raised creatinine, GSH conjugates	Pancreatic abnormalities, kidney damage
	Serum/plasma biochemistry	Liver glutathione	Reactive oxygen species
		P450 induction	Polycyclic aromatic hydrocarbons
		hsp 60, hsp 70, hsp90	Cadmium, heat
		Metallothionein	Heavy metals, e.g. cadmium
		Antibodies, e.g. IgG	Antigens
		Dermatitis	Nickel
		Chromosomal aberrations, micronuclei	Genotoxic agents
		Heart rate, temperature, sleeping time	Barbiturates
		Breeding patterns, migrations	Climate change
Susceptibility	Phenotype	Acetylator phenotype ( <i>NAT 2</i> )	-
	Oncogenes	Dominant oncogenes ( <i>ras</i> , <i>mic</i> )	-
		Recessive suppressor gene ( <i>p52</i> )	-
	'Cancer' genes	Breast-ovary cancer gene ( <i>BRCA 1</i> )	-



# Other examples

# Toxicity biomarkers

**Table 9.2** Availability of biomarkers in blood

Biomarker	Blood	Tissue of choice	Comment
AChE inhibition	+?	Brain	Effects in blood more transient
Neurotoxic esterases	-	Brain	Enzyme is limited to brain
Biogenic amines	-	Brain	Changes in blood too transient
DNA			
Strand breakage	?	Wide range	Nucleated avian red blood cells are possible
Adduct formation	+	Wide range	Haemoglobin is good substitute for DNA
SCE	+	Wide range	Blood lymphocytes can be used
Degree of methylation	?	Wide range	Nucleated avian red blood cells are possible
MFO	-	Liver	Western blotting technique on leucocytes is possible
Thyroid	+	Thyroid	Circulating levels of T <sub>3</sub> and T <sub>4</sub> are sensitive
Retinols	+	Liver	Advances to use plasma are being made
Porphyryns	+?	Liver	Advances to use plasma are likely
ALAD	+	Blood	Tissue of choice
Enzymes	+	Blood	Tissue of choice
Immunotoxic	-	Lymphatic cells, bone marrow	Limited number of tests available for blood

