Centrum pro výzkum toxických látek v prostředí

# BIOMARKERS AND TOXICITY MECHANISMS

# 10 – BIOMARKERS

# Introduction

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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

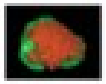
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

- 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“**

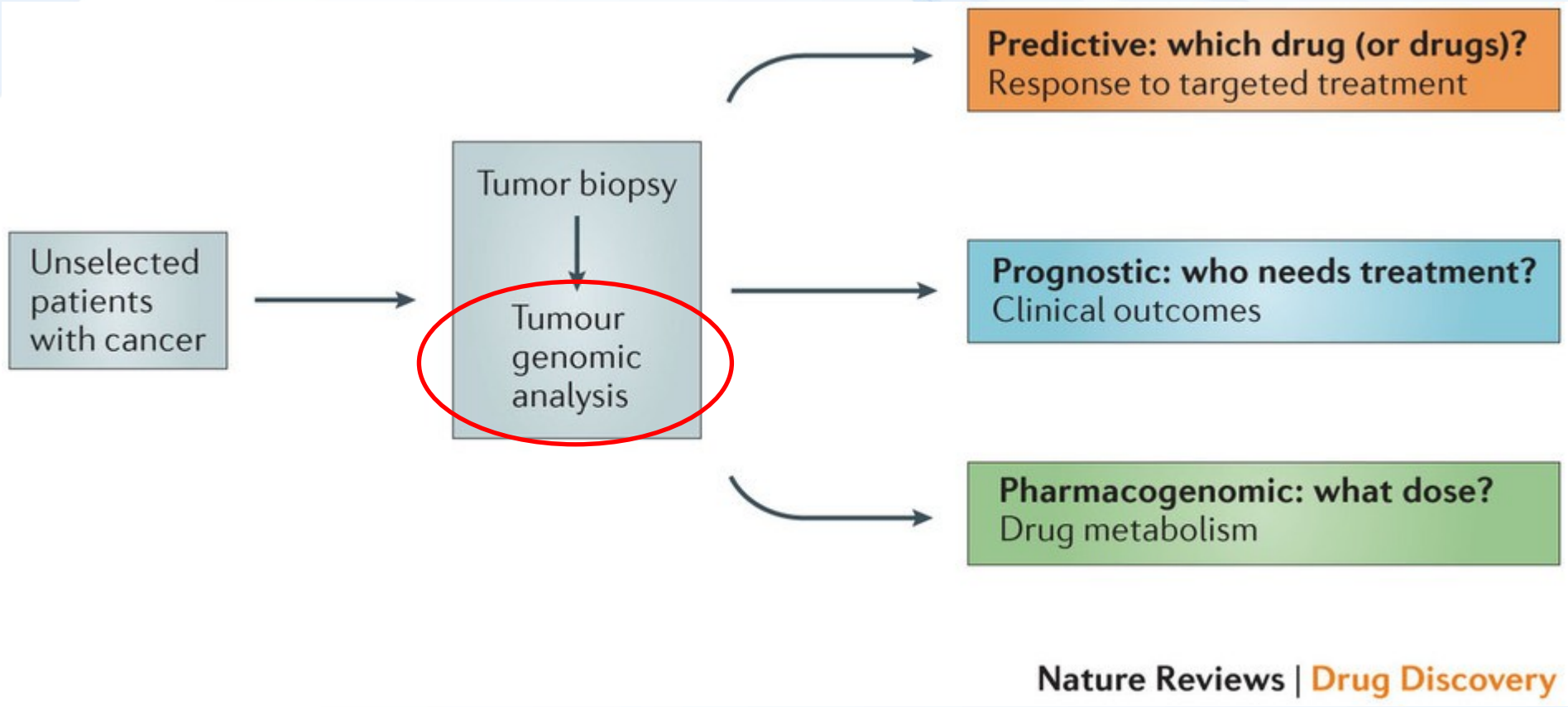
- Ecology / Geology
- Human health and diseases
- **Toxicology** (special focus in this class)

# Biomarkers in ECOLOGY / GEOLOGY

| Molecular Biomarker | Known or postulated source | Application |
|---|---|---|
| Alkenones  | Haptophyte Algae  | $U^{K}_{37}'$ → Sea surface and lake temperatures<br>$\delta^{13}C$ → Paleo-$pCO_2$<br>$\delta D$ → Hydrography, salinity |
| Isoprenoidal GDGTs  | Thaumarchaeota  | $TEX_{86}$ → Sea surface and lake temperatures<br>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<br>MBT → Terrestrial Temperature (MAT)<br>CBT → pH |
| Plant Waxes  | Higher Land Plants  | Land plant organic matter inputs.<br>$\delta^{13}C$ → Changes in carbon cycle/ reservoirs<br>$\delta D$ → P/E, hydrography, paleotopography |
| Hopanes  | Soil bacteria  | $\delta^{13}C$ → Changes in methanogen populations |

# Biomarkers in HUMAN HEALTH

**Examples of biomarker applications in human health:**



# Biomarkers in TOXICOLOGY

- **Identification of markers that inform/predict about long-term risks**
  - **Human**: chronic health –e.g. early stages of liver steatosis, carcinogenesis
  - **Ecotoxicology:** early markers of ecotoxic effects

- BIOMARKER
  - change which occurs as a response to "stressors“ (xenobiotics, disease, temperature...) **extending the adaptive response beyond the normal range**

- **In vivo biomarkers:**
  - changes measured in stressed organisms, i.e. in vivo ("classical biomarkers“ in toxicological research)

- ***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 - 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 a toxicant bound to nucleotide*

*? biomarker of exposure (proof of toxicant)*

*? biomarker of response or effect (modified nucleotide = effect)*

# 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

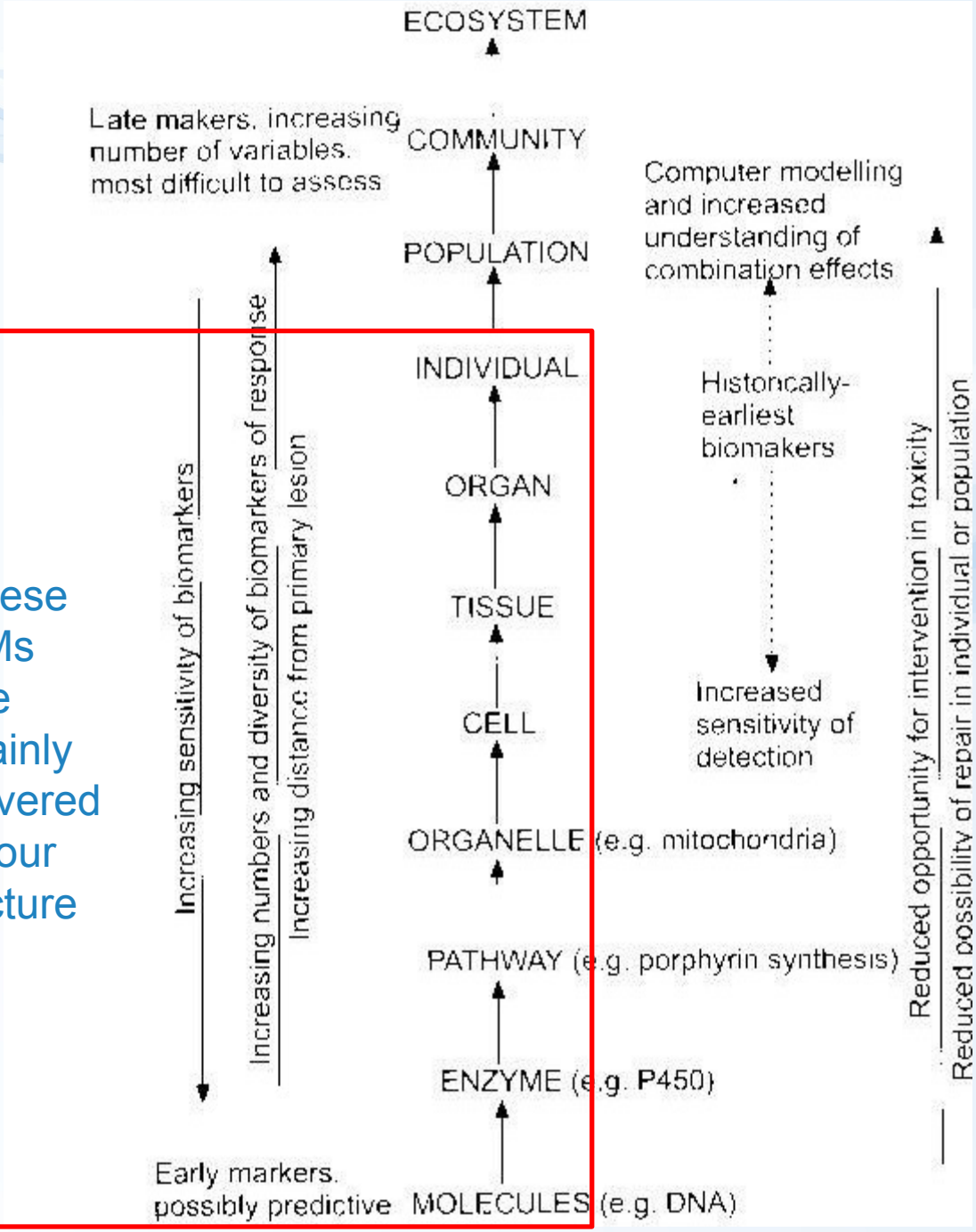
  - multiple biomarkers need to be measured in parallel when searching for a cause of intoxication

- **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" (chemicals, temperature, salinity ...)

# Biomarkers at different levels of biological organisation




These BMs are mainly covered in our lecture

# Sampling biological materials for biomarker analyses

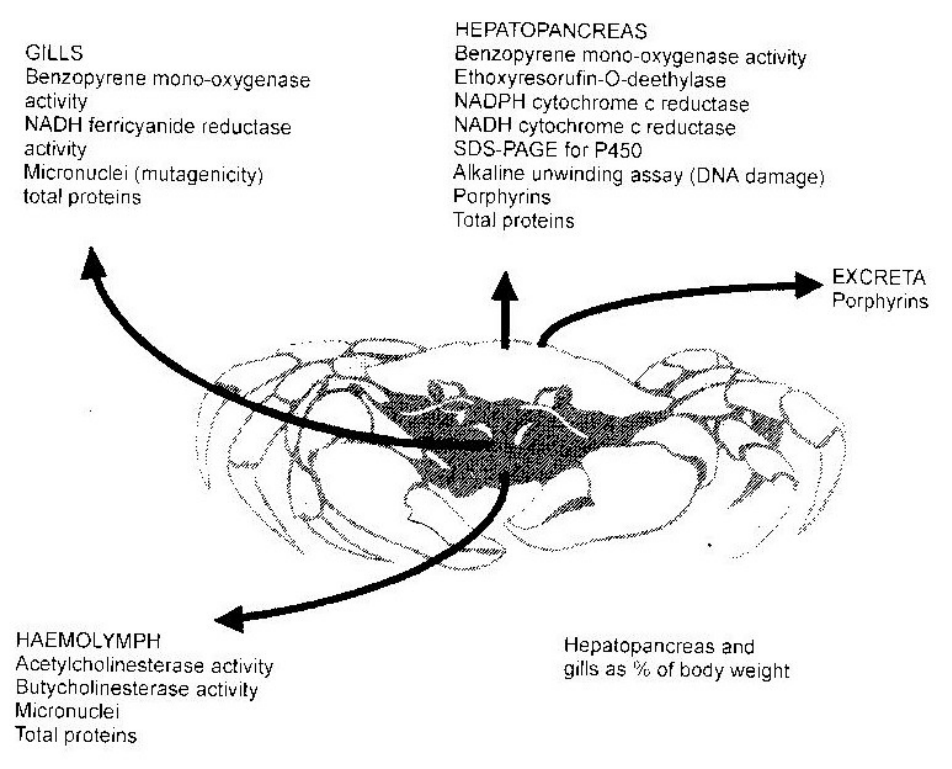
- **Non-destructive (non-invasive)**
  - blood / haemolymph collection & analyses
  - skin, feather, hair, urine …

  (life of the organism not affected)

- **Destructive (invasive)**
  - whole animal

    → research should follow 3R principles (Replacement, Reduction and Refinement)

    → **maximum use of the biological material**
  - multiple biomarker evaluation

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# Biomarkers & Exposure

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

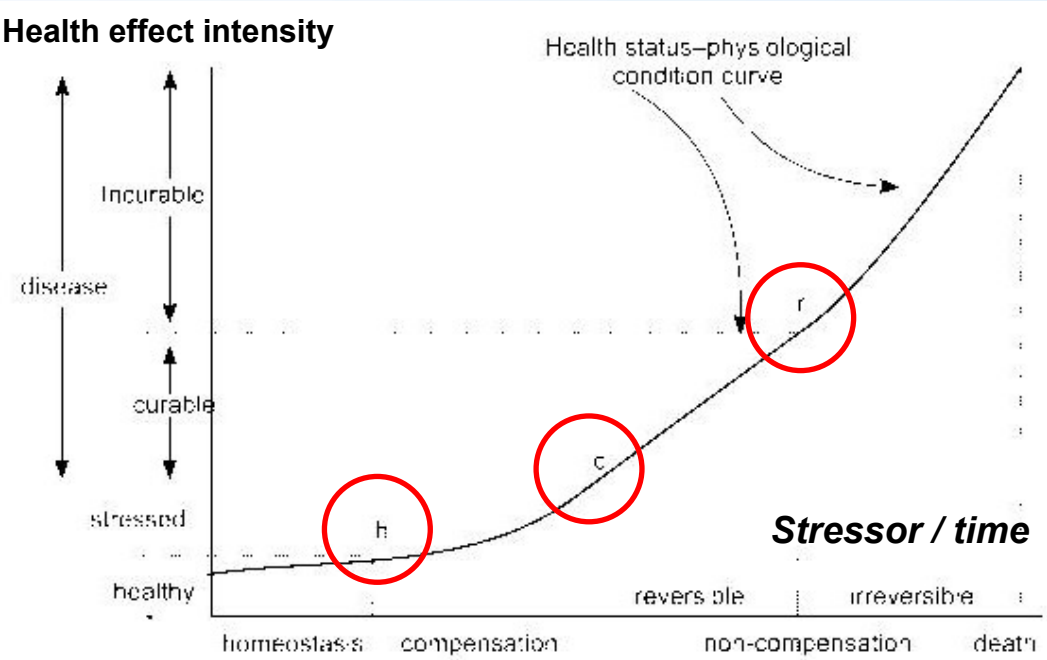
**Various biomarker profiles**

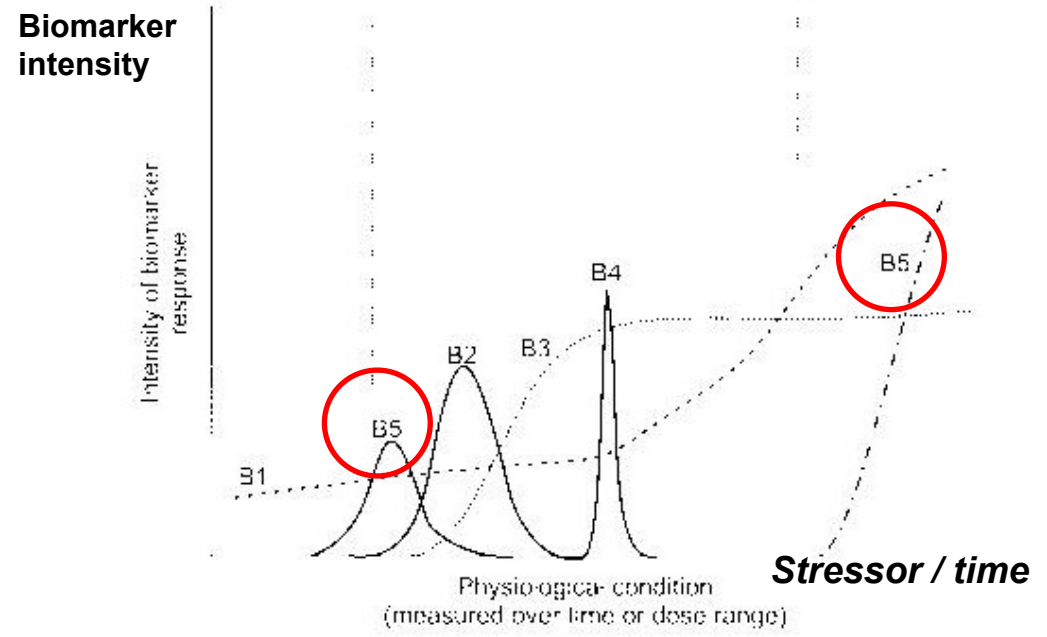
- temporal changes–B2; B4

- repeated occurrence (**B5**)

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

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

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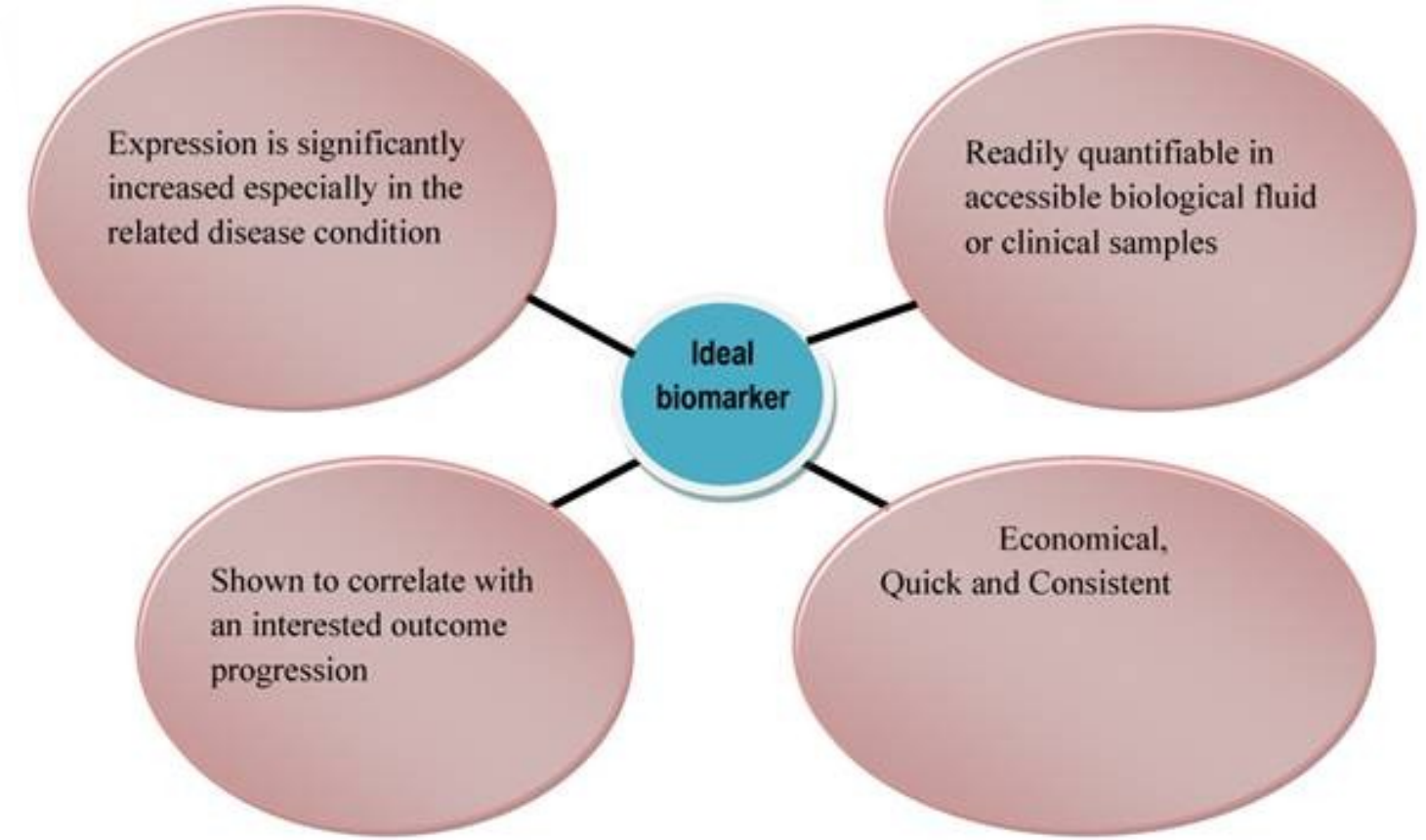
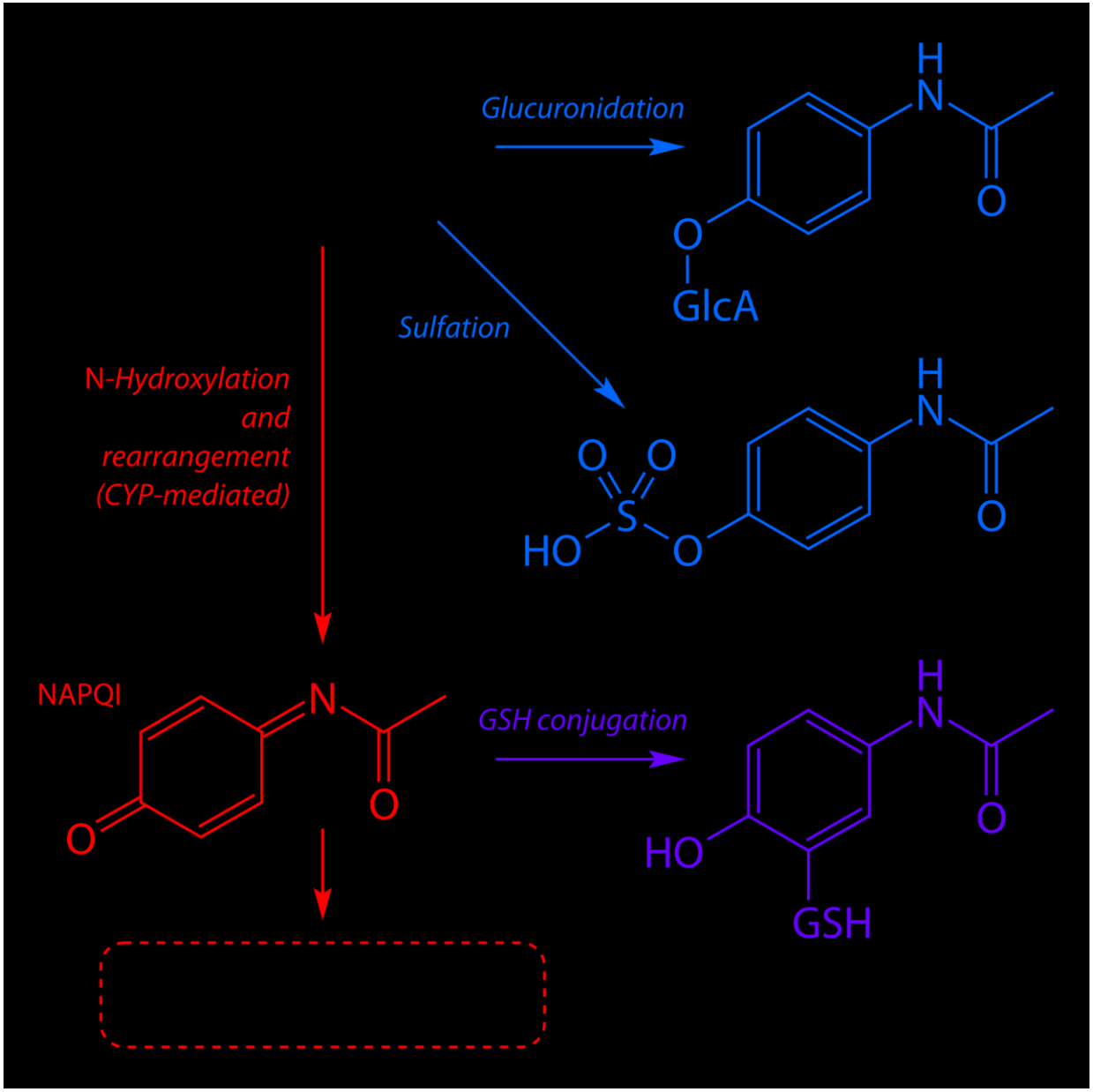
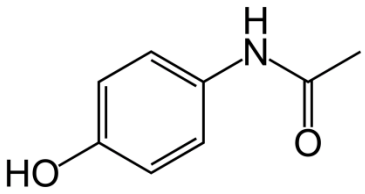
# Ideal biomarker

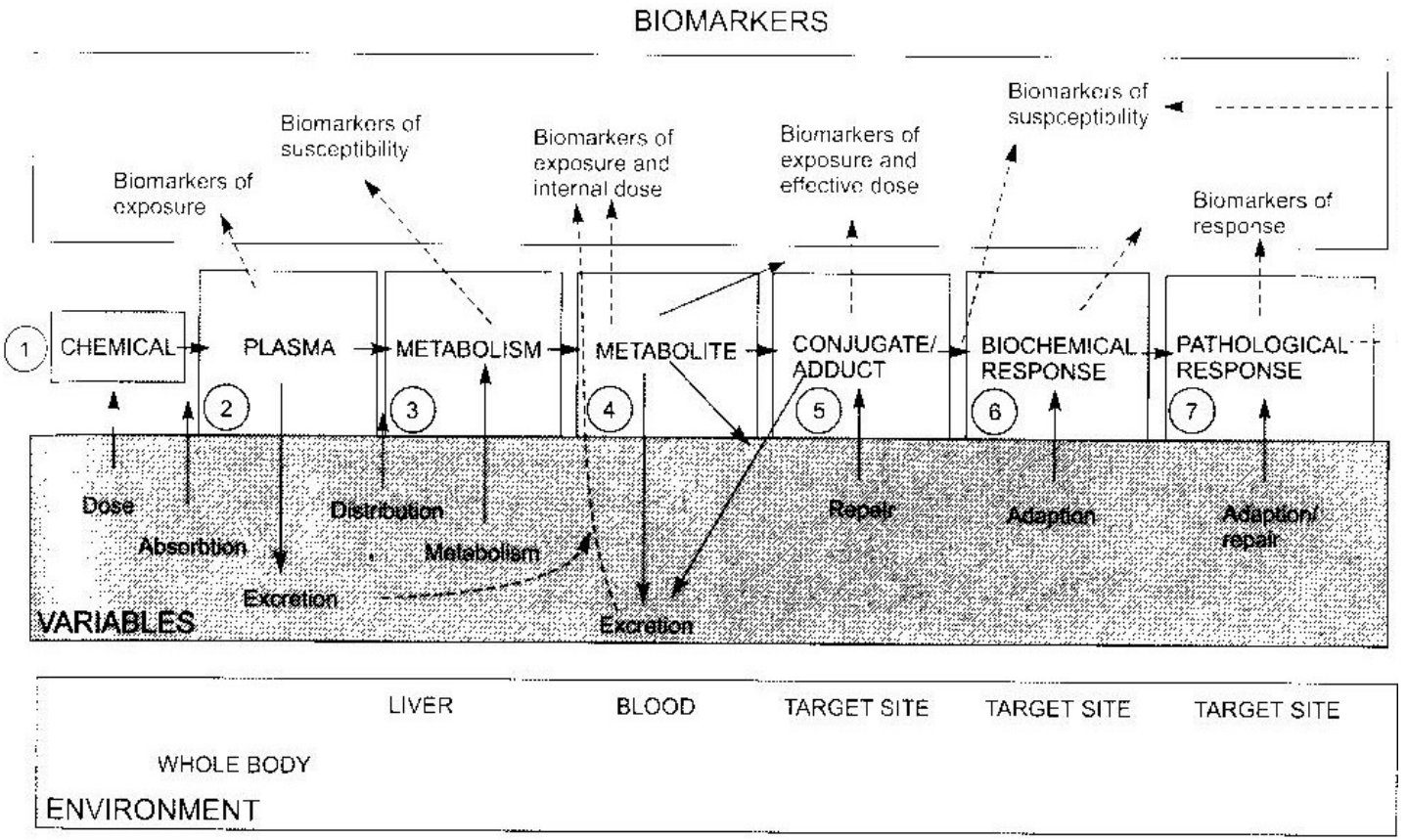


EXAMPLE
- Paracetamol



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**

# Biomarkers in toxicology – examples / overview
(some are discussed in detail in following lectures)

**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*) | – |