BIOMARKERS AND TOXICITY MECHANISMS
11 – BIOMARKERS
of EXPOSURE and SUSCEPTIBILITY

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OP Vzdělávání
pro konkurenceschopnost

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 = CHEMICAL-SPECIFIC

Adducts with DNA
    styrene-oxide-O6-guanine
    N7-guanyl-aflatoxin B1

Hemoglobin-pesticides adducts

Methods of analyses:

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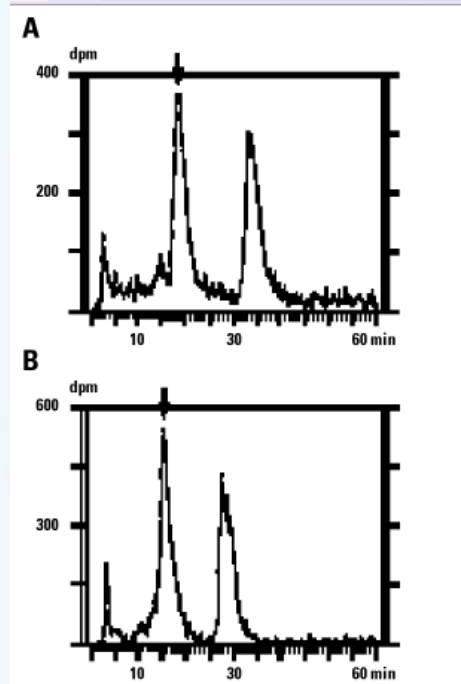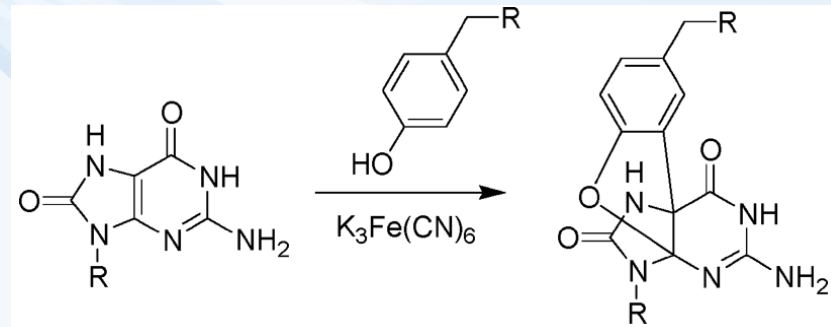   - chemical determination by HPLC or GC (or ELISA)

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Table 1 Reported human haemoglobin adduct levels for various xenobiotics

Chemical (type of exposure)	Adduct/analyte	Method	Adduct level (nmol g $^{-}$ 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)	BPDE–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)
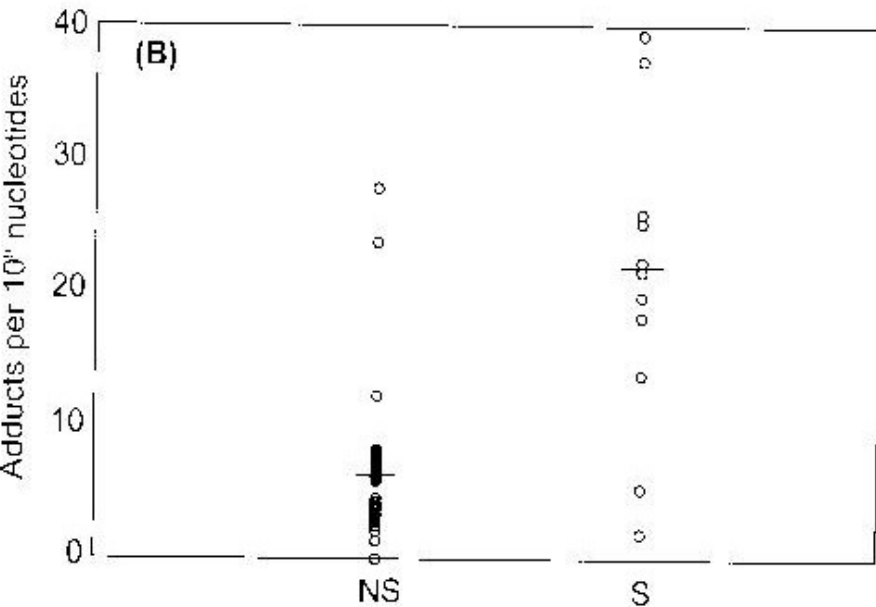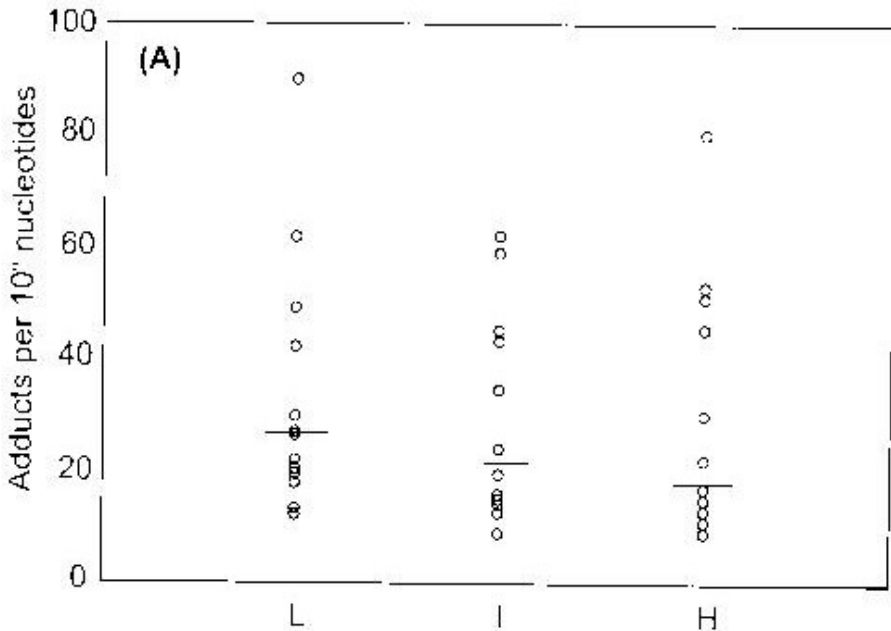
PAH-DNA adducts
PAH (polycyclic aromatic hydrocarbons)
* often high variability
* may have difficult interpretation

Occup. exposure
(Low / Intermed. / High)

Occupational

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

– binding of toxicant to macromolecule (DNA, proteins) with no further information on the structure of actual adduct (i.e. causative agent is not clear but the „exposure" clearly happened)

Typical nonselective biomarker of exposure methods - for DNA damage:

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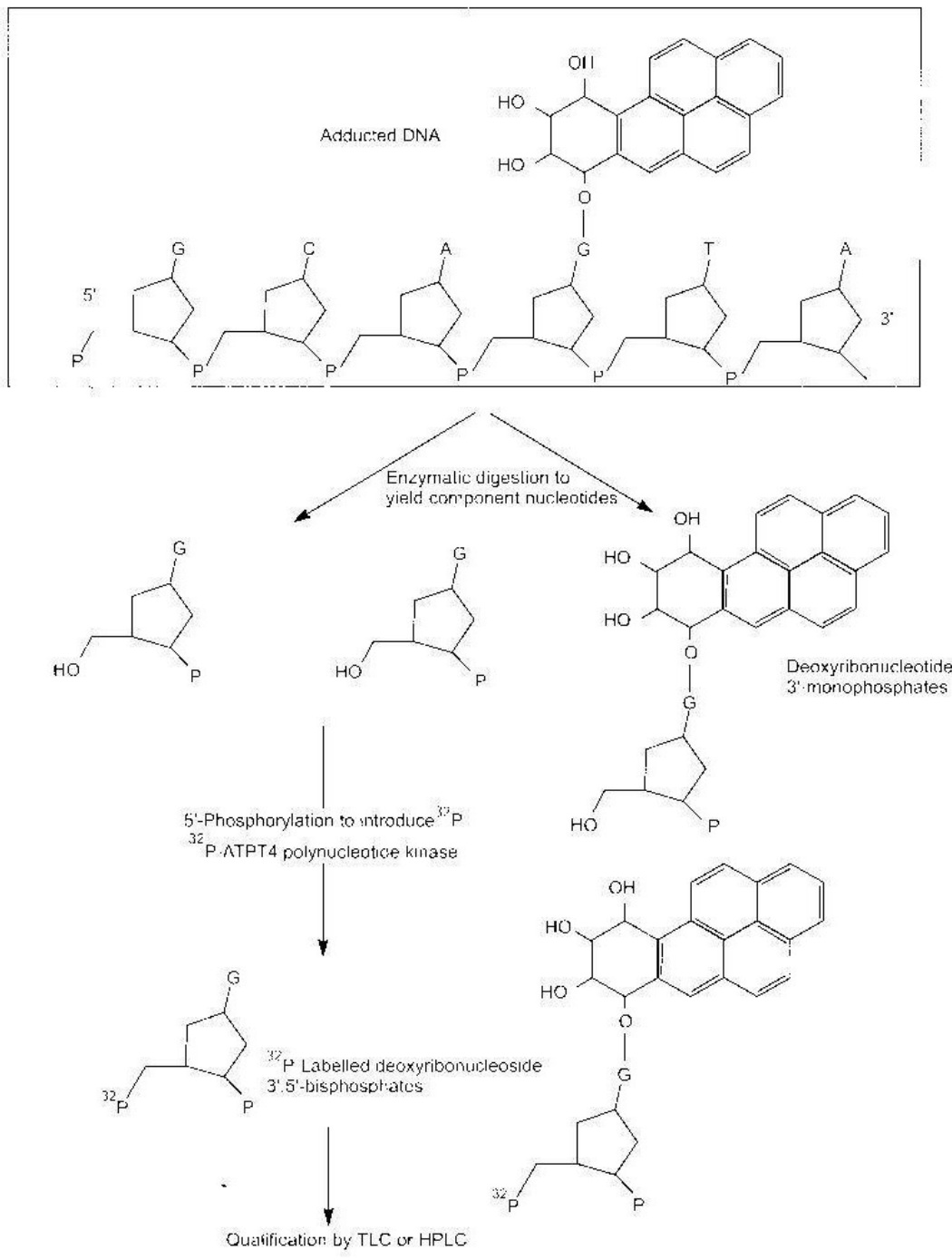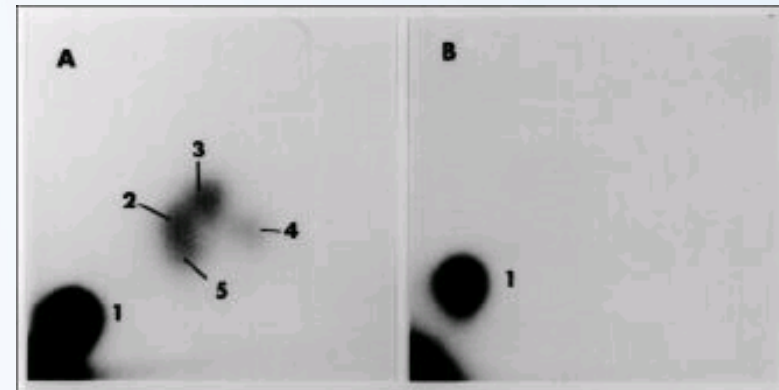
- oxidized DNA: 8-hydroxy-2´-deoxyguanosine

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<superscript>32</superscript>P-postlabelling assay principle

Adducted DNA

Enzymatic digestion to yield component nucleotides

Deoxyribonucleotide 3'-monophosphates

5'-Phosphorylation to introduce <superscript>32</superscript>P
<superscript>32</superscript>P-ATPT4 polynucleotide kinase

<superscript>32</superscript>P Labelled deoxyribonucleoside 3',5'-bisphosphates

Quatification by TLC or HPLC

•Digestion of NA
•Enzymatic labelling with 32P (kinase)
•TLC (or HPLC) analyses of the product

(Not an absolute method
= comparison with controls always needed)
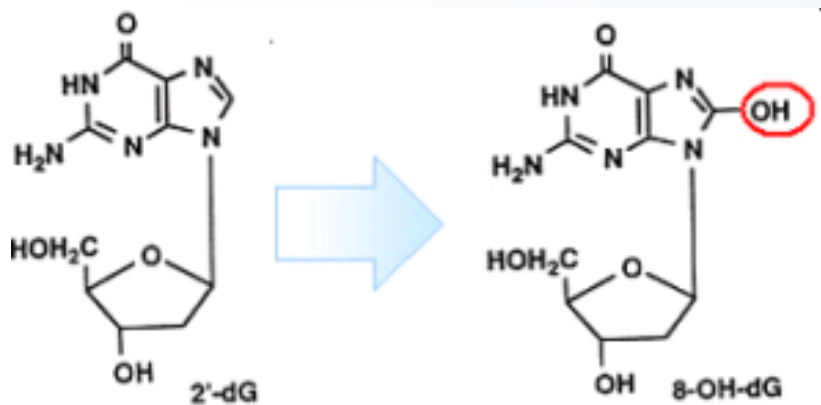
TLC result
(thin layer chromatography)

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**
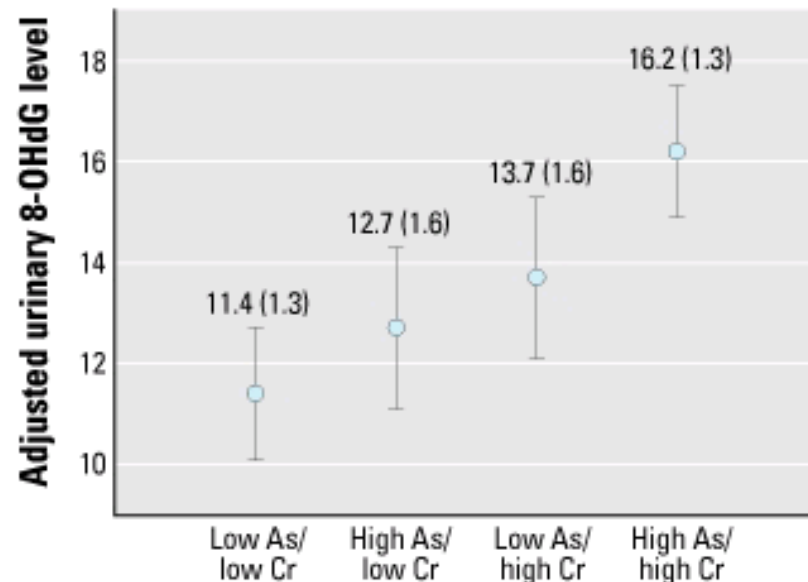- immunochemistry-based methods **ELISA**

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.

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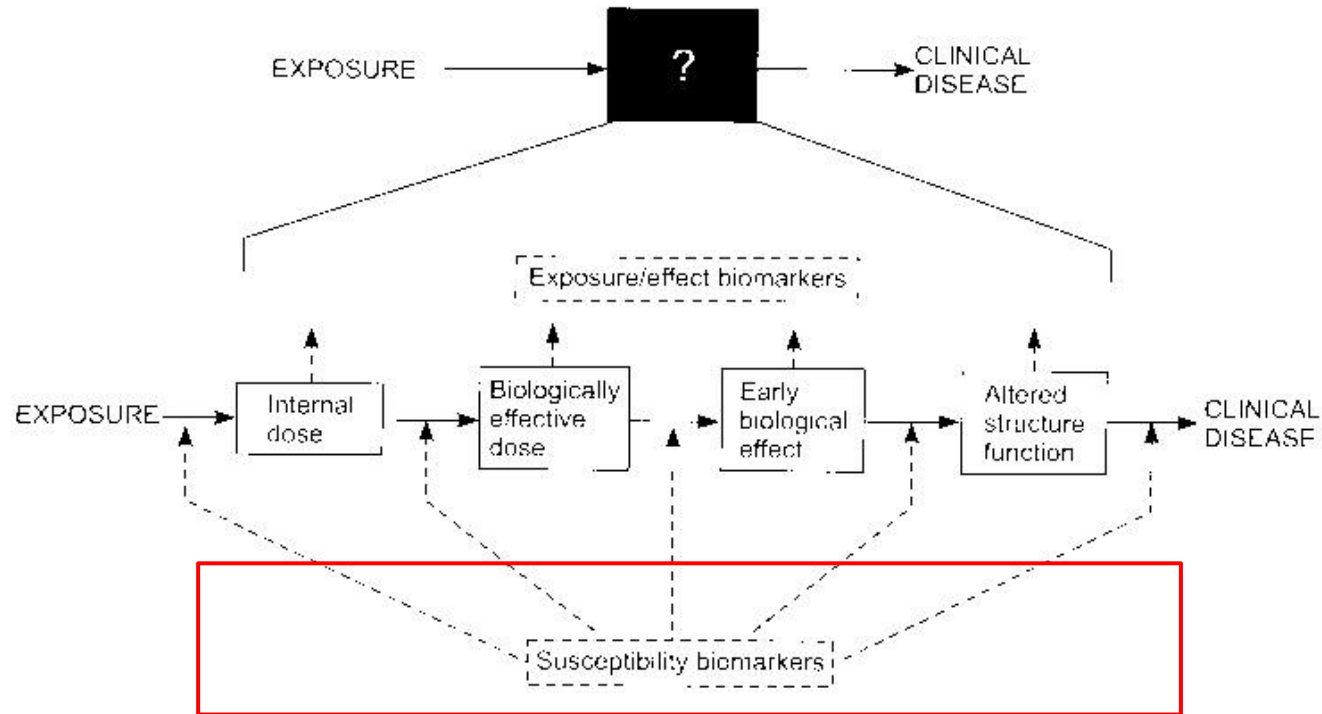
Biomarkers of susceptibility

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.
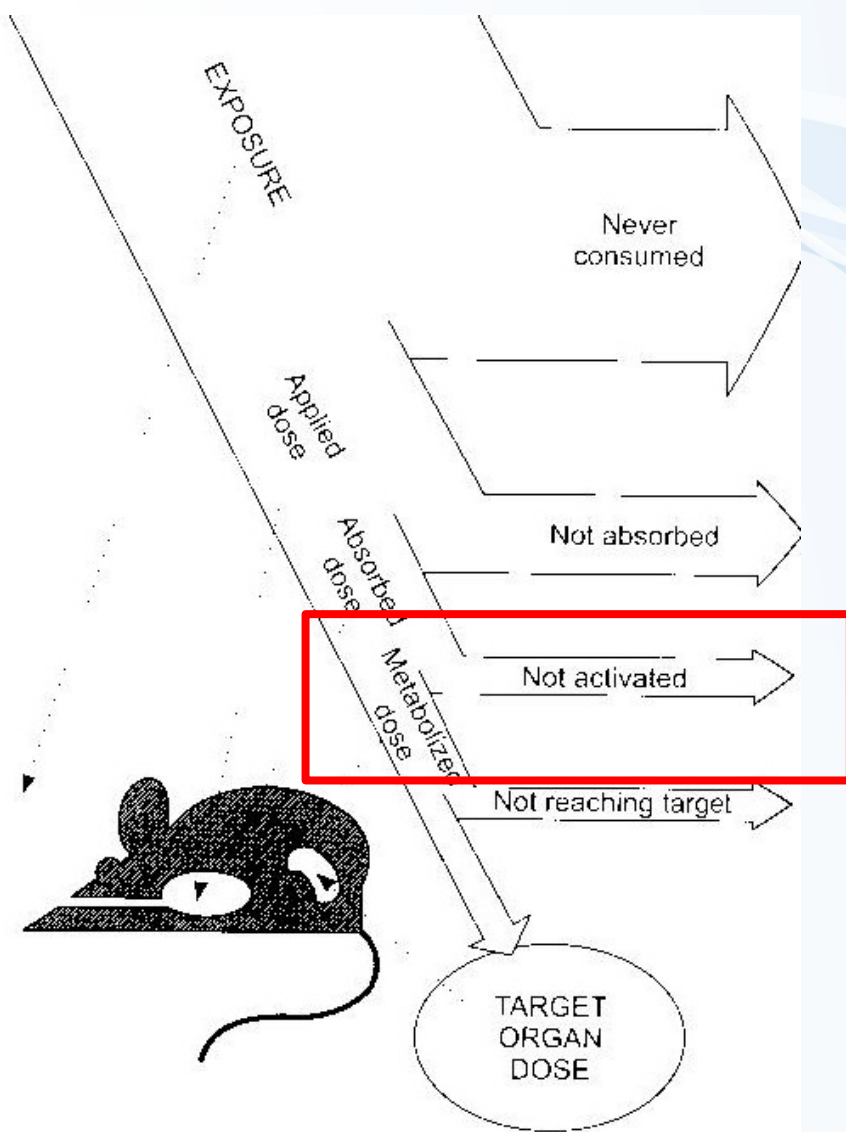
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Figure 2 Representation of the relationships between ambient exposure and critical target dose and the progressive decrease in effective exposure due to various biological barriers. Source: *Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives*, 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 a BM of susceptibility
(mostly known for prototypical enzymes, like CYPs, for prototypical chemicals such as DRUGS…)

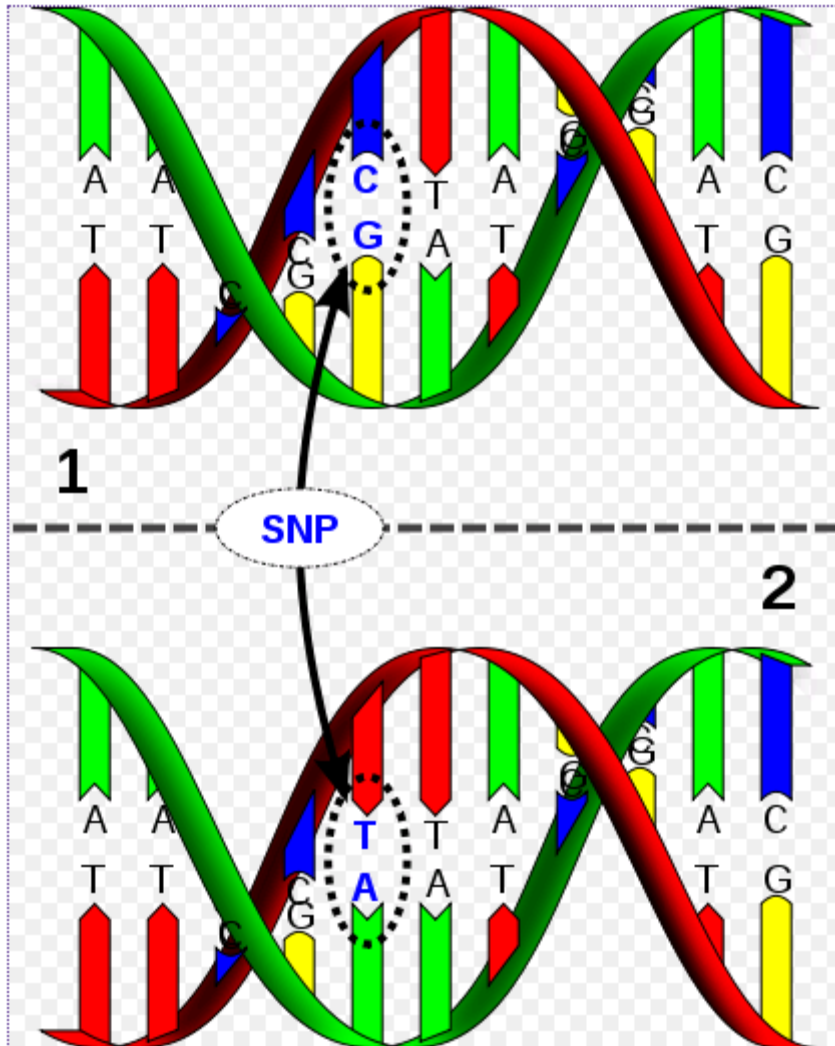**Susceptibility depends on genotype >> metabolism**

    - genetic polymorphism in detoxification enzymes

    - variability in specific isoenzymes

→ Example: susceptibility to „activate" toxicants:

    example: N-acetylation of arylamines (NAT2 gene)

    →   susceptibility to genotoxins

    →   family cancers

    →   susceptibility to drugs (including anticancer drugs)

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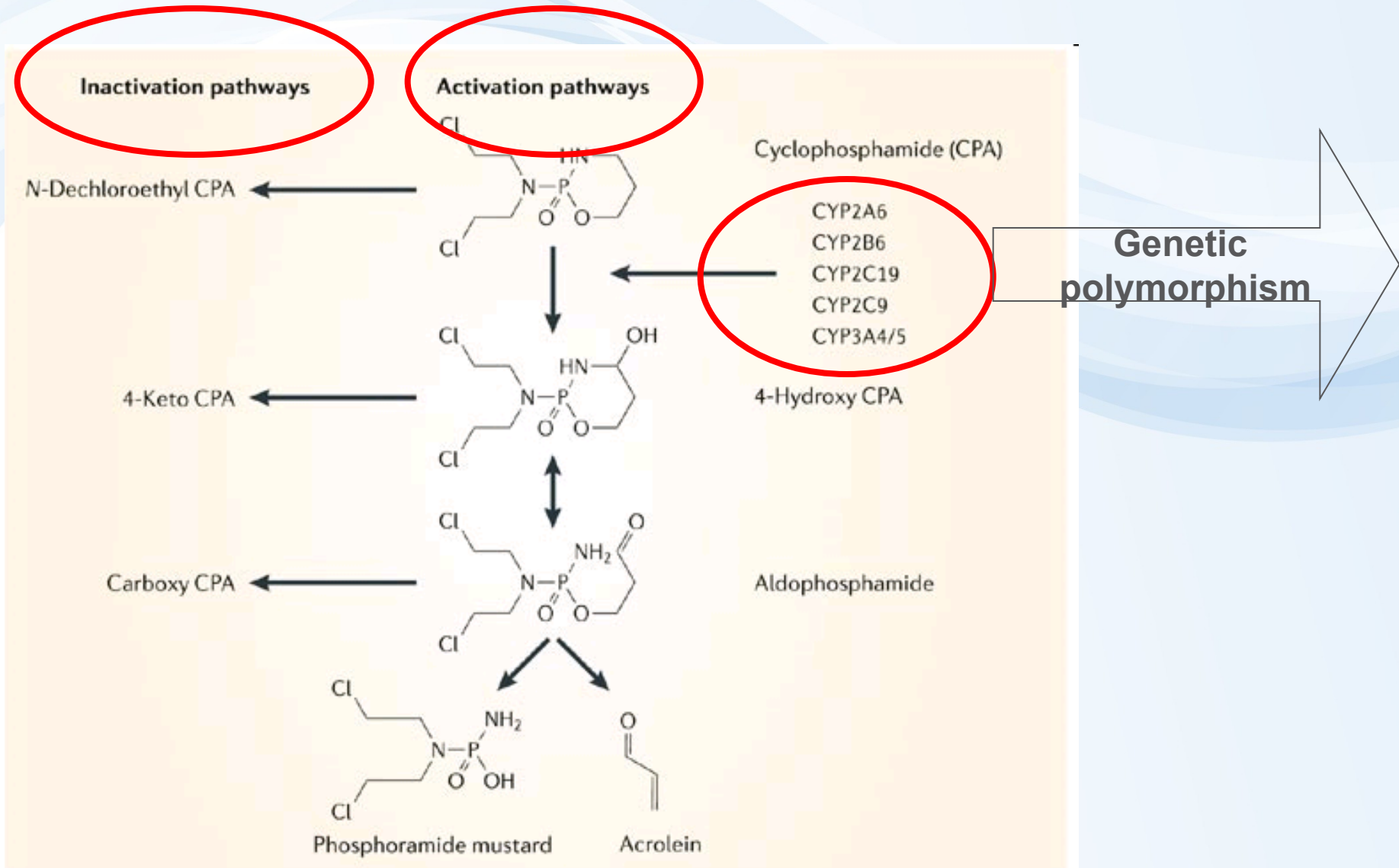toxických látek
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SNPs
→ affects protein functions

→ in specific cases (see example) some SNPs identified

To identify SNP as a biomarker

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

→ **application:**
PERSONALIZED MEDICINE

Cyclophosphamide (anticancer drug) and its toxicity

Inactivation pathways

Activation pathways

N-Dechloroethyl CPA

Cyclophosphamide (CPA)

CYP2A6
CYP2B6
CYP2C19
CYP2C9
CYP3A4/5

Genetic polymorphism

4-Keto CPA

4-Hydroxy CPA

Carboxy CPA

Aldophosphamide

Phosphoramide mustard

Acrolein

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Example: genetic polymorphism

CYP450 Enzymes and Polymorphisms

Roche

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

Diagnostics

Alleles known to be involved in polymorphism

DNA molecule

Version 1 C T A A G T A
Version 2 C T A C G T A
Version 3 C T A G G T A
Version 4 C T A T G T A
          SNP

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Personalized medicine: tailored treatments

Medicine of the present: one treatment fits all

Cancer patients with e.g. colon cancer

Therapy

Effect   No effect   Adverse effects
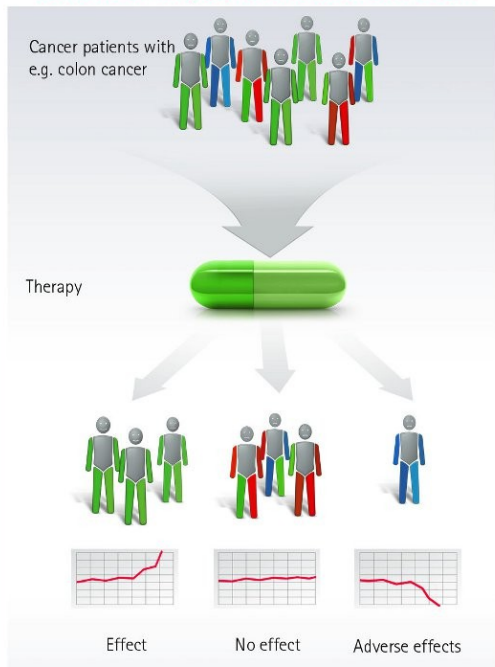
Medicine of the future: more personalized diagnostics

Cancer patients with e.g. colon cancer

Blood, DNA, urine and tissue analysis
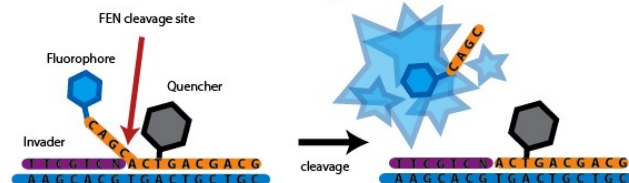
Biomarker diagnostics

Therapy

Effect
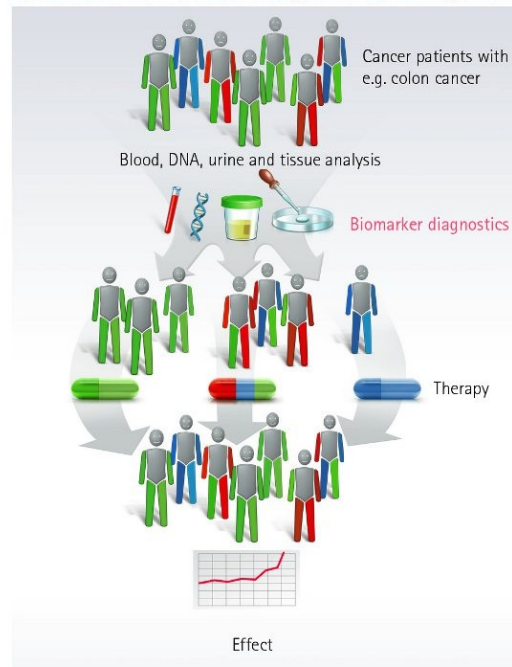
Different people respond differently to the same therapy: while one treatment brings about the desired success in one group of patients with e.g. colon cancer, it does not change the condition of other groups at all, or even leads to adverse effects (left). The reason: the genetic makeup and metabolic profile of each individual patient influences the effect of a drug. Personalized medicine takes these individual patterns of cellular and metabolic products into account in the diagnostic phase: biomarker diagnostics separates patients into groups with similar characteristics, and provides information on the best individual treatment. This should enable all patients to benefit from their own, "personal" therapy.

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

Invader assay in which probe complements the SNP resulting in fluoescene

FEN cleavage site

Fluorophore

Quencher

Invader

cleavage

Invader assay in which probe mismatches at the SNP location preventing cleavage from occuring

no FEN cleavage site

Fluorophore

Quencher

Invader

no cleavage and no fluorescence
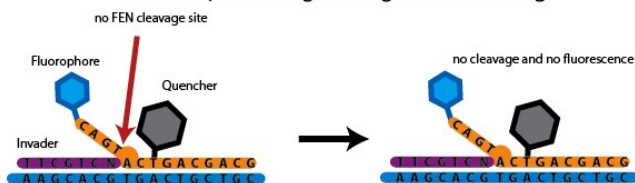
Reference: Based on Olivier M. 2005. The Invader assay for SNP genotyping.