Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

10 – BIOMARKERS

Introduction

Luděk Bláha, PřF MU, RECETOX
www.recetox.cz

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

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

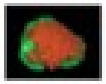
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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

Various definitions and applications of „biomarkers“

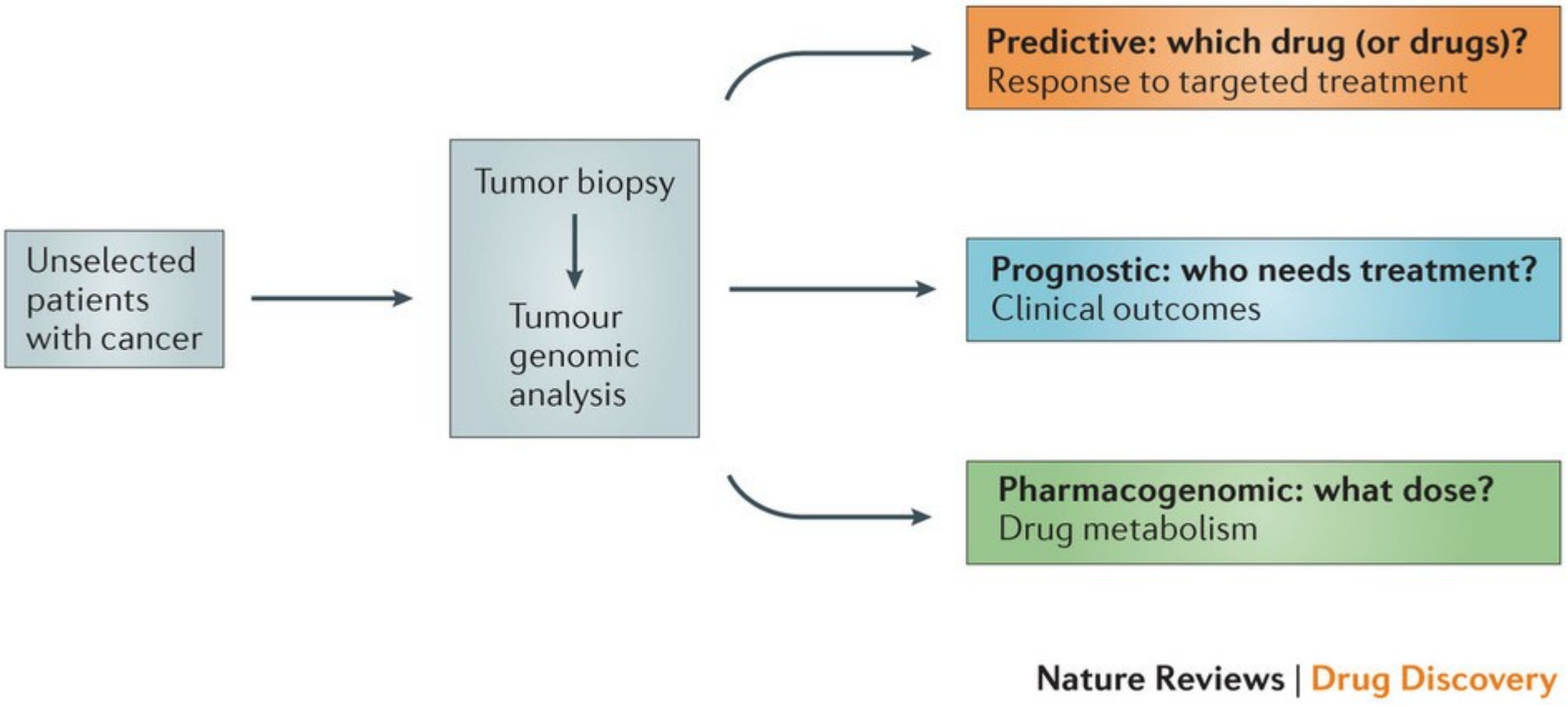
- Ecology / Geology
- Human health and diseases
- **Toxicology** (special focus in this class)

Biomarkers in ECOLOGY / GEOLOGY

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

Biomarkers in HUMAN HEALTH

Potential applications of biomarkers:



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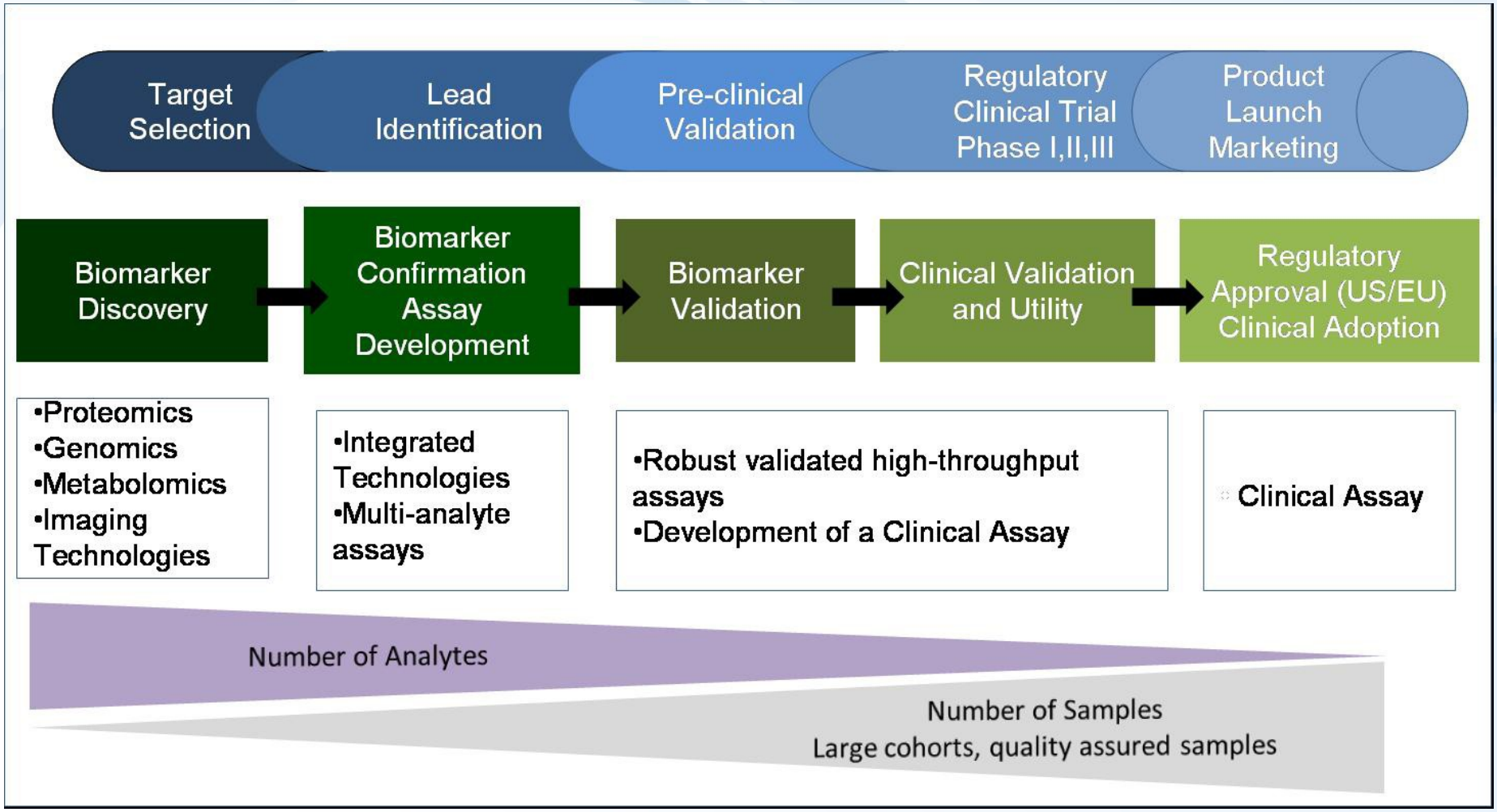
Biomarkers in HUMAN HEALTH … a lot of work



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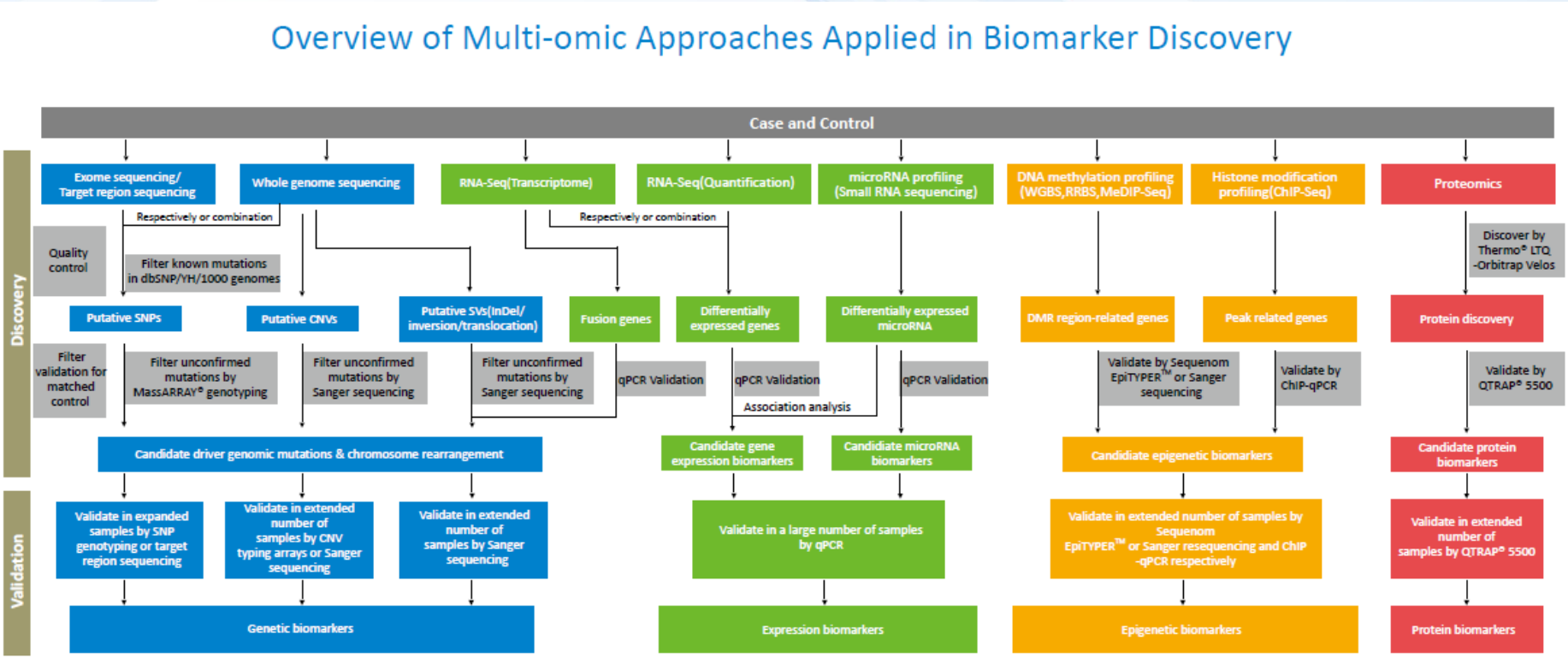

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Biomarkers in TOXICOLOGY

- **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

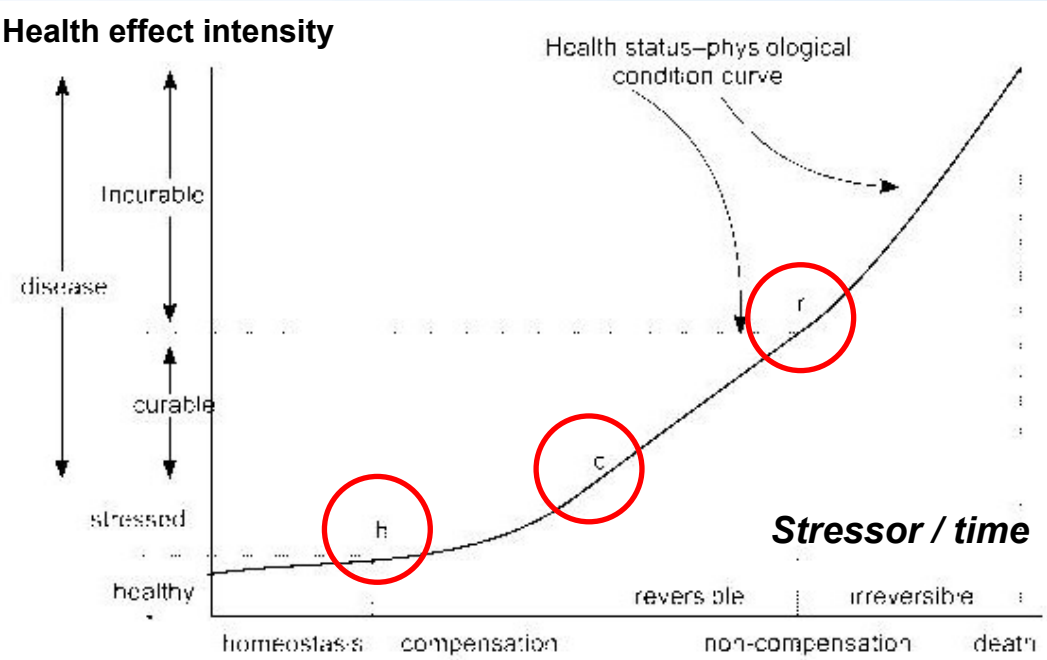
Various biomarker profiles

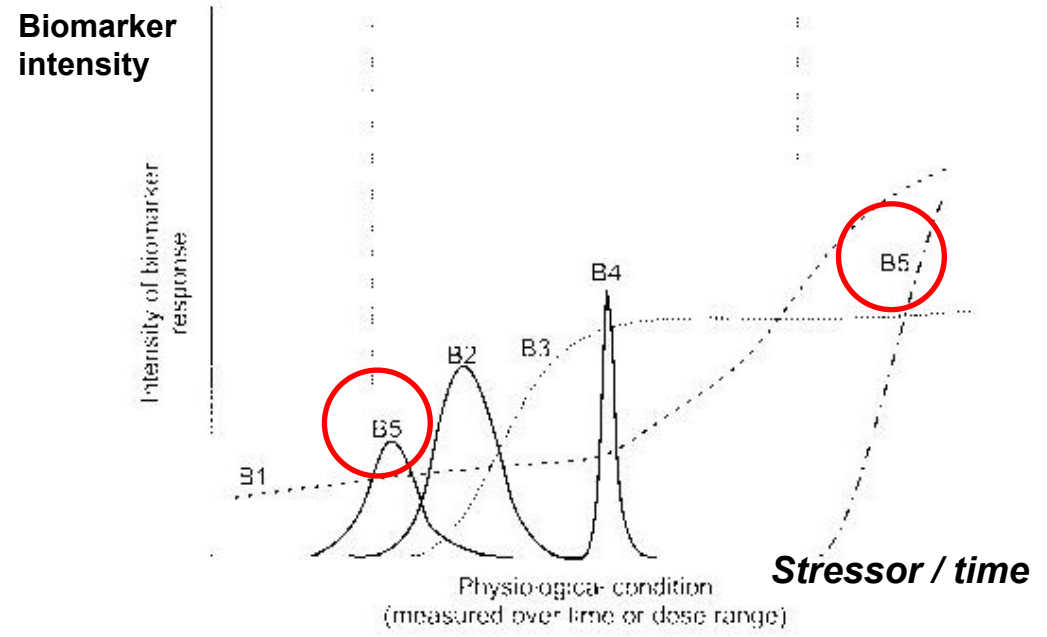
- temporal changes–B2; B4

- repeated occurrence (**B5**)

- continuous increase (B1)
- increase with maximum (B3)

: B1 + B3 are candidate biomarkers !





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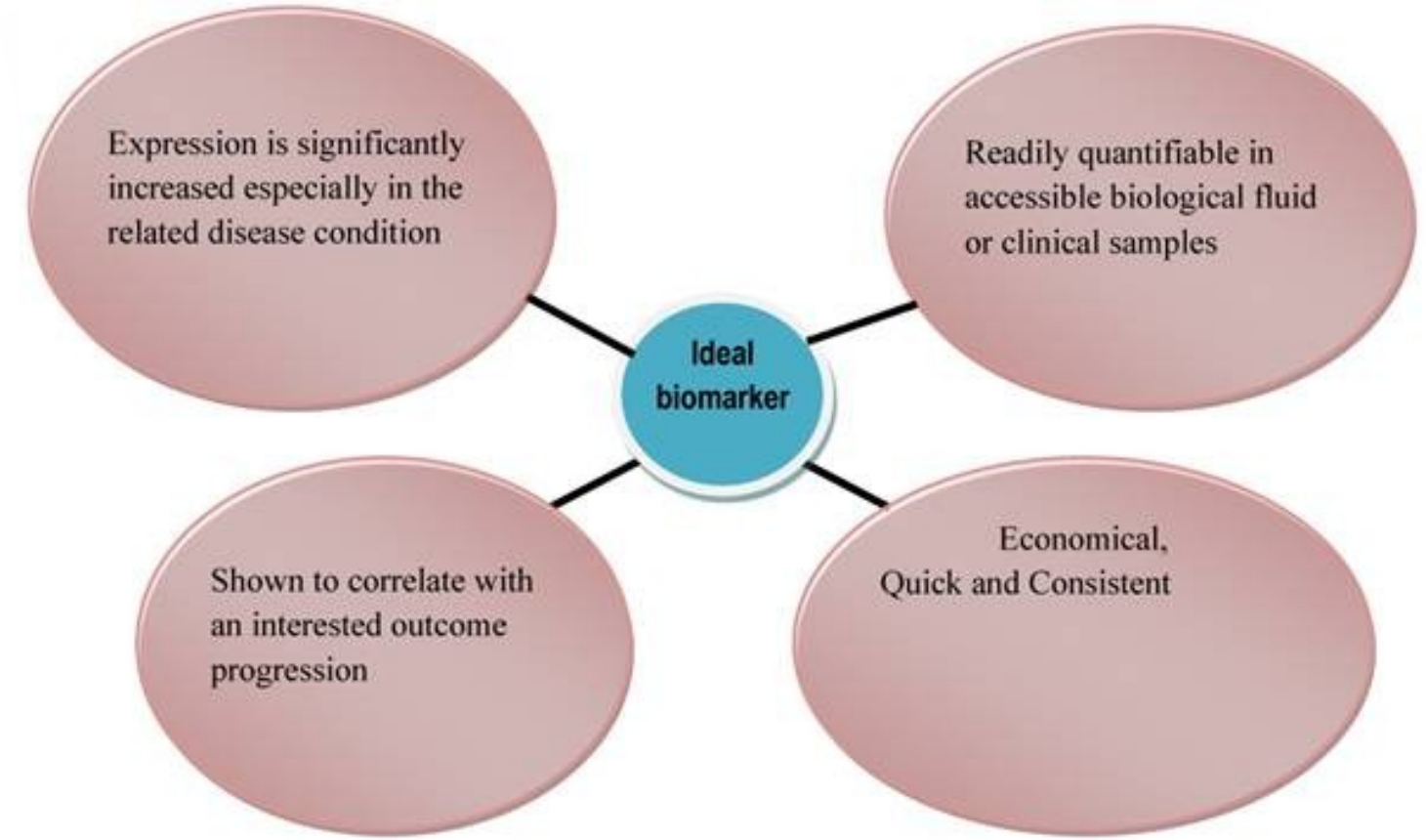
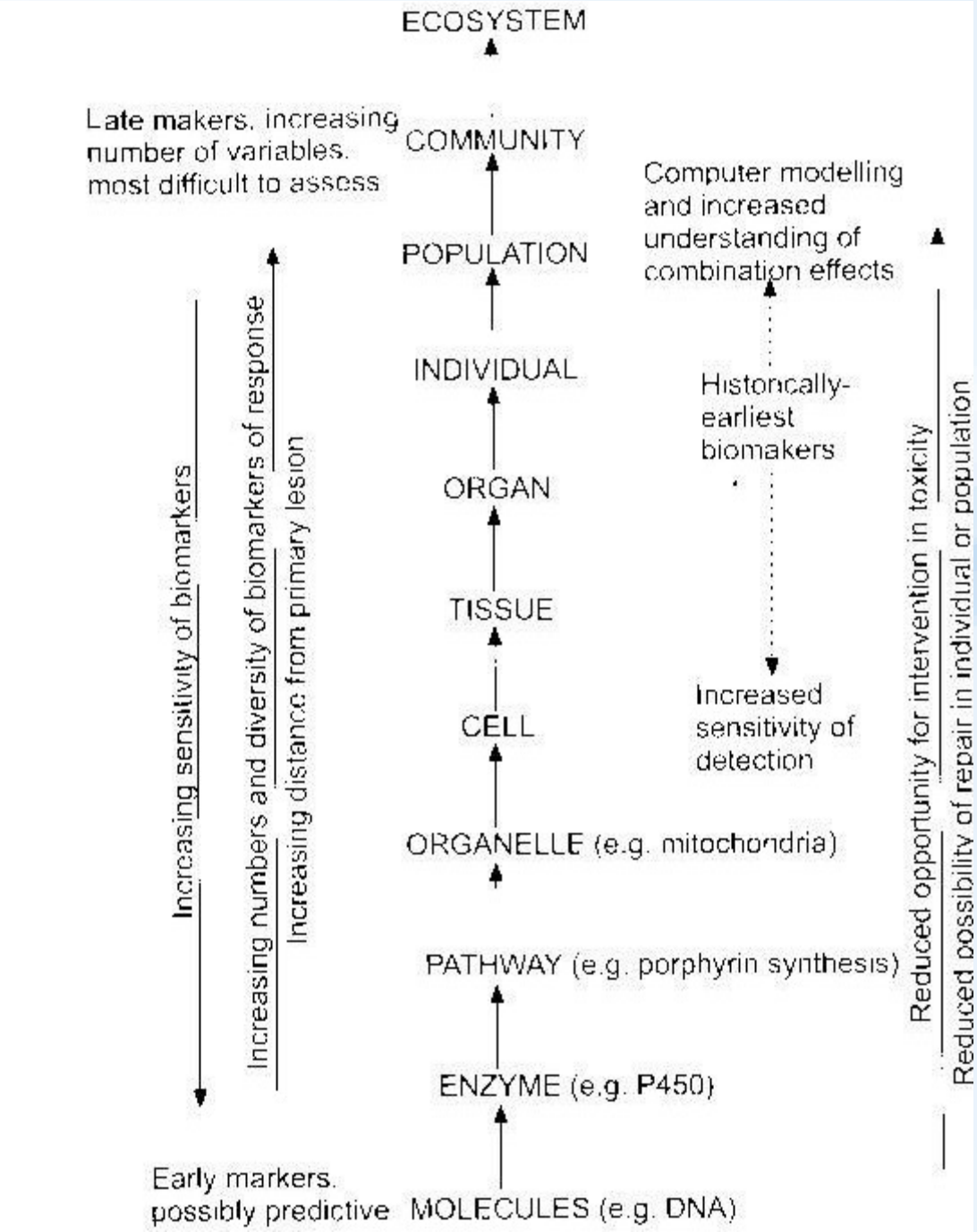
Ideal biomarker



Biomarkers at different levels of biological organisation



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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)

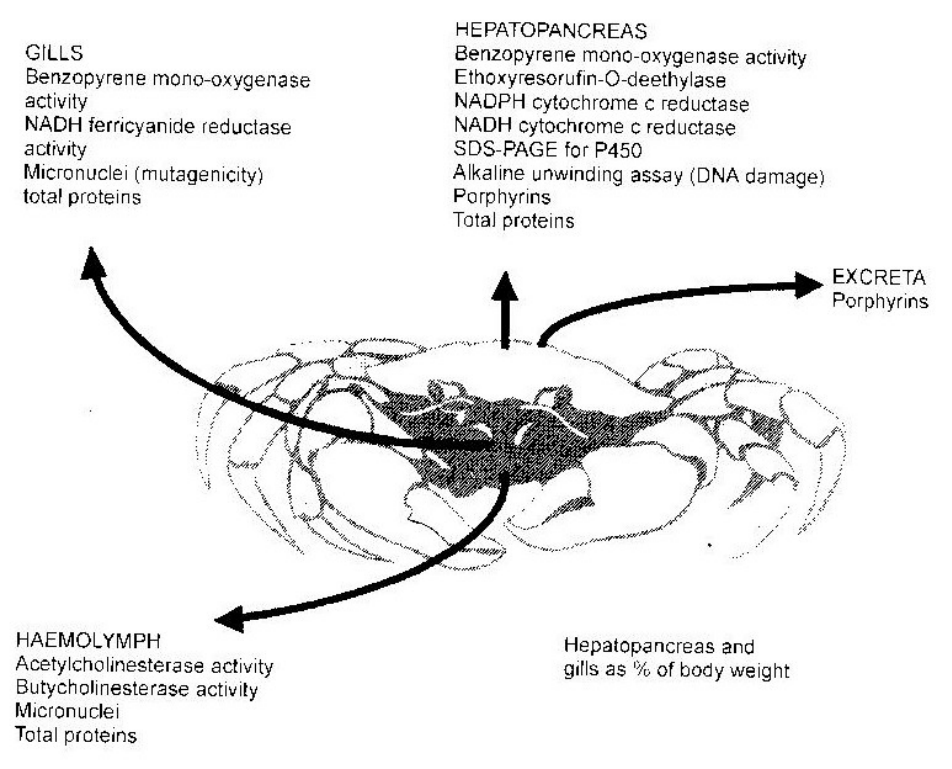
  + general information about stress
  - sensitive to many "stressors" (temperature, salinity ...)

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



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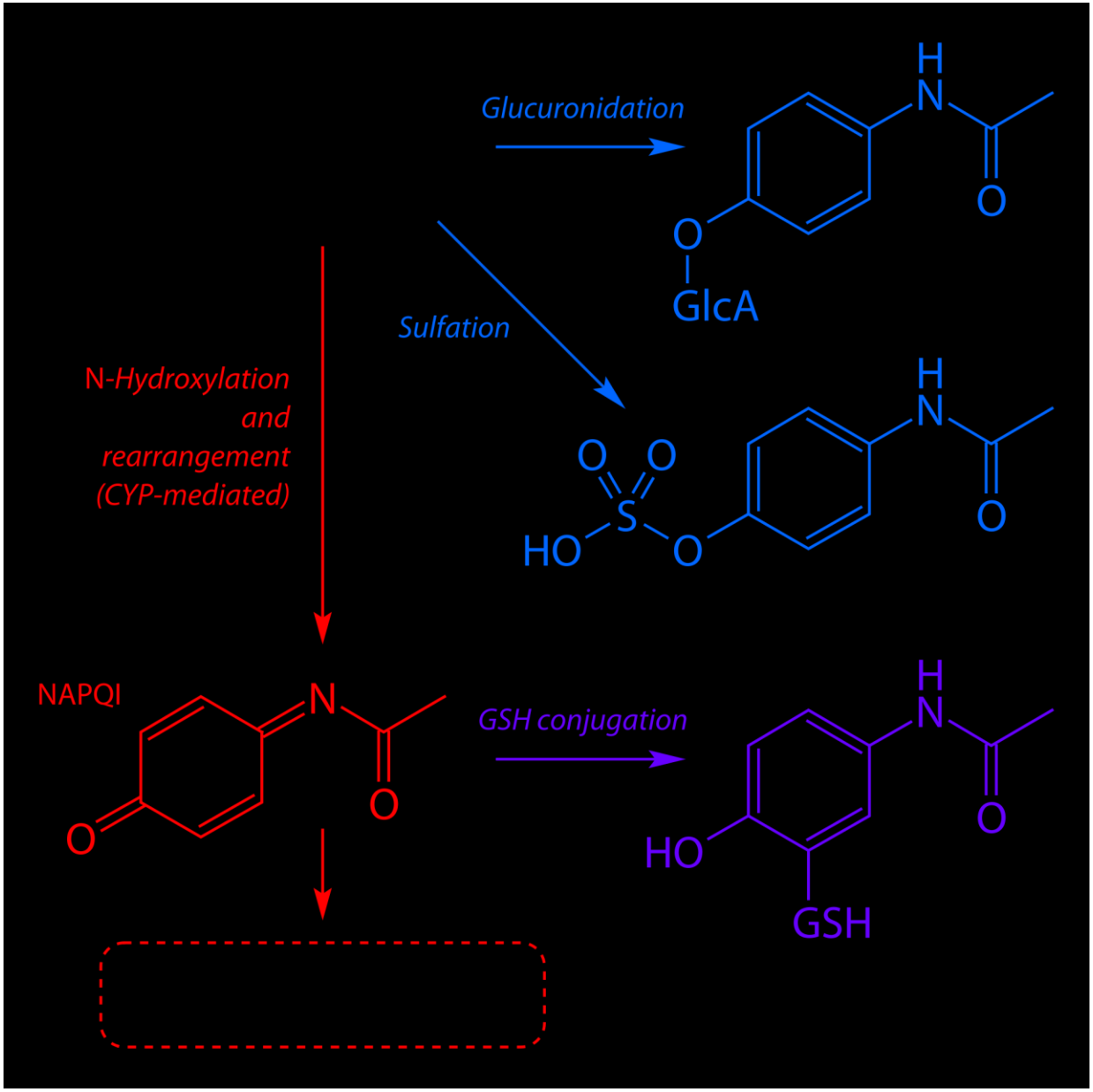
EXAMPLE
- Paracetamol



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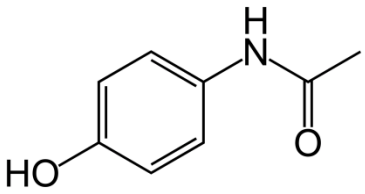

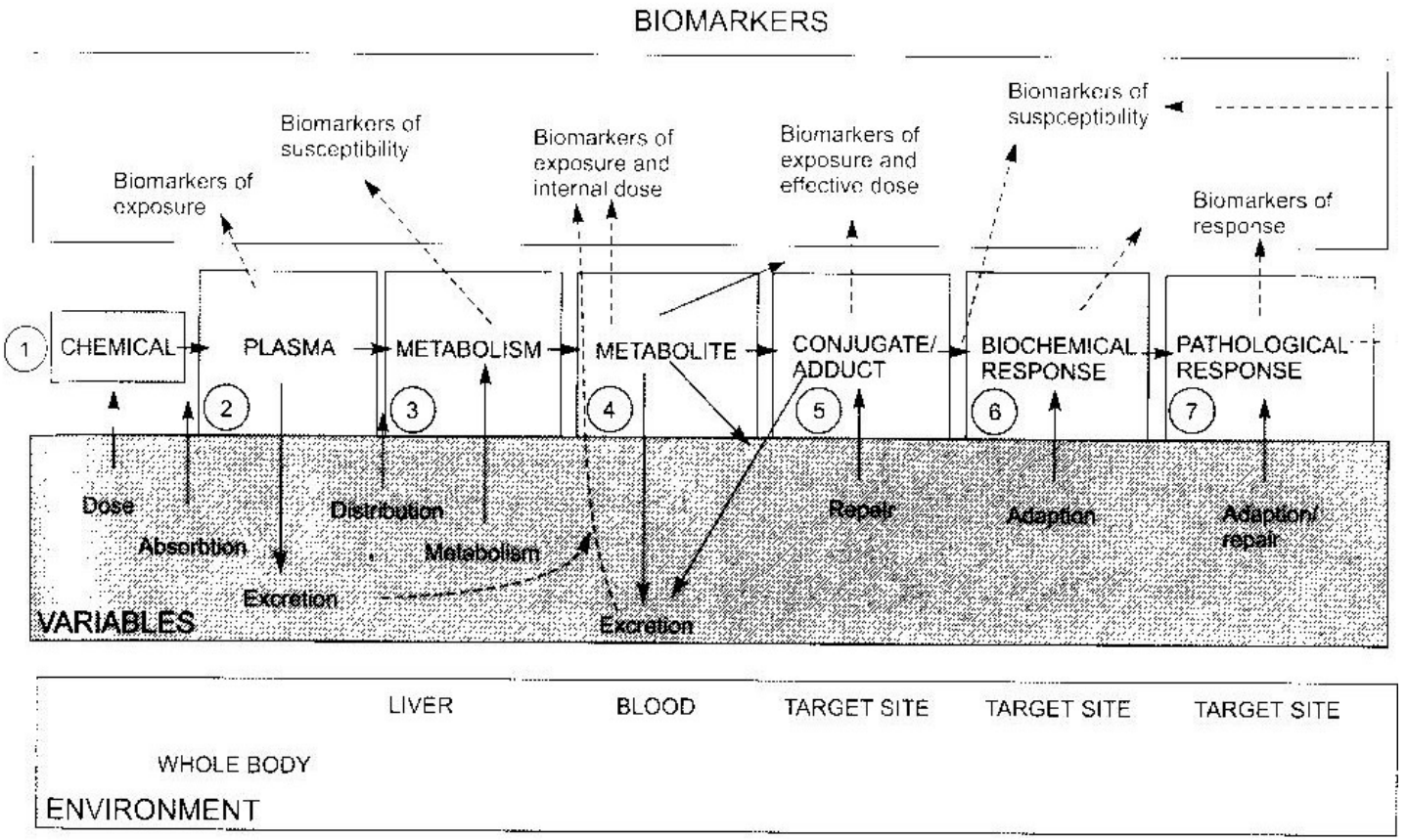

(1) paracetamol

(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-O^6 guanine	Styrene exposure
	Protein adduct	N^7-Guanyl-aflatoxin B_1	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
	Serum/plasma biochemistry	Urea (changes)	Hepatotoxic and nephrotoxic compounds
		Protein (reduced, e.g. albumin)	Hepatotoxic compounds
		Bilirubin	Liver injury
	Clotting time	Prothrombin	Warfarin (rodenticide)
	Urinary metabolites	Glucose, raised creatinine, GSH conjugates	Pancreatic abnormalities, kidney damage
	Raised antioxidant levels	Liver glutathione	Reactive oxygen species
	Enzyme induction	P450 induction	Polycyclic aromatic hydrocarbons
	Stress proteins	hsp 60, hsp 70, hsp90	Cadmium, heat
	Protective proteins	Metallothionein	Heavy metals, e.g. cadmium
		Antibodies, e.g. IgG	Antigens
	Allergic response	Dermatitis	Nickel
	Histology	Chromosomal aberrations, micronuclei	Genotoxic agents
	Clinical observations	Heart rate, temperature, sleeping time	Barbiturates
	Population studies	Breeding patterns, migrations	Climate change
Susceptibility	Phenotype	Acetylator phenotype (*NAT 2*)	–
	Oncogenes	Dominant oncogenes (*ras, mic*)	–
		Recessive suppressor gene (*p52*)	–
	'Cancer' genes	Breast–ovary cancer gene (*BRCA 1*)	–

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_3 and T_4 are sensitive
Retinols	+	Liver	Advances to use plasma are being made
Porphyrins	+?	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