Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

12 – BIOMARKERS of EXPOSURE and SUSCEPTIBILITY

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

Biomarkers of Exposure

Biomarkers of internal and effective dose

depends on toxicokinetics

Biomarkers of internal dose (short / long term)

– examples: Cd in urine, DDE in fat tissues

- should be easy to sample (urine, breath)
- instrumental analytical methods (analyses of toxicant)

Biomarkers of effective dose

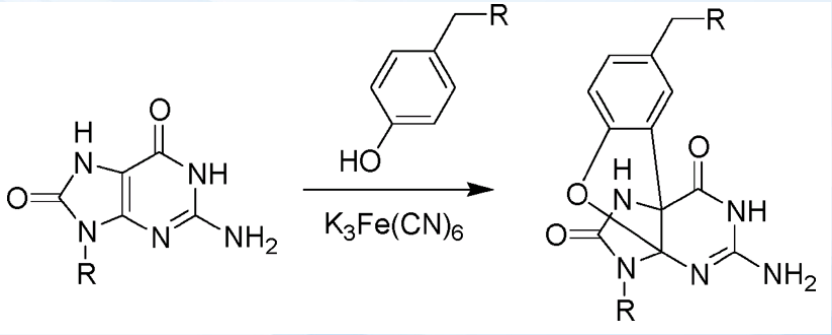
- the chemical interacted with the biological target

→ analyses of ADDUCTS

Two types of adducts: **selective and non-selective**

SELECTIVE ADDUCTS OF TOXICANTS with BIOMOLECULES

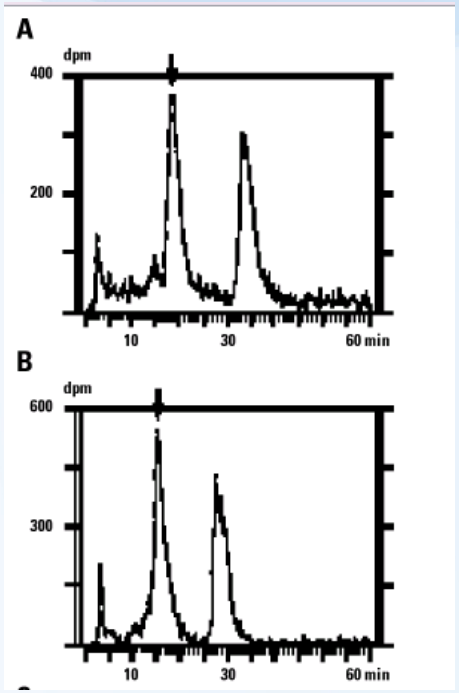
SELECTIVE = CHEMICAL-SPECIFIC



Adducts with DNA

styrene-oxide-O6-guanine
N7-guanyl-aflatoxin B1

Hemoglobin-pesticides adduct



Methods of analyses:

- analytical chemistry
  - extraction from biological sample
  - chemical determination by HPLC or GC

Centrum pro výzkum toxických látek v prostředí

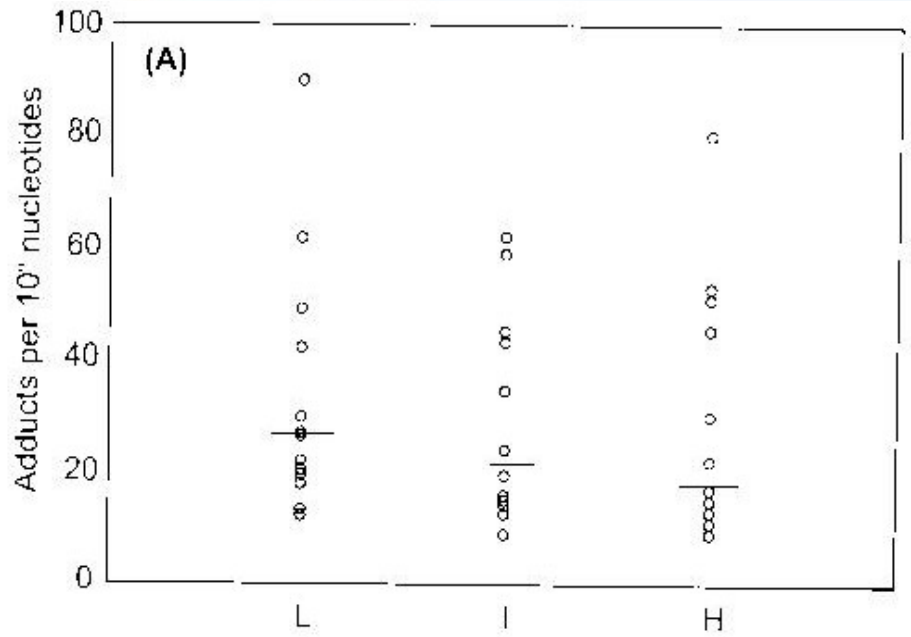
Table 1 Reported human haemoglobin adduct levels for various xenobiotics

Chemical (type of exposure)	Adduct/analyte	Method	Adduct level (nmol g^{-1} haemoglobin)
N, *N*-Dimethylformamide (occupational)	3-Methyl-5-isopropylhydantoin	Hydrolysis; GC–MS	75–1000 (exposed) 4–12 (control)
Epichlorohydrin (occupational)	*N*-(2, 3-Dihydroxypropyl)valine	Modified Edman; GC–MS	0.020 (exposed smokers) 0.007 (exposed non-smokers) 0.013 (control smokers) 0.007 (control non-smokers)
Acetaminophen (drug overdose)	3-(Cystein-*S*-yl)acetaminophen	Immunoassay	100–4100
PAHs (occupational)	BPDF–Hb	Spectrofluorimetry	0.005–0.139
Ethylene oxide (occupational)	*N*-Hydroxyethylvaline	Modified Edman; GC–MS	5–20 (exposed) 0.1–0.5 (control smokers) 0.01–0.1 (control non-smokers)
Ethene (occupational)	*N*-Hydroxyethylvaline	Modified Edman; GC–MS	0.02
Propylene oxide (occupational)	*N*-Hydroxypropylvaline	Modified Edman; GC–MS	0.05–3.5 (exposed) < 0.02 (unexposed)
Acrylonitrile (smoking)	*N*-Cyanoethylvaline	Modified Edman; GC–MS	0.09
NNK (smoking)	4-Hydroxy-1-(3-pyridyl) butan-1-one	Hydrolysis; GC–MS	0.0015 (smokers) 0.0005 (non-smokers)
4-ABP (smoking)	4-ABP-cysteine	Hydrolysis; GC–MS	0.00025–0.0025 (smokers) 0.00005–0.0005 (non-smokers)
Acrylamide (occupational, smoking)	*N*-(2-Carbamoylethyl)valine	Modified Edman; GC–MS	9.5 (production workers) 0.054 (laboratory workers) 0.116 (smokers) 0.031 (non-smokers)
Butadiene (occupational)	*N*-(2,3,4-Trihydroxybutyl)valine	Modified Edman; GC–MS	0.010–0.014 (exposed) 0.002–0.003 (control)
Styrene (occupational)	2-Phenylethanol	Cleavage with Raney nickel, GC–MS	3.7–8.0 (exposed) 2.0–8.6 (control)

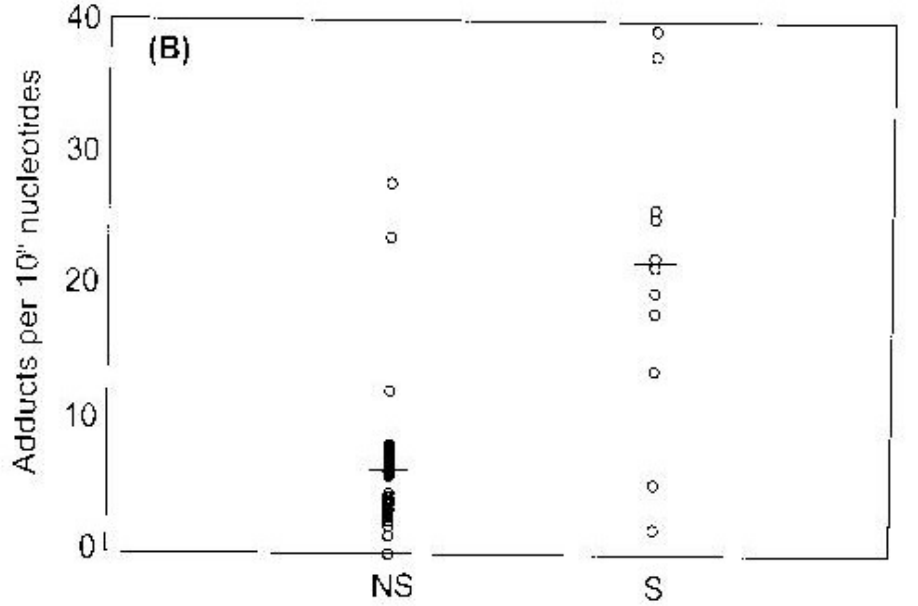
PAH-DNA adducts

PAH (polycyclic aromatic hydrocarbons)

* often high variability
* may have difficult interpretation



Occup. exposure
(Low / Intermed. / High)



Occupational
Non-exposed (NS)
vs.
Exposed (S)

Non-selective adducts

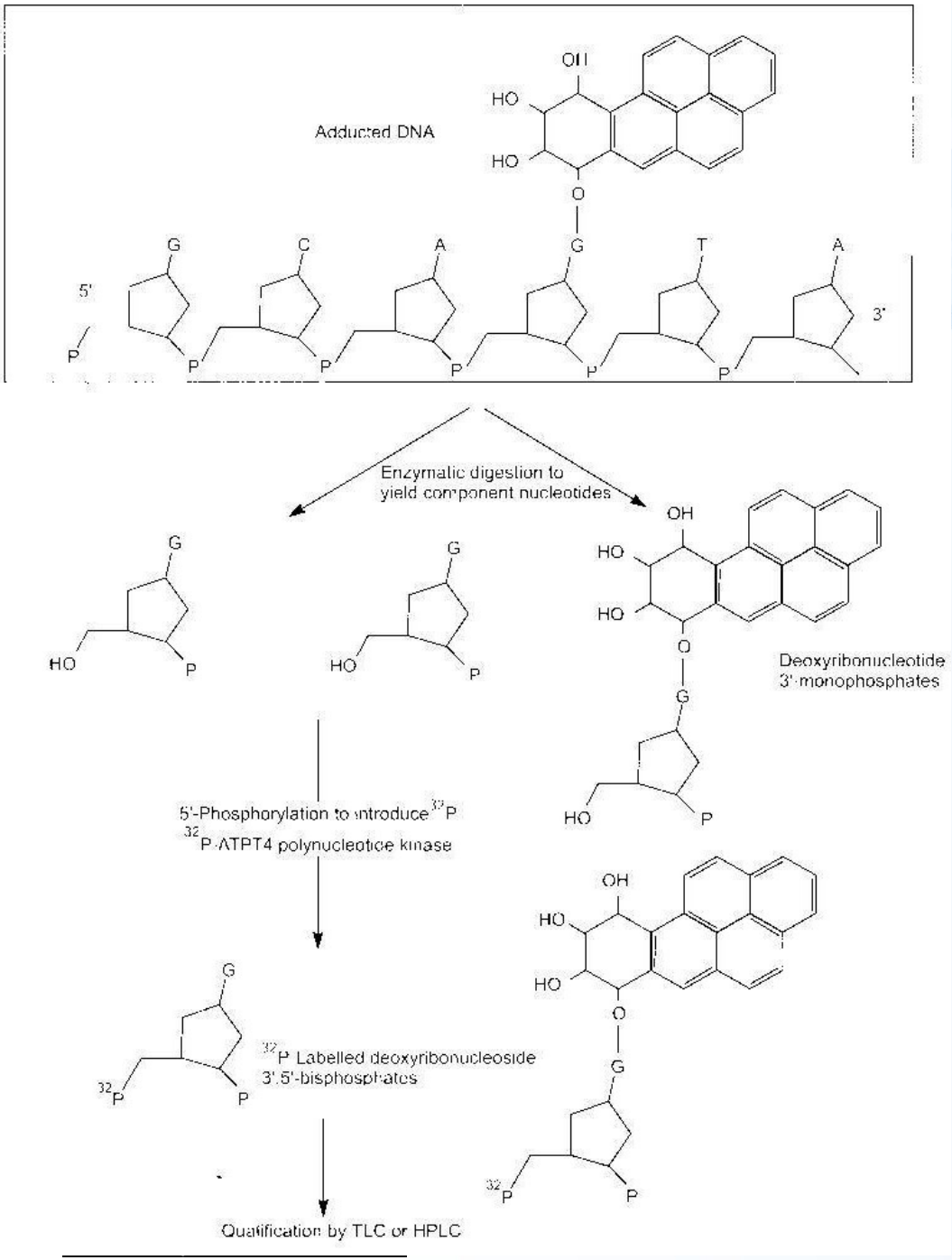
– binding with macromolecules (DNA, proteins) with no further information on the structure of actual adduct (i.e. causative agent not clear)

Typical nonselective biomarker of exposure methods (for DNA damage)

- ^{32}P-postlabelling assay
- oxidized DNA: 8-hydroxy-2´-deoxyguanosine

^{32}P-postlabelling assay principle

- Digestion of NA
- Enzymatic labelling with 32P (kinase)
- TLC or HPLC analyses of products

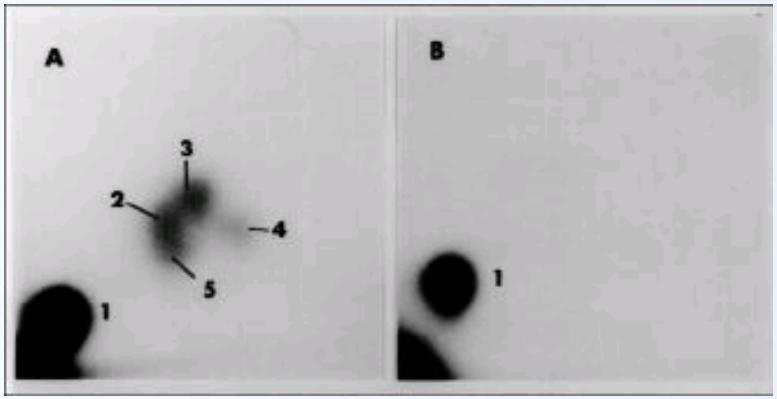


TLC result

(thin layer chromatography)

A - 2-5 = various adducts

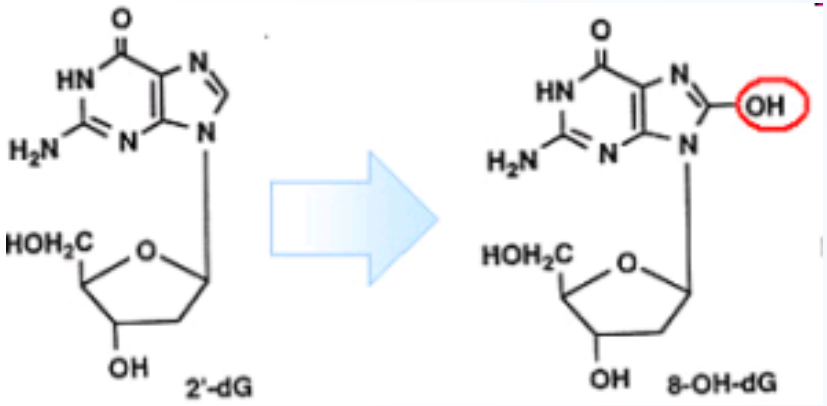
B - controls



8-hydroxy-2 -deoxyguanosine analysis

Oxidative damage to DNA

- many causes → 8-OH-dG is the most common marker of DNA oxidation



Analysis: analytical chemistry methods

- **HPLC**
- immunochemistry (ELISA)

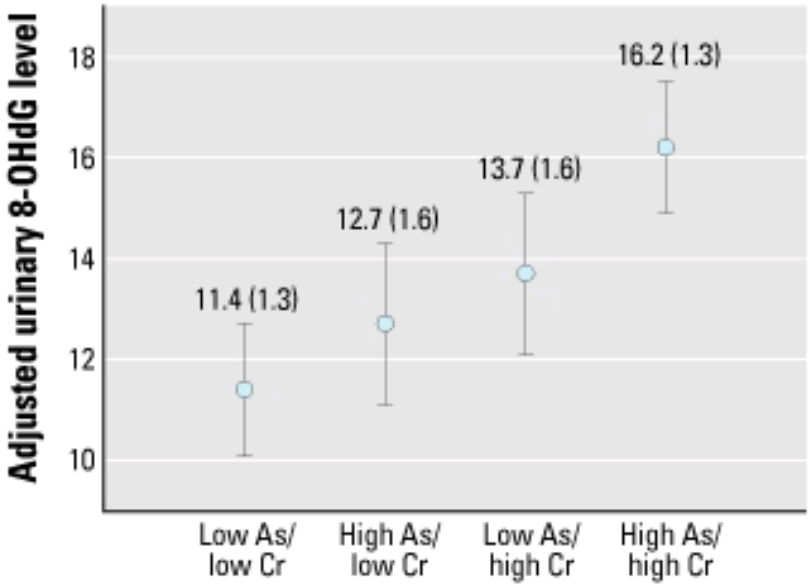


Figure 1. Adjusted urinary 8-OHdG level (ng/mg creatinine) by urinary arsenic and urinary chromium concentrations. Values shown are mean ± SE. Cut points were determined according to medians (arsenic, 7.7 µg/g creatinine; chromium, 2.0 µg/g creatinine) of urinary creatinine-adjusted levels among all subjects.

Centrum pro výzkum toxických látek v prostředí

Biomarkers of susceptibility

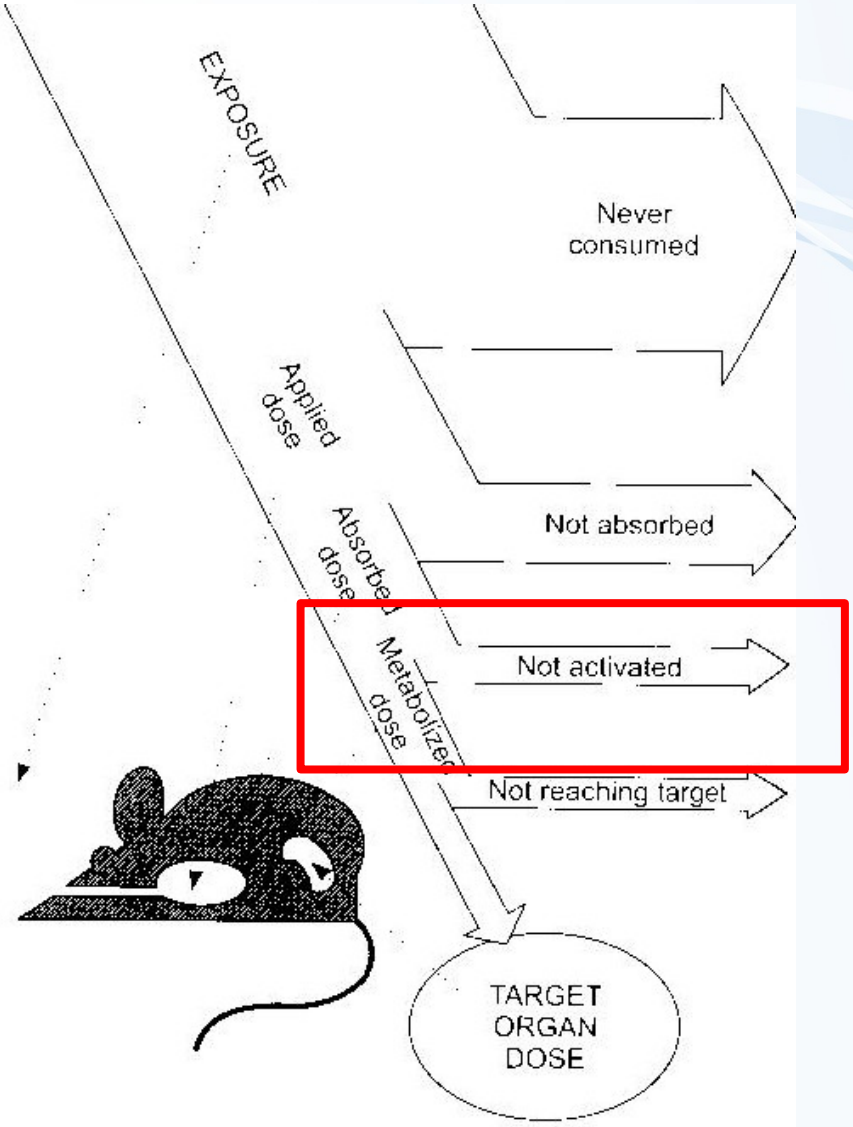


Figure 2 Representation of the relationships between ambient exposure and critical target dose and the progressive decrease in effective exposure due to various biological barriers. Source: *Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives*, p. 188. Used with permission. © 1995 International Life Sciences Institute, Washington, DC, U.S.A.

Toxicokinetics

determines susceptibility of an individual at various levels

→ Biomarkers of susceptibility

Will the individual be sensitive?
Will patient respond to a drug?

Esp. METABOLISM is important and used as BM of susceptibility
(mostly known for prototypical enzymes, like CYPs, for prototypical chemicals such as DRUGS…)

Importance of susceptibility biomarkers

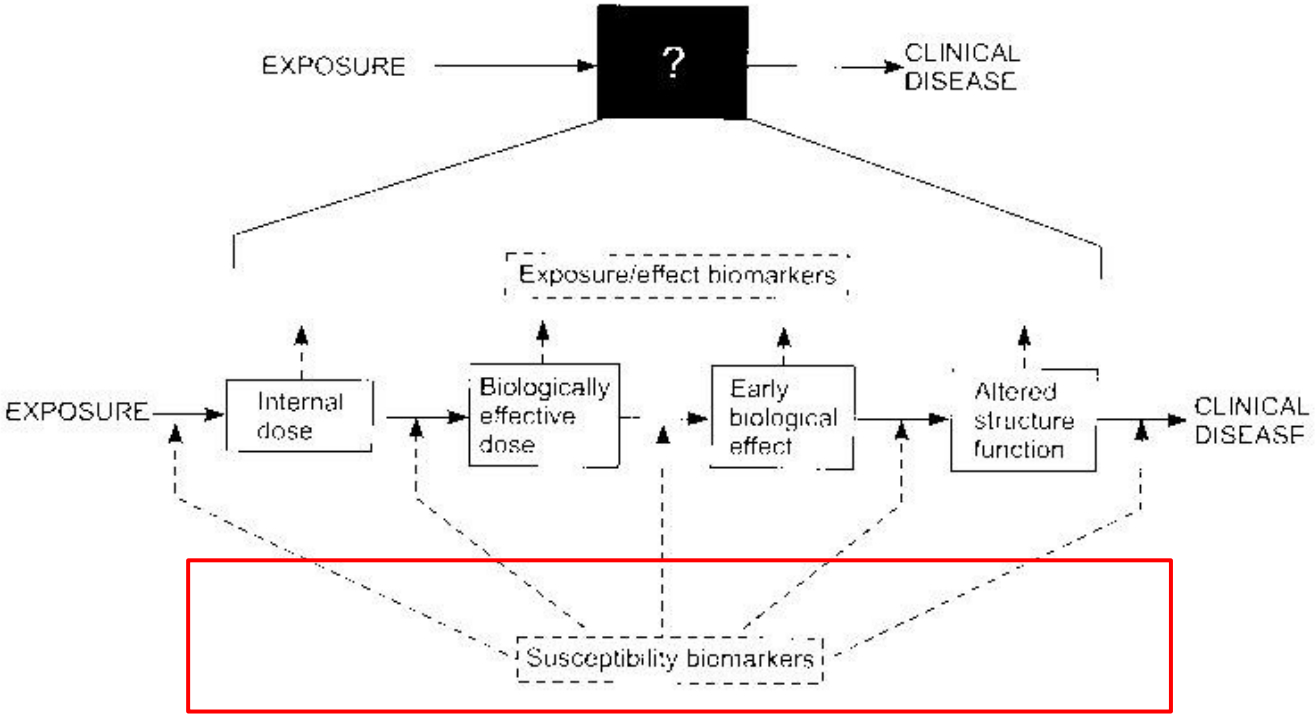


Figure 1 The biomarker paradigm linking exposure with disease and showing expansion of the classical epidemiological 'black box' to reveal discrete mechanistic stages. Reprinted with permission from *Environ. Sci. Technol.* (1997) **31**, pp. 1837–1848. Copyright 1997 American Chemical Society.

Biomarkers of susceptibility

Susceptibility depends on genotype and metabolism

- genetic polymorphism in detoxification enzymes
- variability in specific isoenzymes

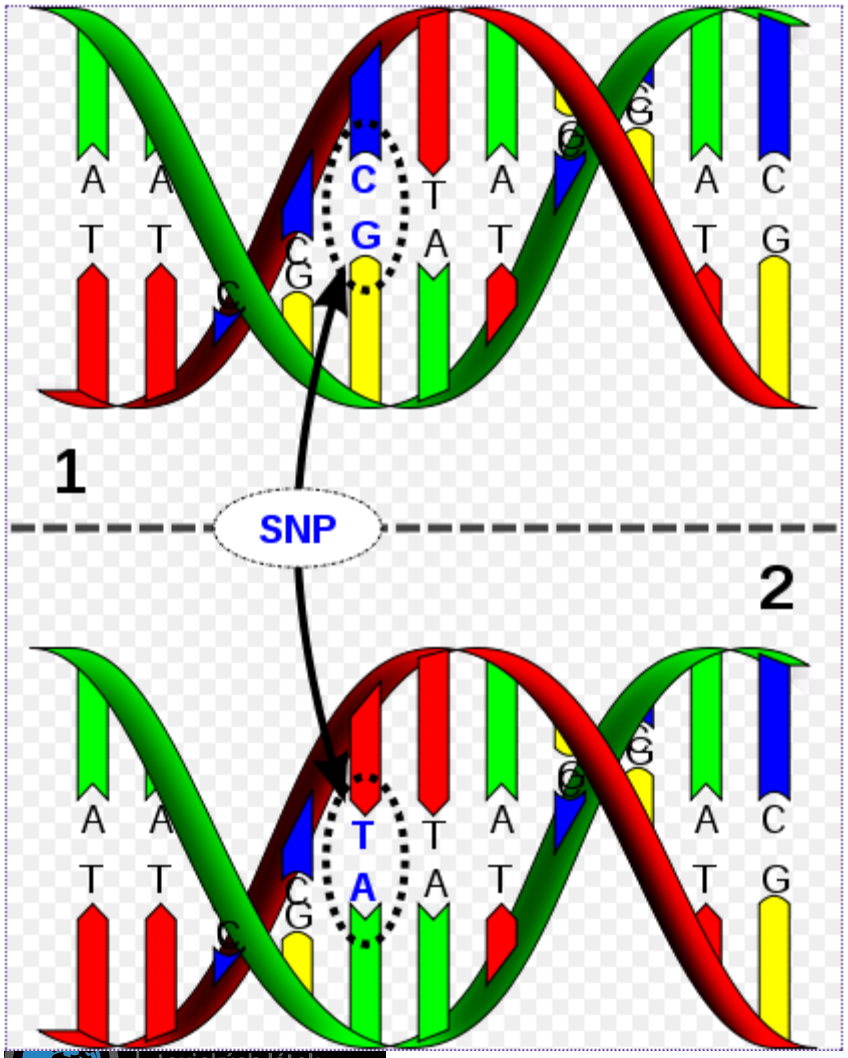
→ susceptibility to „activate“ toxicants:
example: N-acetylation of arylamines – NAT2

→ susceptibility to genotoxins

→ family cancers

→ susceptibility to drugs (including anticancer drugs)

Example: genetic polymorphism
SNPs - single nucleotide polymorphism



SNPs

→ affects protein functions

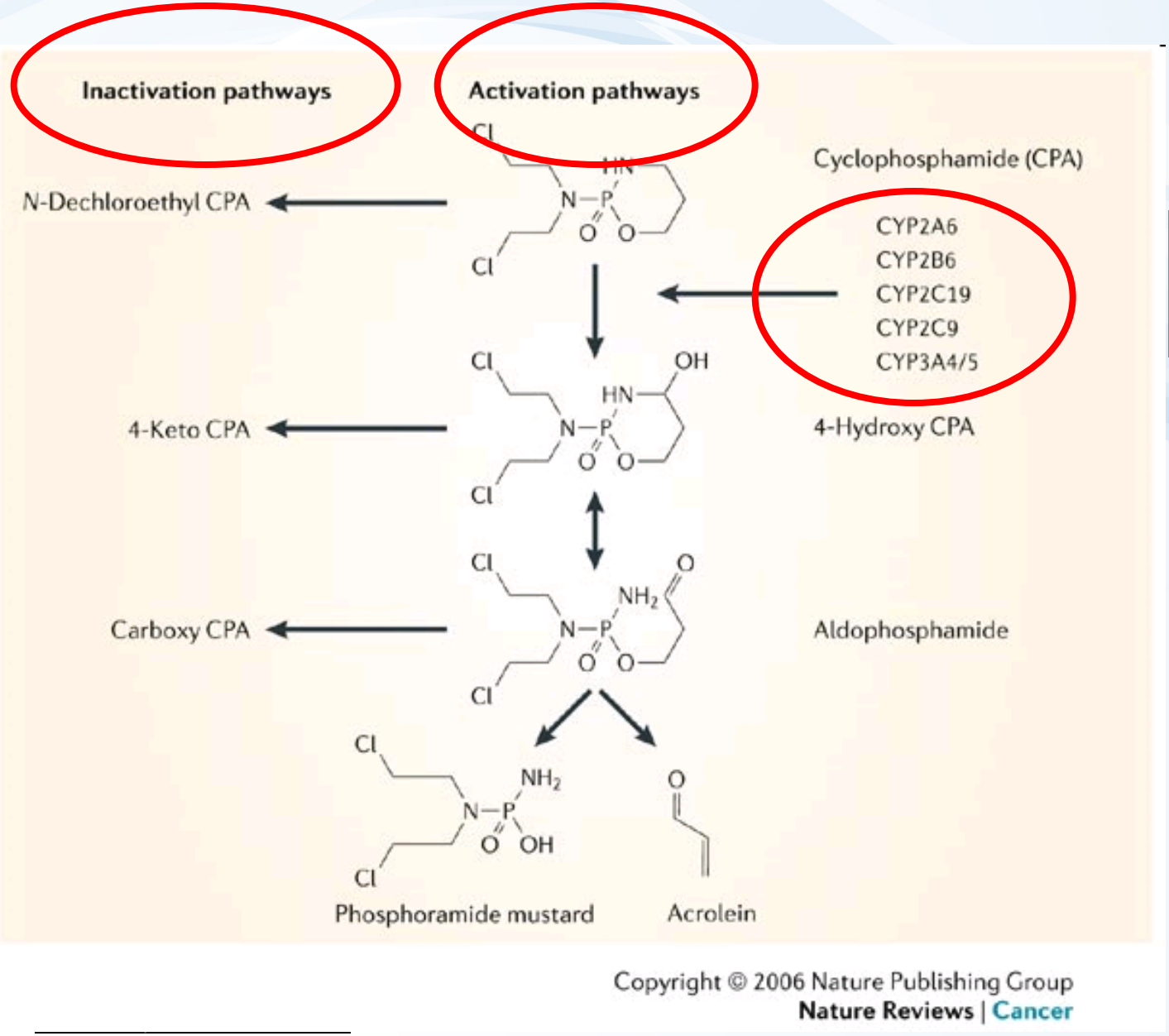
→ in specific cases (see example) some SNPs identified

→ PERSONALIZED MEDICINE

To identify SNP as a biomarker

Many **genotypes** (from many individuals) must be sequenced and compared with **phenotype** (e.g. responsiveness to certain drug)

Cyclophosphamide (anticancer drug) and its toxicity



Genetic polymorphism

Example: genetic polymorphism

CYP450 Enzymes and Polymorphisms

Roche Diagnostics

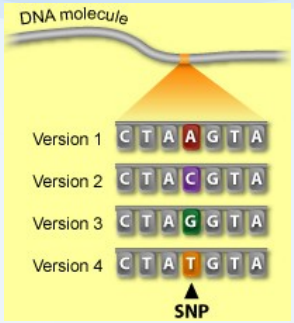
Enzyme	Fraction of drug metabolism	Major polymorphisms
CYP3A4	40-45%	Rare
CYP2D6	20-30%	*2xn, *4, *10, *17, *41
CYP2C9	10%	*2, *3
CYP2C19	5%	*2, *3
CYP1A2	5%	*1K
CYP2B6	2-4%	-
CYP2E1	2-4%	-
CYP2A6	2%	*4, *9
CYP2C8	1%	*3
CYP3A5	<1%	*3

The CYP 2D6 gene is extremely polymorphic with more than 70 allelic variants described so far [1]

Ingelman-Sundberg, TRENDS in Pharmacological Sciences, Vol. 25 No.4 April 2004
[1] Dahl, Clin. Pharmacokinet 2002; 41 (7): 453-470

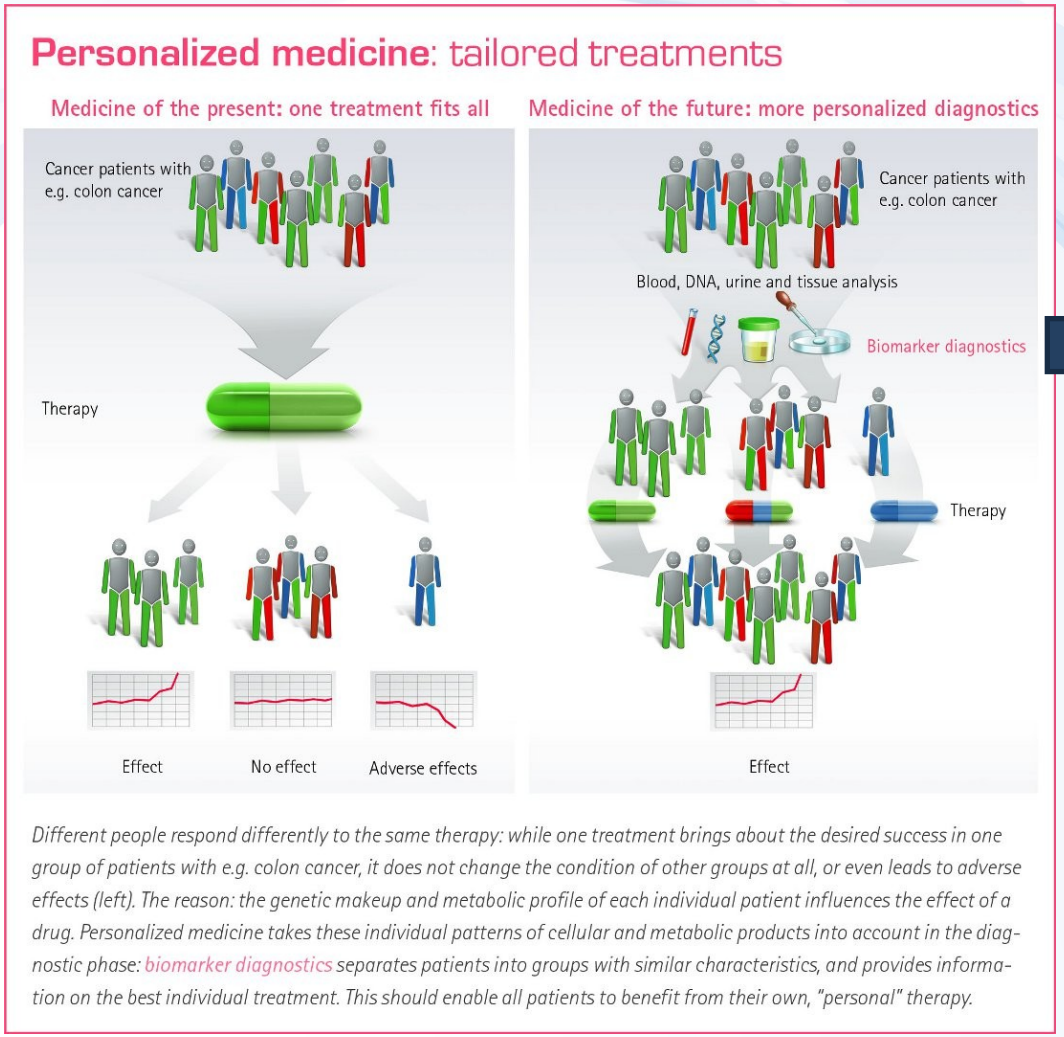
AMPLICHIP

Alleles known to be involved in polymorphism



Centrum pro výzkum toxických látek v prostředí

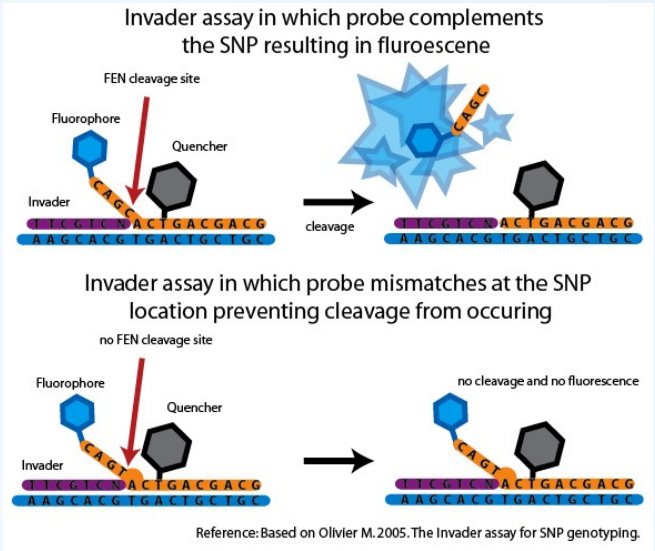
Personalized medicine



SNP diagnostics:

1) DNA isolation
2) Multiplication of specific gene eg. CYP

3) SNP identification
... Molecular biology methods such as
* NA sequencing
* Probe pairing ... number of variants



Centrum pro výzkum toxických látek v prostředí