Biomarkers

Biomarkers

- 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.

Toxicology – present status**:**

- identification of markers of long-term risks
  : human (health, toxicology and carcinogenesis)
  : ecotoxicology – early markers of toxic effects

Biomarkers - summary

Biomarker:

change which occurs as response to "stressors" (xenobiotics, disease, temperature...) which **extend the adaptive response beyond the normal range**

In vivo biomarkers:

changes measured in stressed animals ("classical biomarkers")

In vitro biomarkers

in vitro testing to characterize potencies of xenobiotic to induce specific biological activity (genotoxicity, estrogenicity, dioxin-like activity, tumor promotion ...)

= biological potencies (markers) of potential hazards

Biomarkers & Exposure

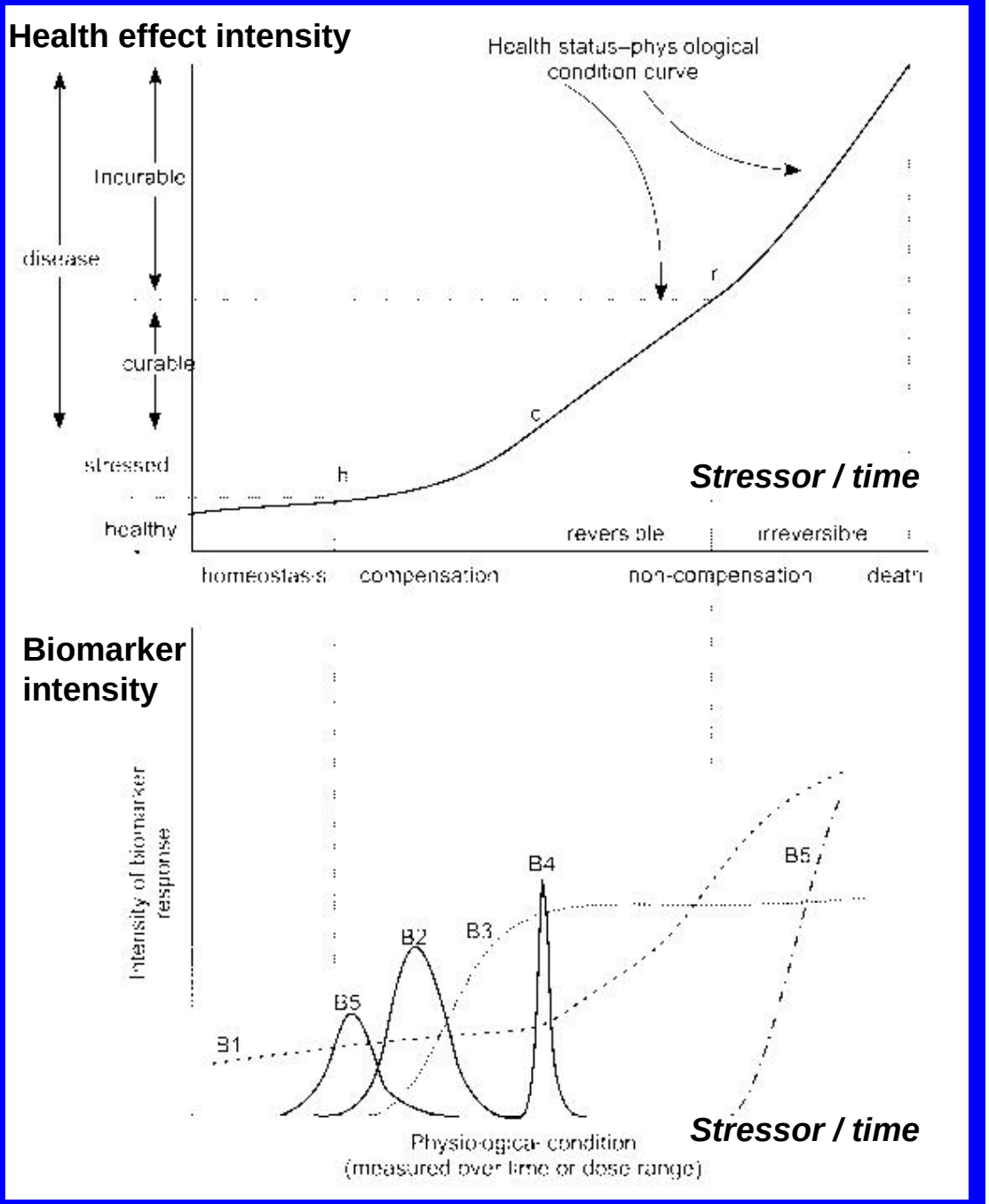
h: homeostatic conditions
c: reversible stage
r: irreversible effects of pollutants

<u>Biomarkers:</u>

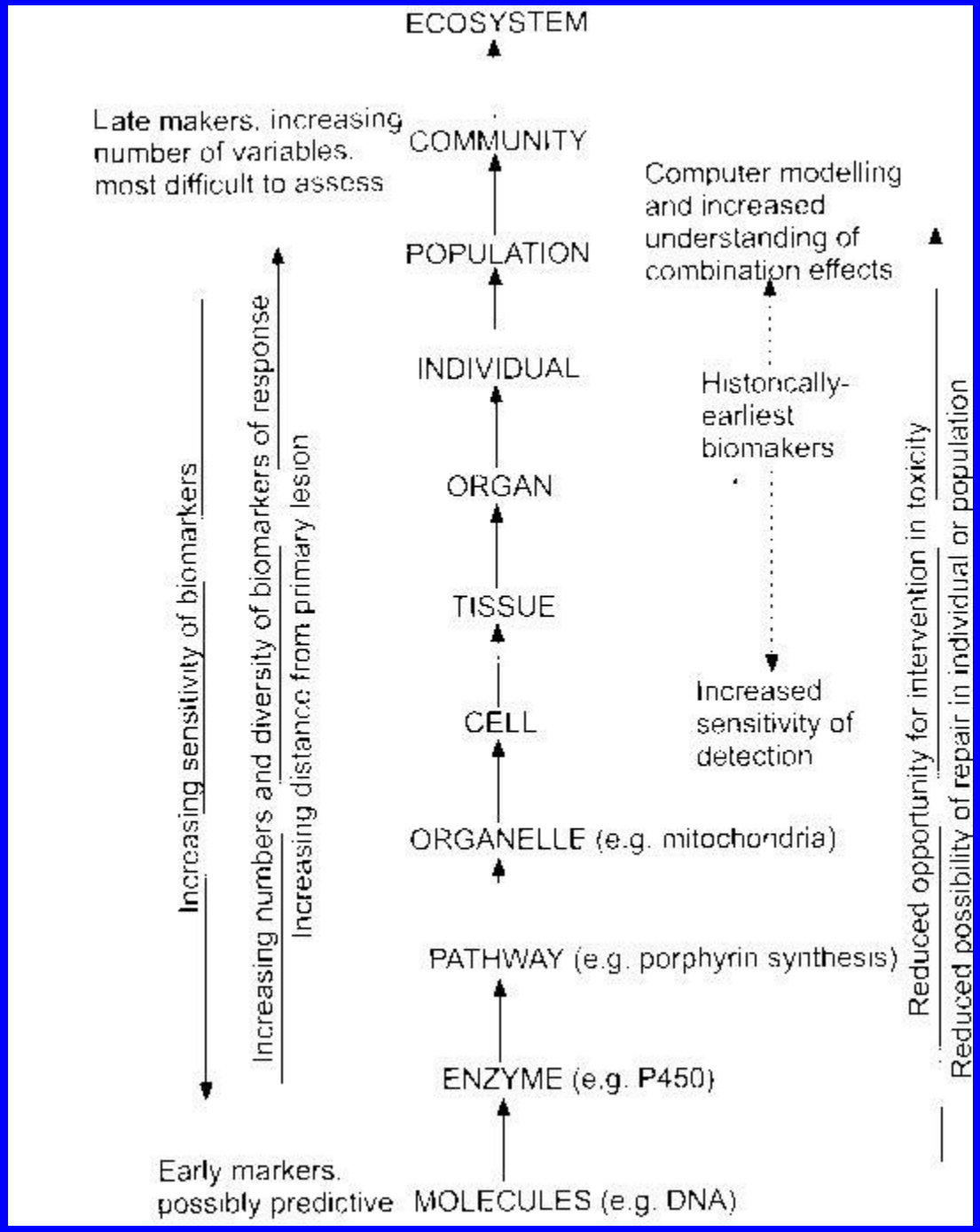
- **temporal change**
  – B5, B2; short period: B4

- **continuous increase – B3**

- **repeated occurrence (B5)**
  – irreversible change



Biomarkers at different levels of biological organisation



Biomarkers - classification

Categorization US National Academy of Sciences

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

Continuum exists among biomarkers

example: adducts of toxicant with DNA

? biomarker of exposure / ? response

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

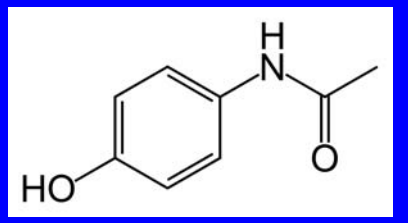
- sensitive to many "stressors" (temperature, salinity ...)

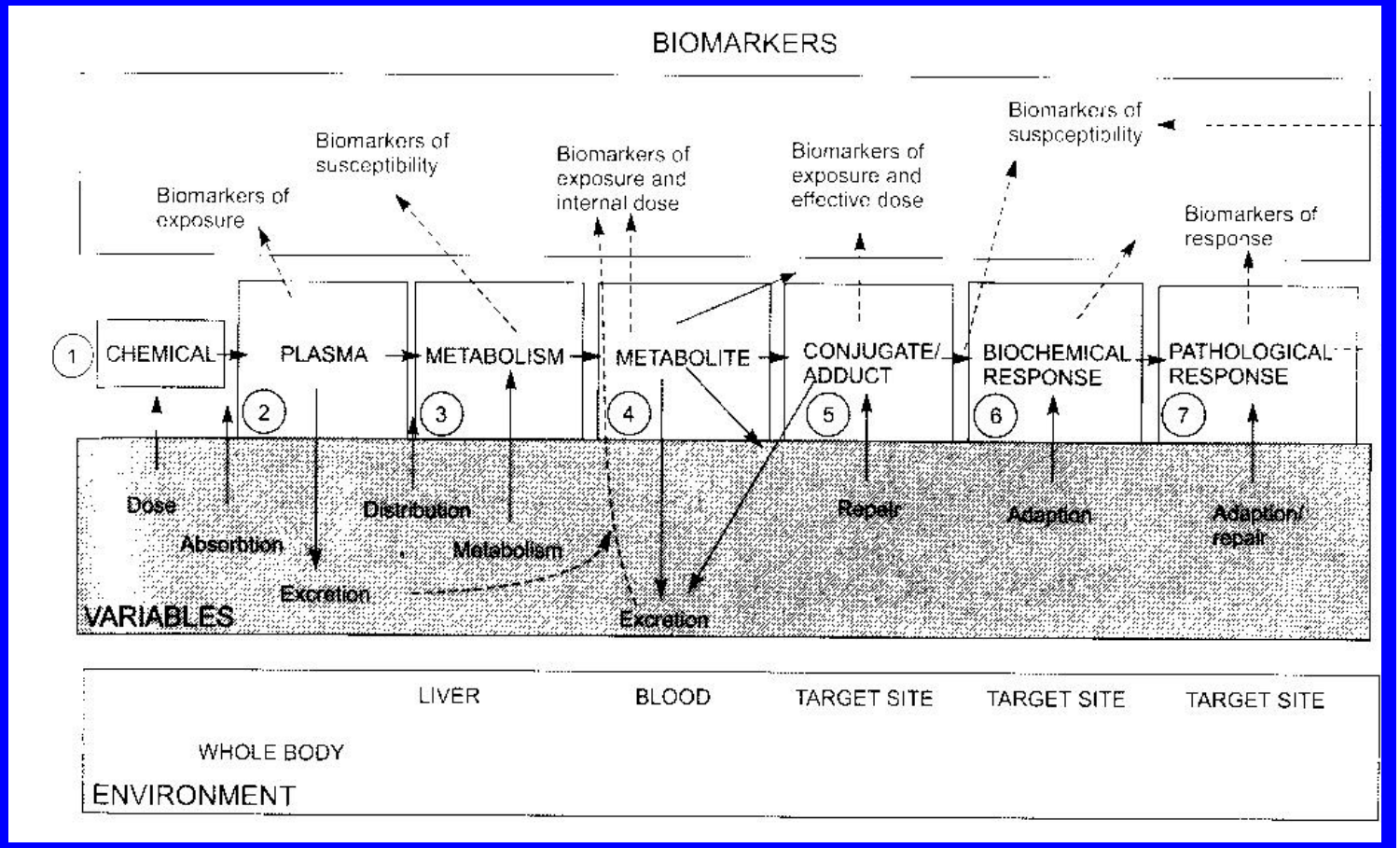
In vivo biomarkers - sampling

- Non-destructive (non-invasive)
  : blood / haemolymph collection & analyses
  : skin, feather, hair …
  : life of the organism not affected

- Destructive (invasive)
  : whole animal -> multiple biomarker evaluation

EXAMPLE
- Paracetamol





(1) paracetamol

(2) parent compound measurement - *biomarker of exposure*

(3) activation to reactive metabolite (N-ac-p-benzoquinone, NAPQI) by CYPs; reaction with GSH / measurement – levels of CYPs; levels of GSH – *susceptibility*)

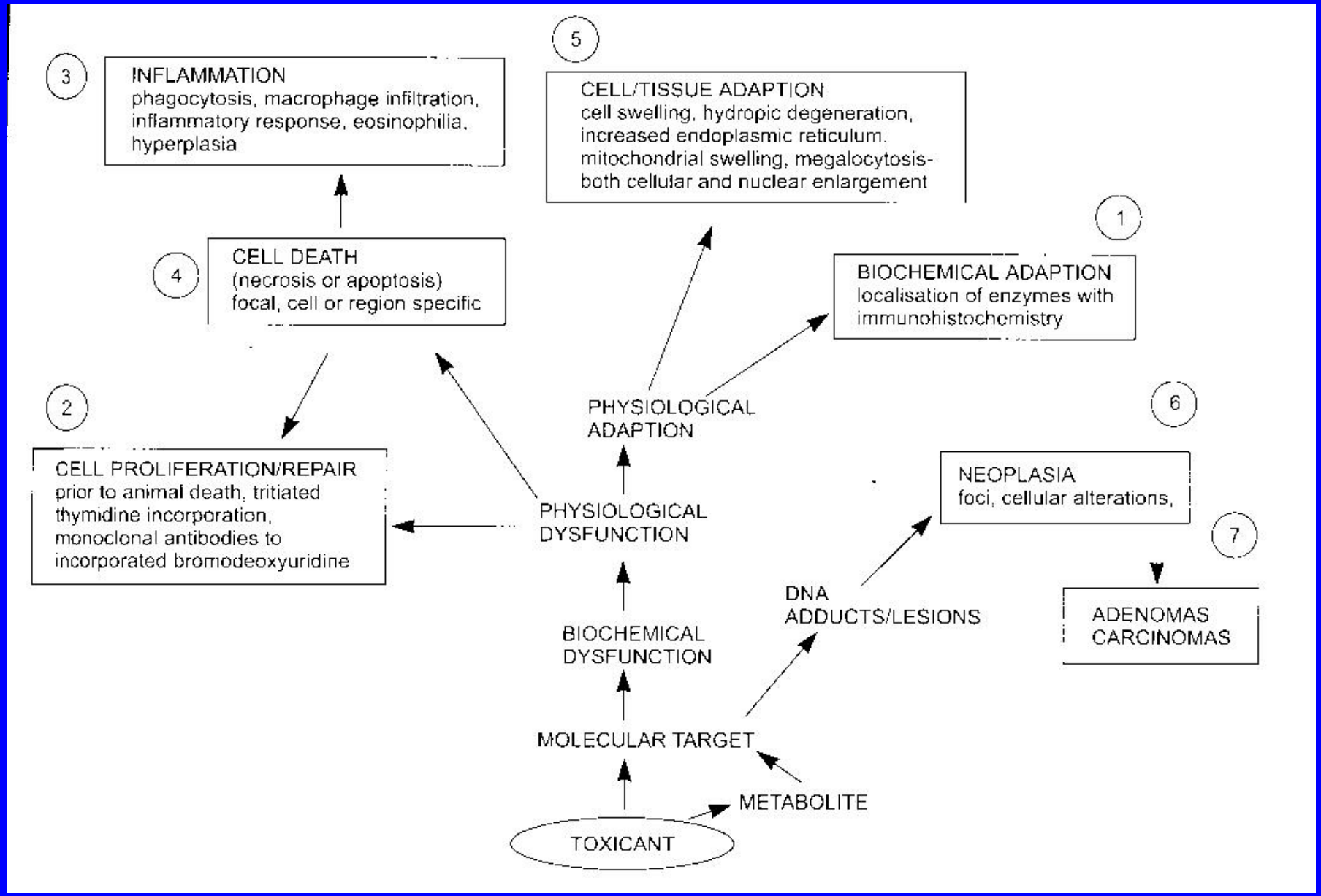
(4) GSH-NAPQI conjugate – *exposure, susceptibility*

(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 bile acids, bilirubin in plasma; start of inflamation in degraded tissue – *response / effect*

Human biomarkers – example



Human biomarkers – example

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*)	–

Further 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

What kind of biomarkers to measure ?

Do we know possible exposure (toxicant) ?

: specific biomarkers

? estrogenic effects in effluents

? dioxin-like effects, mutagenicity in urban areas

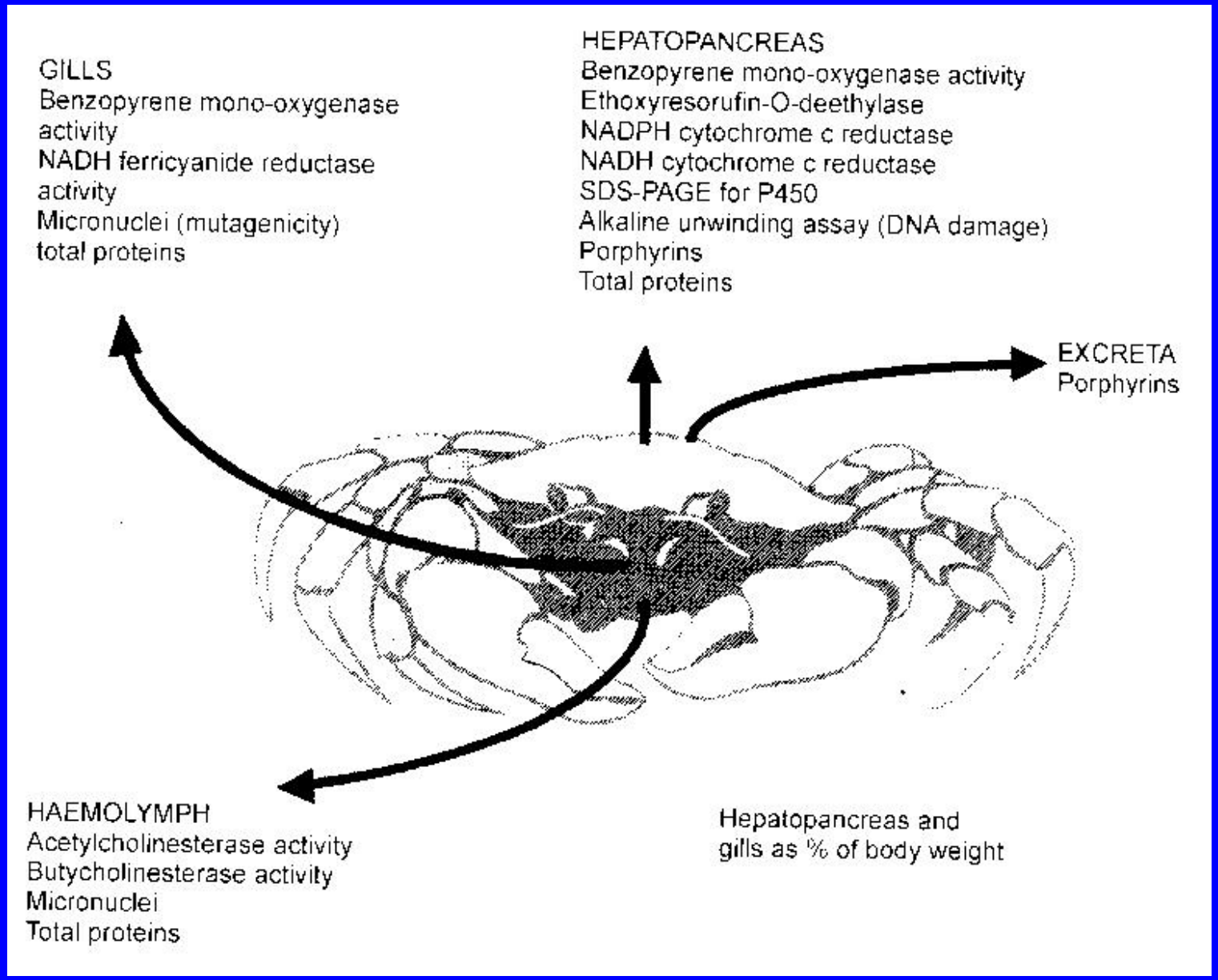
? neurotoxicity (AcChE) in rural areas

Do we expect complex exposures/contamination ?

- integrated approach needed

- nonspecific biomarkers (*hsp*) …

Multiple biomarker evaluation



Biomarkers of susceptibility

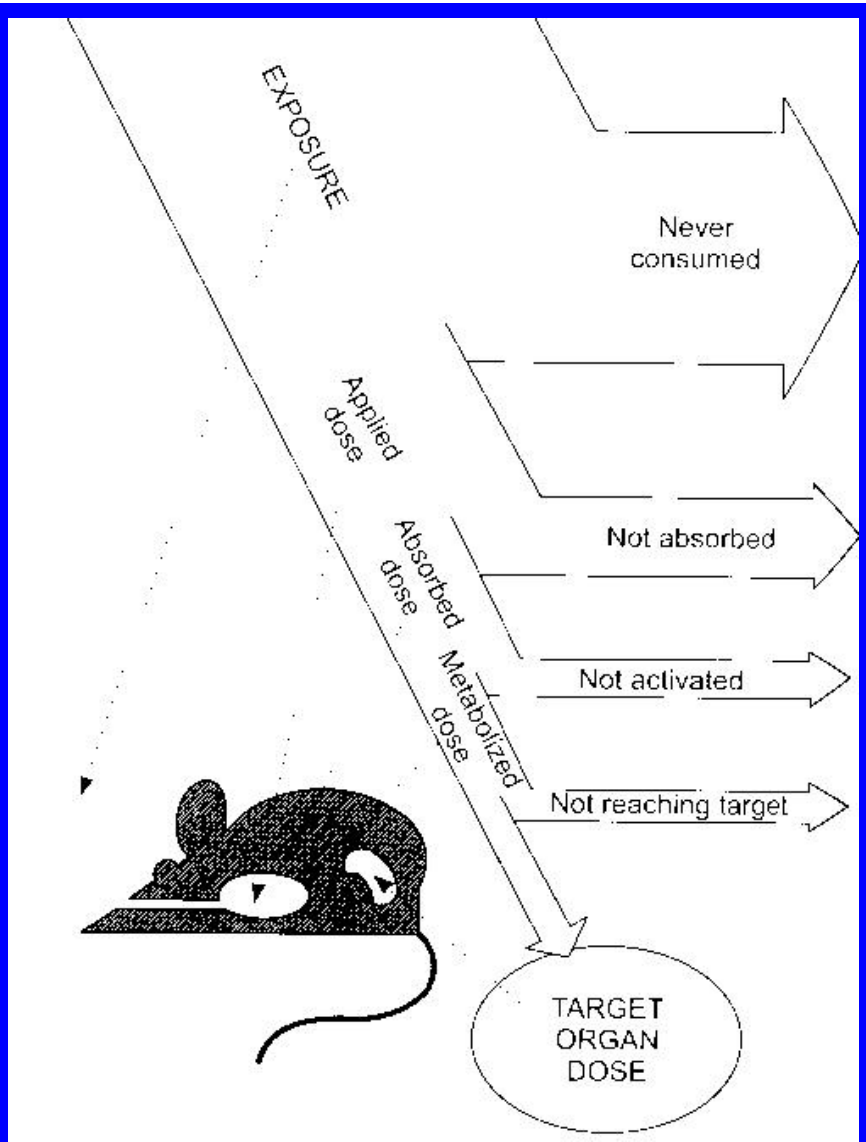


Figure 2 Representation of the relationships between ambient exposure and critical target dose and the progressive decrease in effective exposure due to various biological barriers. Source: *Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives*, p. 188. Used with permission. © 1995 International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

Toxicokinetics

& Biomarkers of susceptibility

Biomarkers of susceptibility

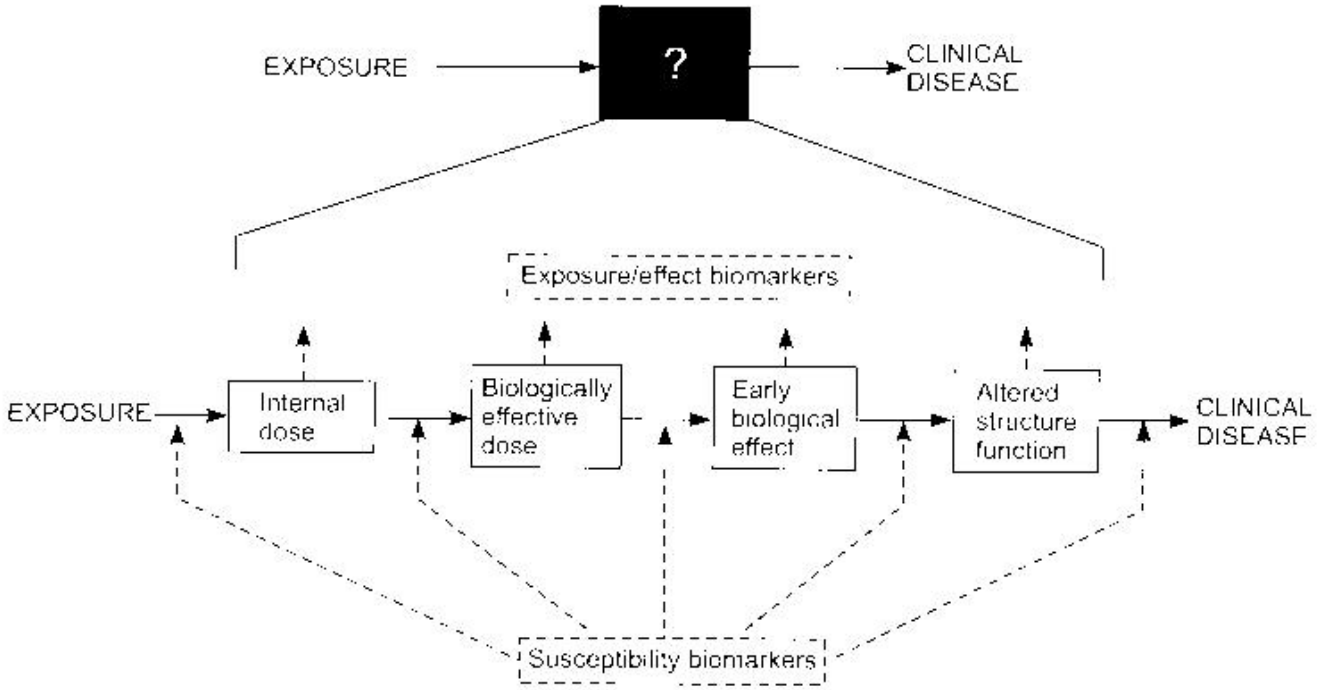


Figure 1 The biomarker paradigm linking exposure with disease and showing expansion of the classical epidemiological 'black box' to reveal discrete mechanistic stages. Reprinted with permission from *Environ. Sci. Technol.* (1997) **31**, pp. 1837–1848. Copyright 1997 American Chemical Society.

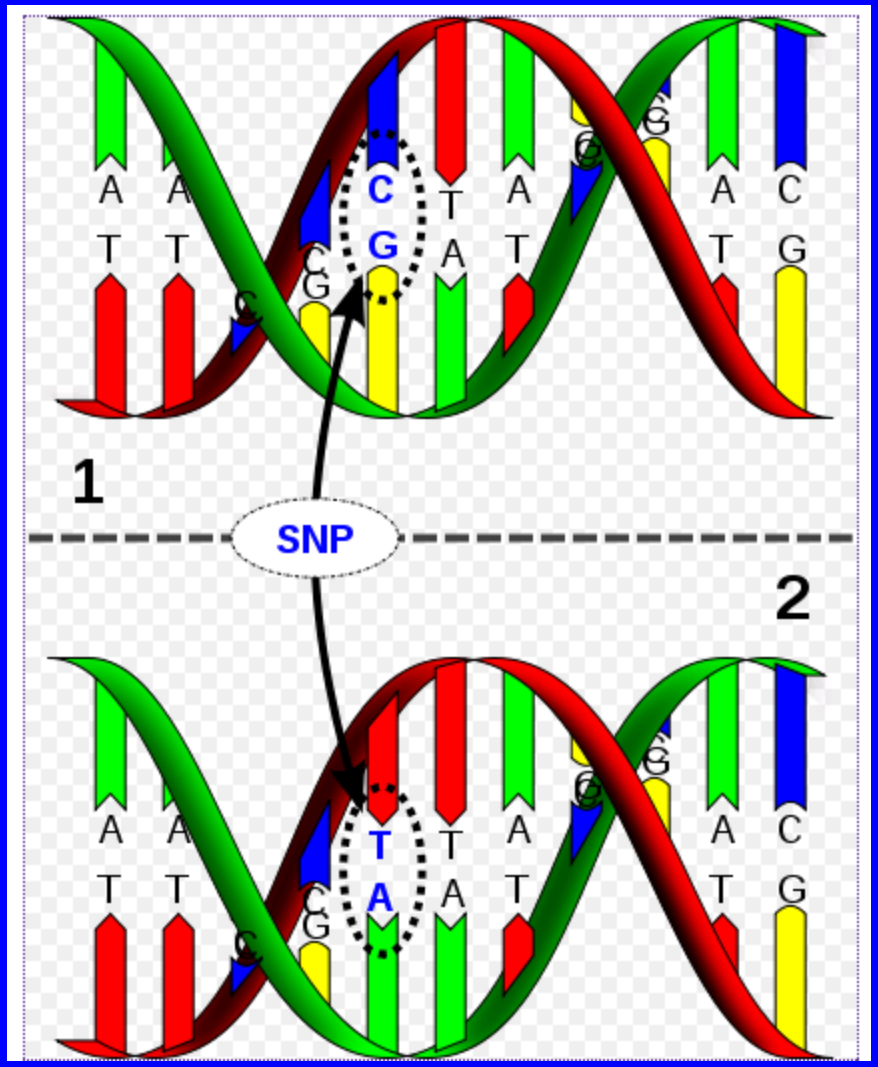
Biomarkers of susceptibility

Metabolism and genotype

- genetic polymorphism in detoxification enzymes
- variability in specific isoenzymes
- susceptibility to „activate“ toxicants:
  example: N-acetylation of arylamines – NAT2

- familial cancers
- susceptibility to genotoxins
- susceptibility to drugs (including anticancer drugs)

Example: genetic polymorphism
SNPs - single nucleotide polymorphism

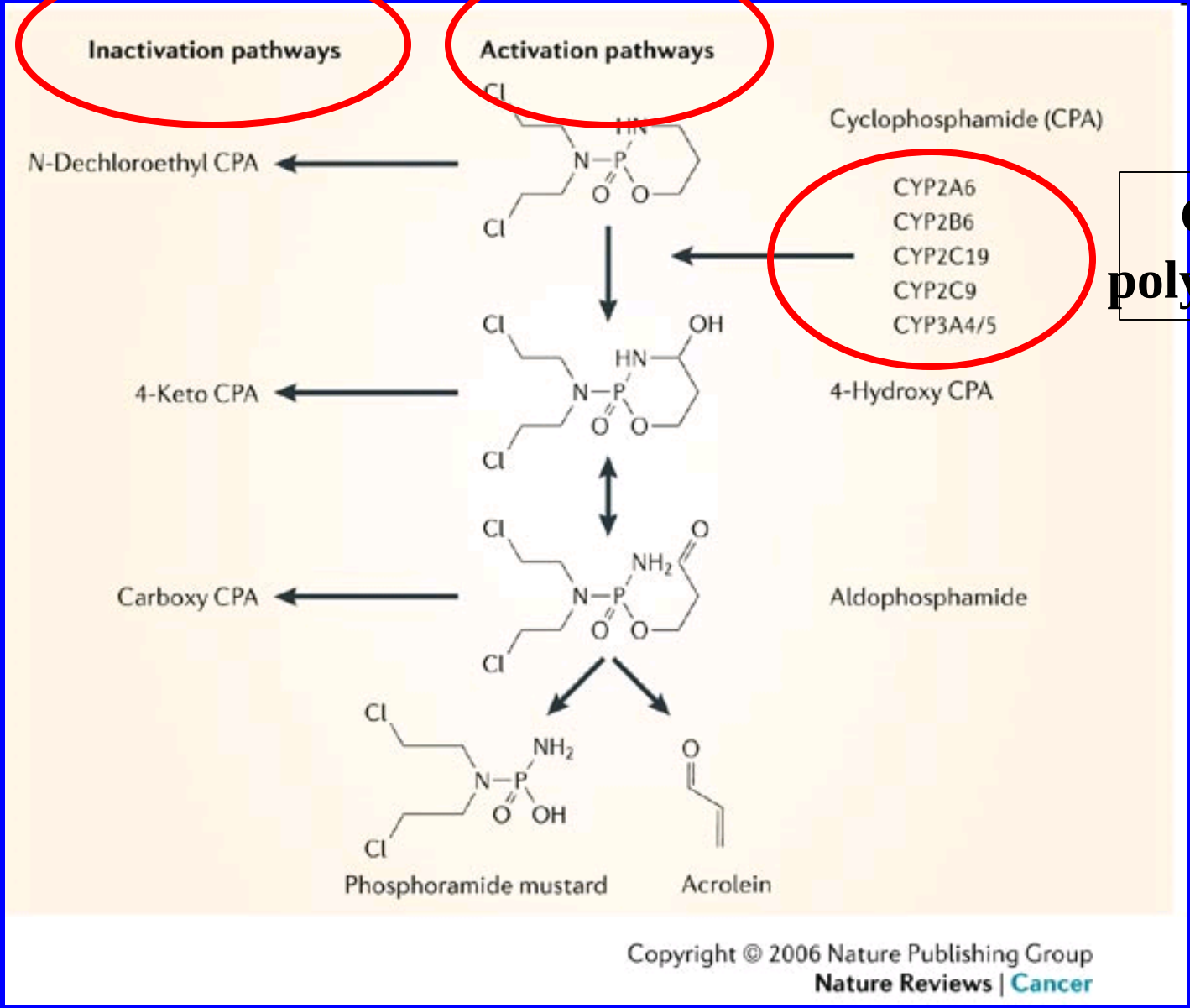


SNP -> affects protein functions

Many genotypes (from many individuals) must be sequenced to identify SNPs

(Some) SNPs identified for some (few) genes

Example: cyclophosphamide 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

Alleles known to be involved in polymorphism

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

Biomarkers of EXPOSURE

Biomarkers of Exposure

Biomarkers of … *internal / effective dose*

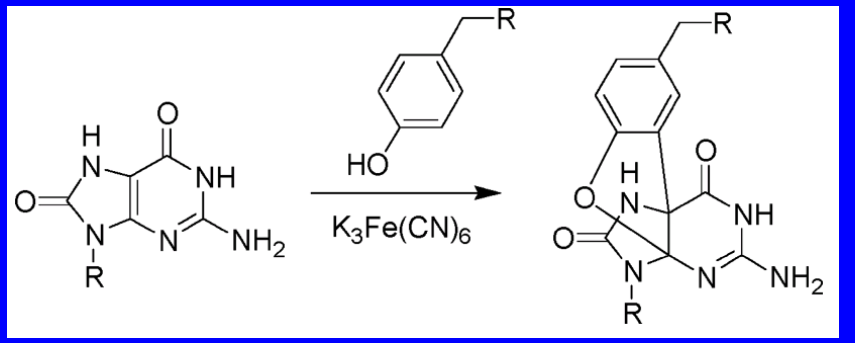
depending on toxicokinetics

- **internal dose** (short / long term)
  - *– Cd in urine, DDE in fat tissues*
  - should be easy to sample (urine, breath)
  - instrumental analytical methods (analyses of toxicant)

- **effective dose**
  - the chemical interacted with the biological target
    = ADDUCTS

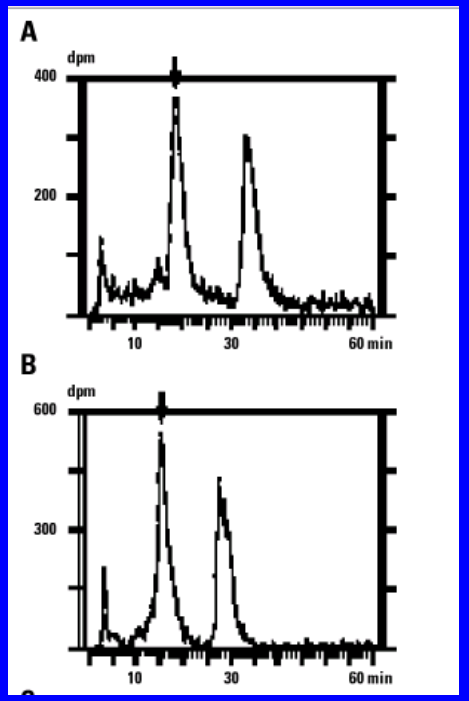
TOXICANT ADDUCTS with BIOMOLECULES

1) Selective adducts
(chemical-specific)



- *DNA aducts:*
  styrene-oxide-O6-guanine;
  N7-guanyl-aflatoxin B1;

- *hemoglobin-pesticides*



- extraction
and chemical determination (HPLC, GC)

2) Non-selective adducts

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)	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)

TOXICANT ADDUCTS with BIOMOLECULES

2) Non-selective aducts

– binding with DNA (*proteins*) but no further information on the structure of aduct (*causative agent*)

- Analysis:

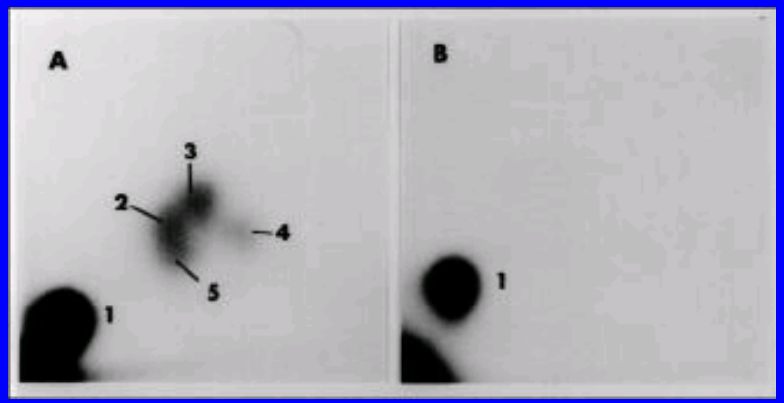
- *32P-postlabelling assay*
- *DNA-strand breaks*
  - *comet assay*
- *identification of oxy-DNA*
  - *8-hydroxy-2´-deoxyguanosine*

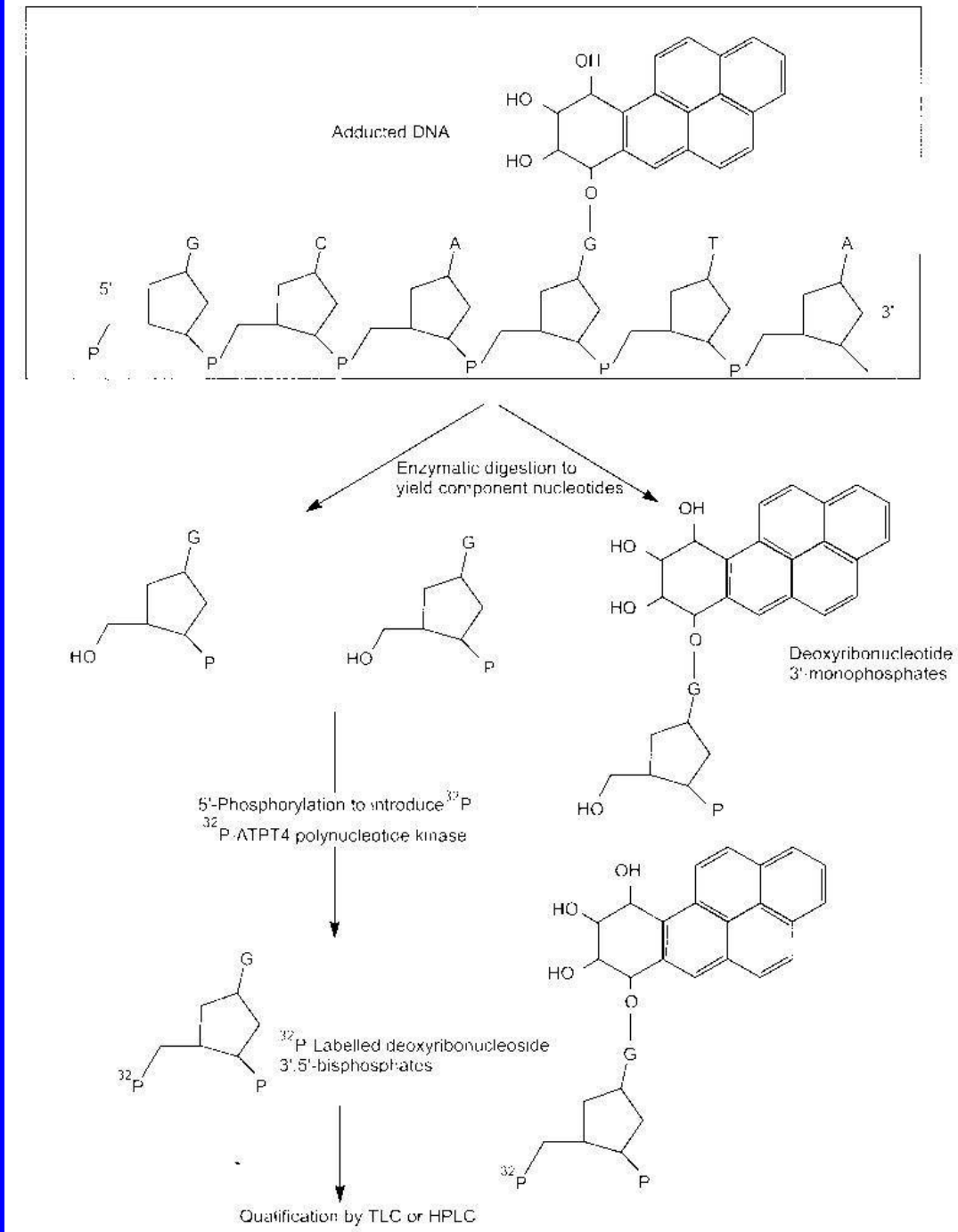
32P-postlabelling assay

TLC result

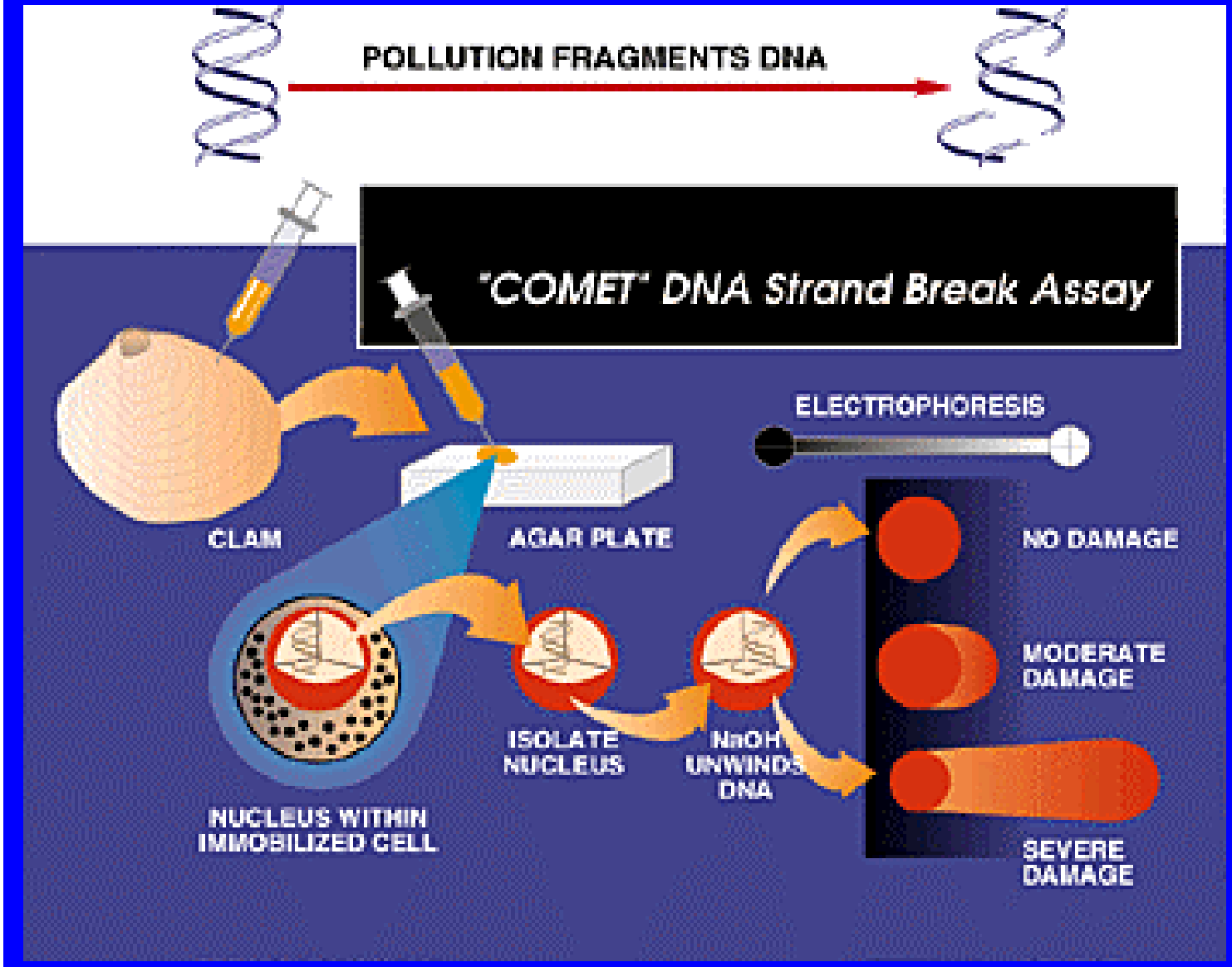
A - 2-5 = various adducts

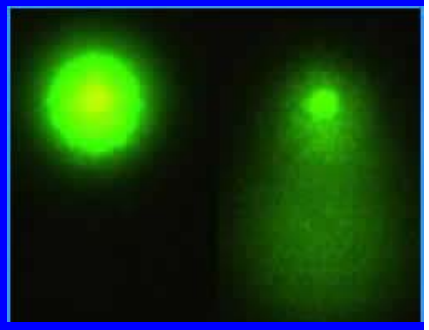
B - controls

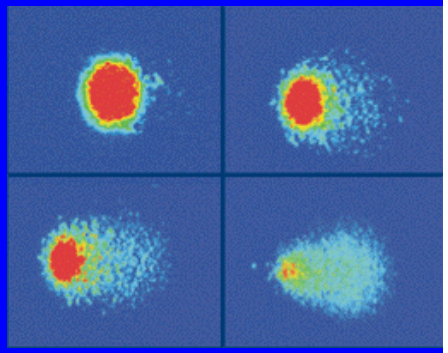




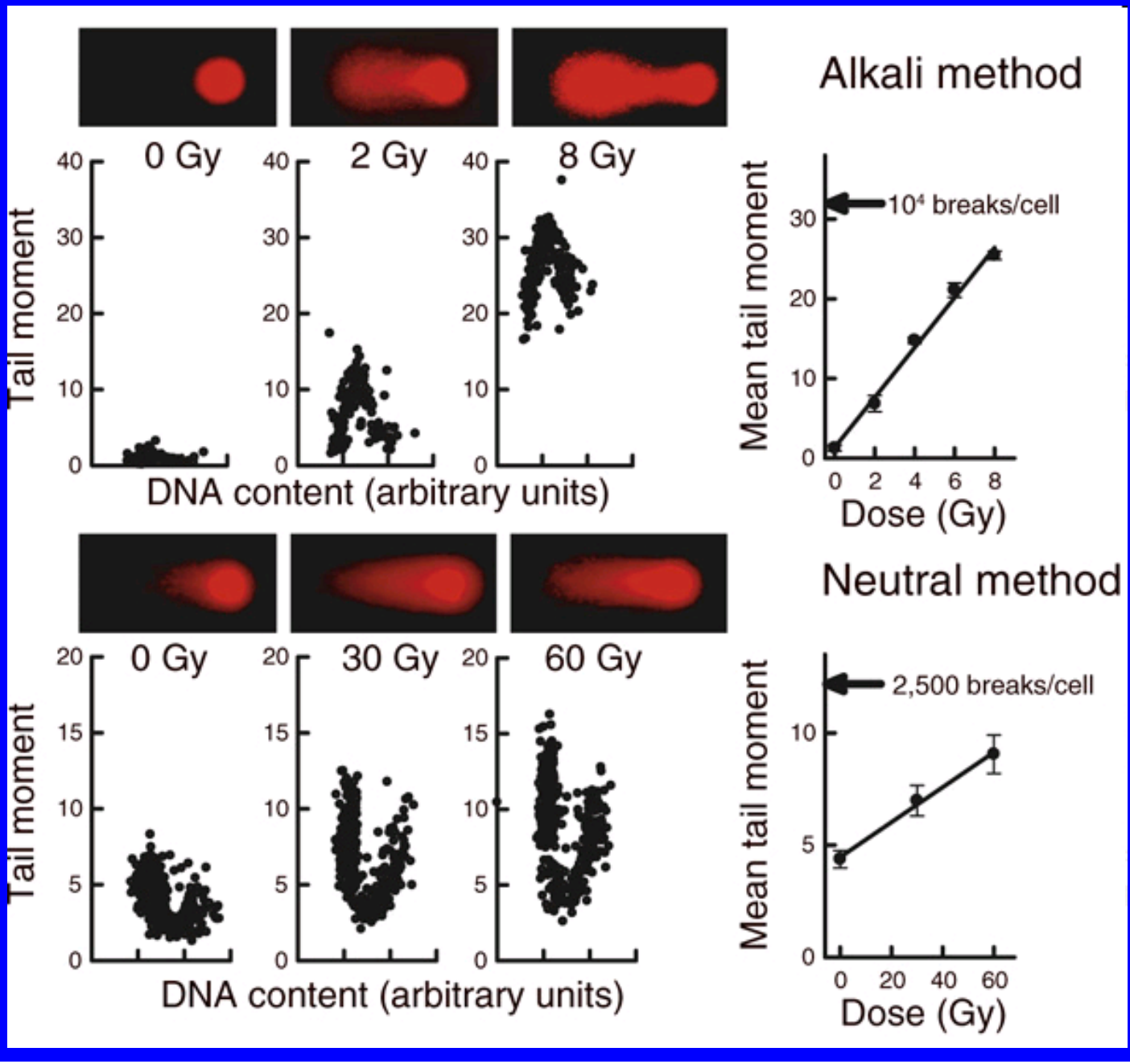
Comet assay


POLLUTION FRAGMENTS DNA
"COMET" DNA Strand Break Assay
ELECTROPHORESIS
CLAM
AGAR PLATE
NO DAMAGE
MODERATE DAMAGE
ISOLATE NUCLEUS
NaOH UNWINDS DNA
NUCLEUS WITHIN IMMOBILIZED CELL
SEVERE DAMAGE





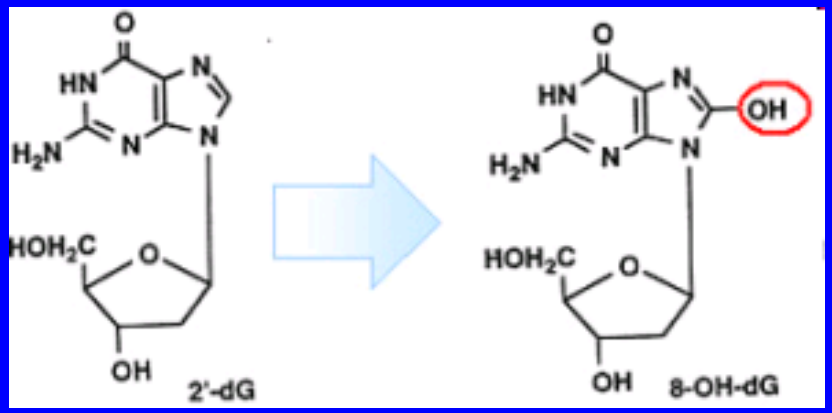
Example results - Comet assay vs. radiation



8-hydroxy-2´-deoxyguanosine analysis

Oxidative damage to DNA

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



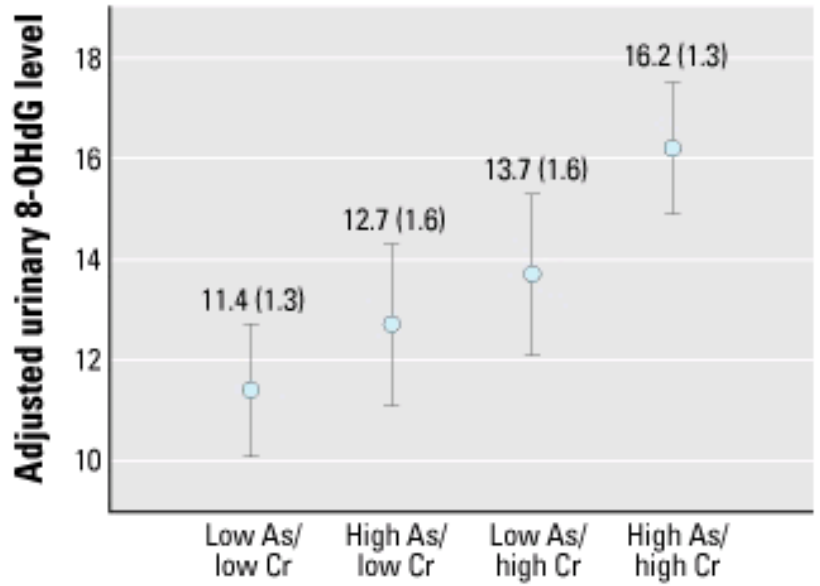
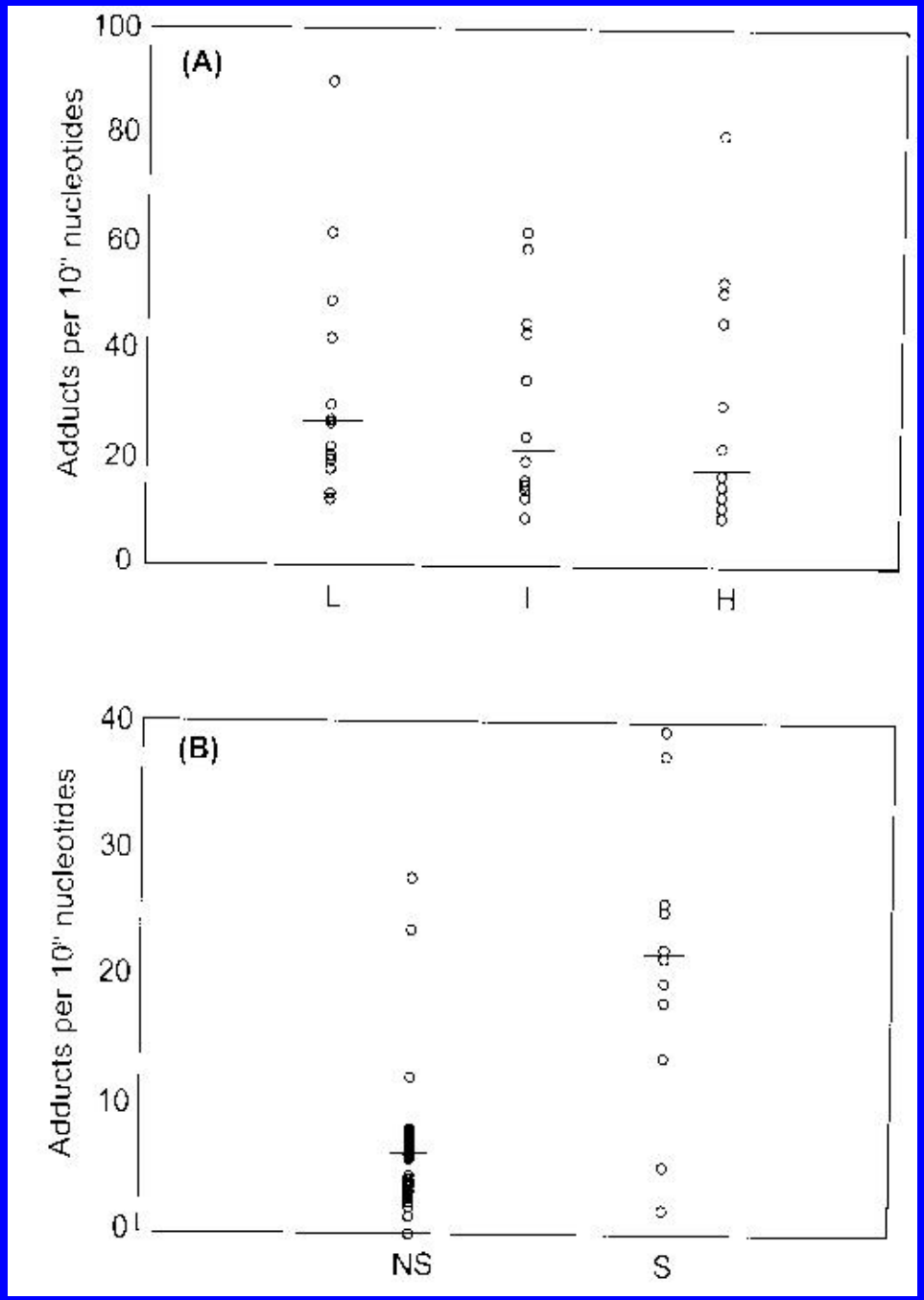


Figure 1. Adjusted urinary 8-OHdG level (ng/mg creatinine) by urinary arsenic and urinary chromium concentrations. Values shown are mean ± SE. Cut points were determined according to medians (arsenic, 7.7 µg/g creatinine; chromium, 2.0 µg/g creatinine) of urinary creatinine-adjusted levels among all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

Analysis:

- HPLC
- immunochemistry (ELISA)

PAH-DNA adducts



Occup. exposure
(Low / Intermed. / High)

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