The late effects of xenobiotics

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The outline of the lecture

1. Definition and characterization of xenobiotics

2. Comparison of toxic and late effects of xenobiotics

3. Late effects of xenobiotics

4. Late effect xenobiotics in the environment

5. Biological monitoring of exposure to xenobiotics
   - methods
   - occupational vs. non-occupational exposure

Xenobiotic – a compound found in the organism which is not produced or expected to be present in it

Xenobiotics
- harmless
- dangerous to human organism

MORE THAN 20,000 CHEMICAL COMPOUNDS ARE CLASSIFIED AS DANGEROUS TO HUMAN ORGANISM

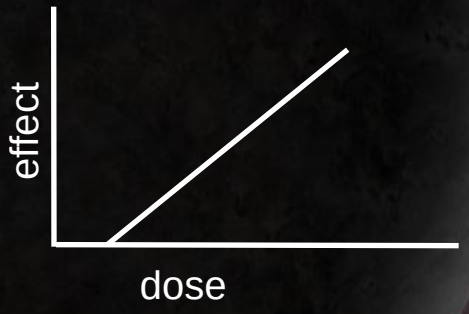
Effects of xenobiotics

TOXIC EFFECTS

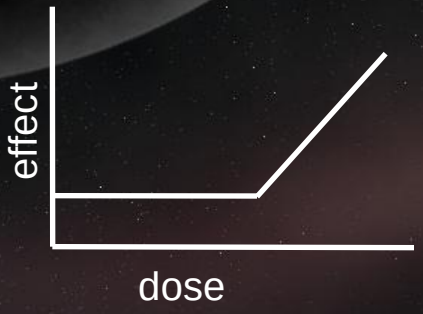
LATE EFFECTS
- congenital deffects
- cancer
- allergies

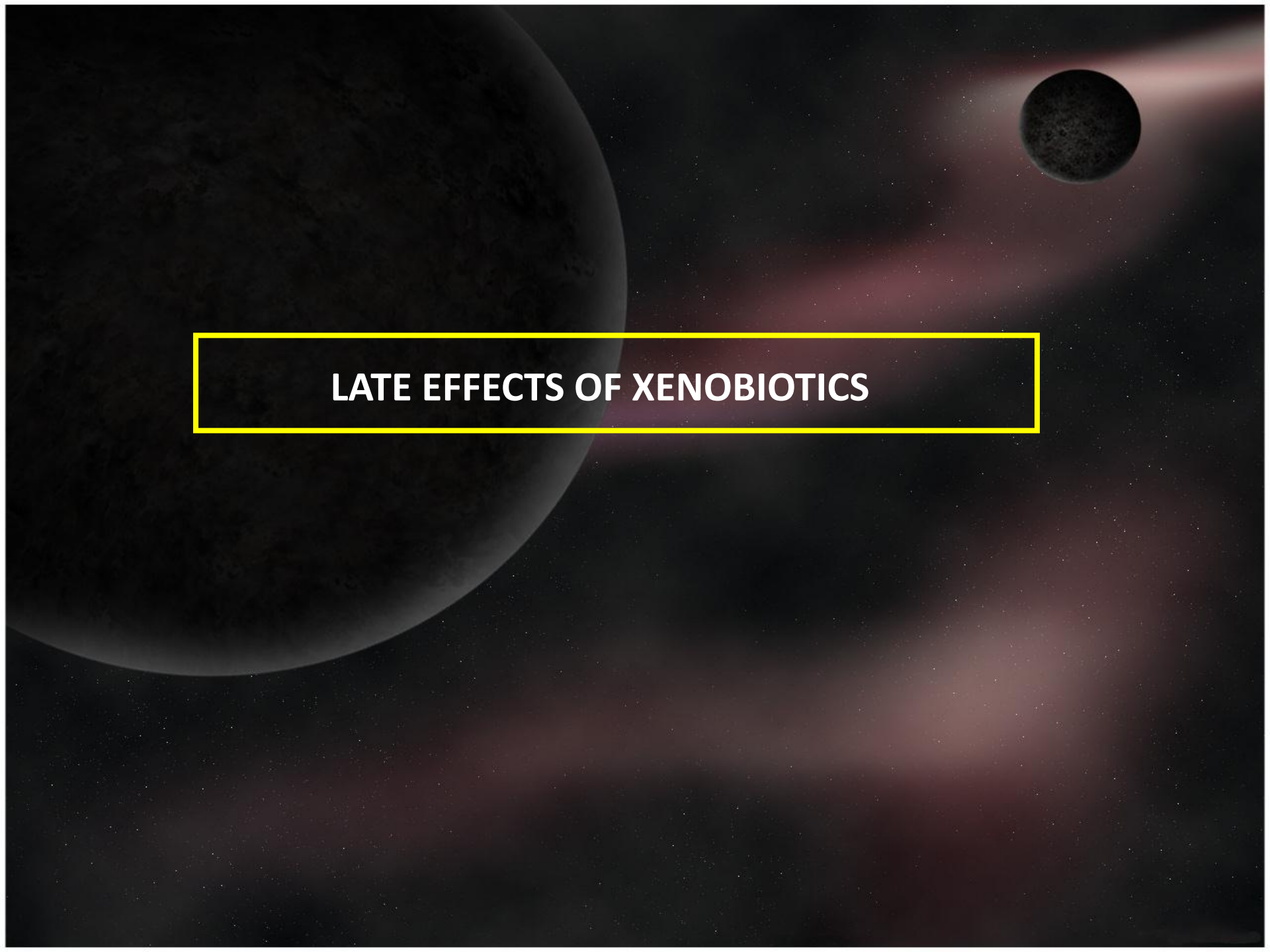
TOXIC VS. LATE EFFECTS

Toxic effects – direct dose-effect association



Late effects – long latency period, usually no threshold




LATE EFFECTS OF XENOBIOTICS

LATE EFFECTS OF XENOBIOTICS

1. mutagenic (genotoxic)

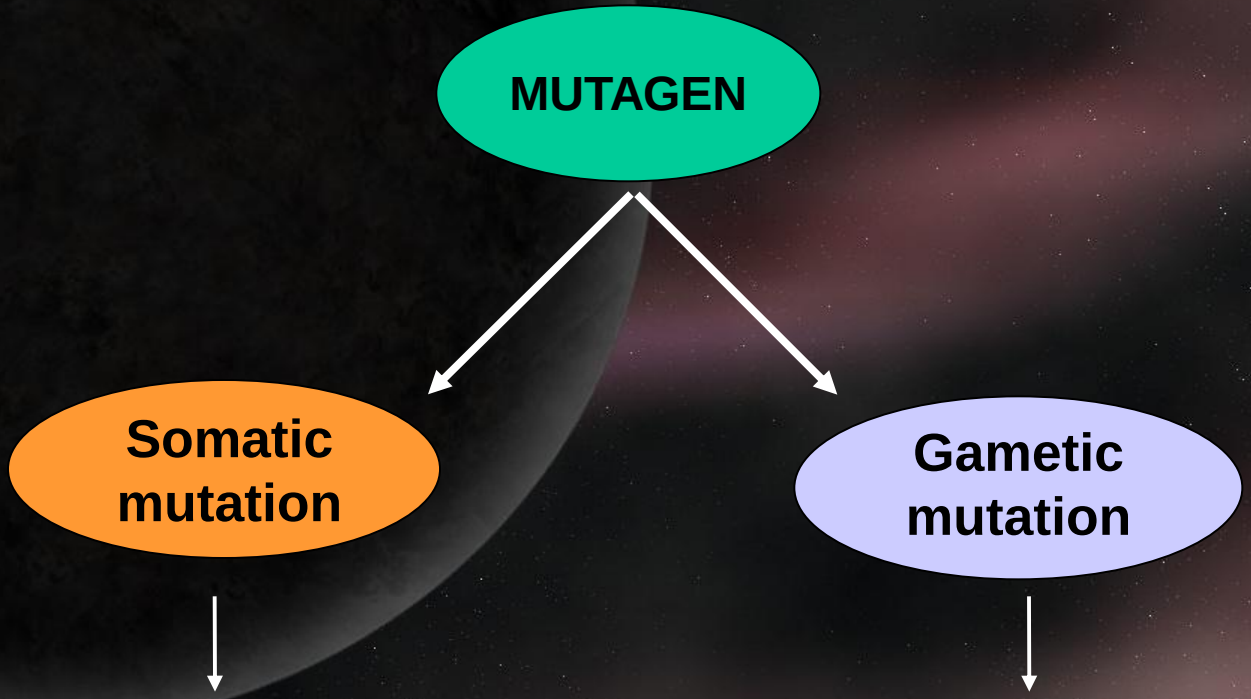
2. carcinogenic

3. teratogenic

4. allergenic

1. MUTAGENIC EFFECTS OF XENOBIOTICS

Mutation – a permanent hereditary change of genetic material of a cell caused by a MUTAGEN



Teratogenic processes

Risk of development of cancer

Aging of cells

Sterility

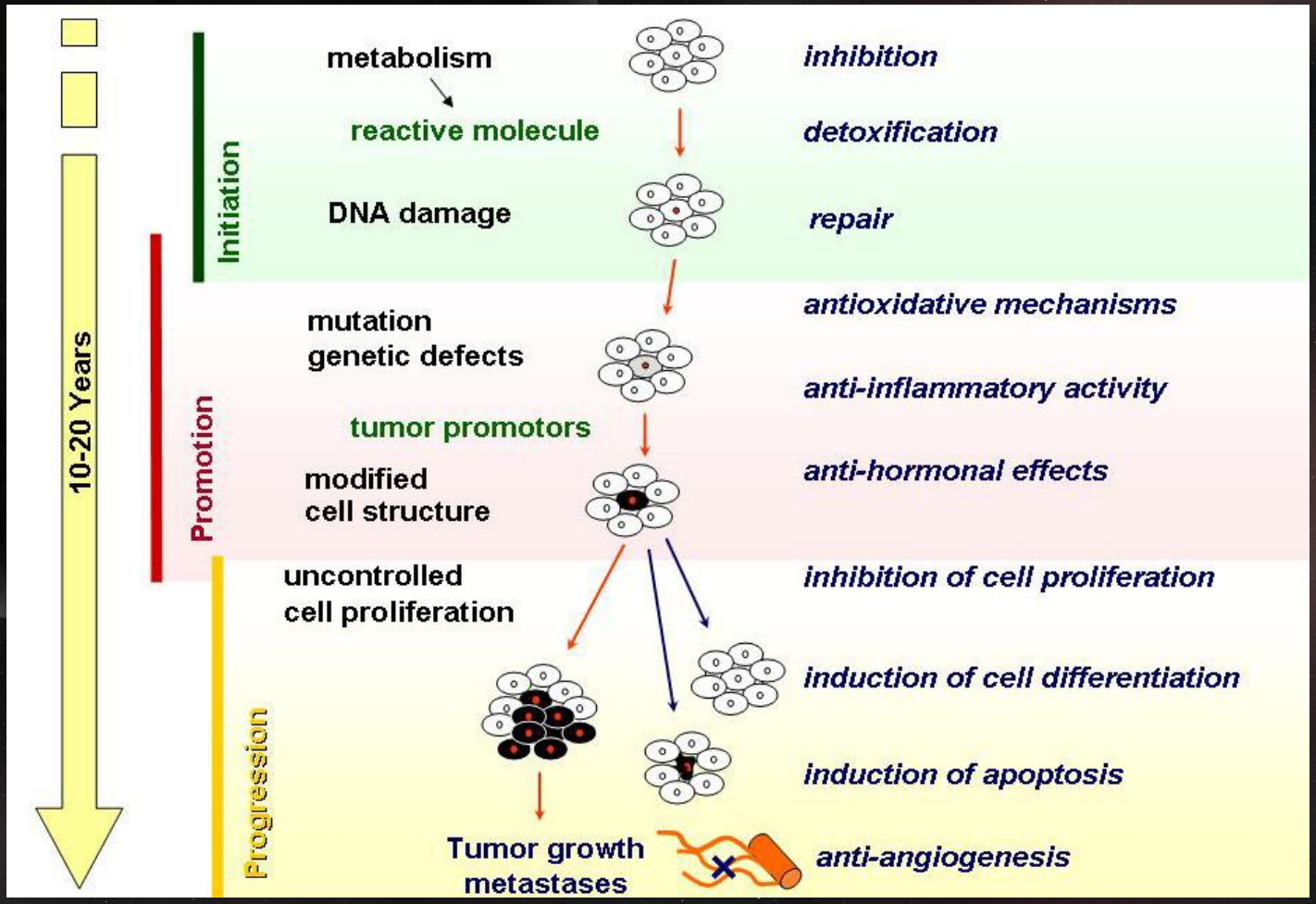
Spontaneous abortions

Congenital defects

Predisposition to cancer

2. CARCINOGENIC EFFECTS OF XENOBIOTICS

Carcinogenesis – a multistep process with a long latency between changes to DNA and the disease (cancer)



Klimo et al., DKFZ, 2010

2. CARCINOGENIC EFFECTS OF XENOBIOTICS

initiation and promotion – enzymatic changes, alteration of diferentiation, increased proliferation

proliferation – changes of cell surface and cytoskeleton, increased invasiveness, induction of chromosomal aberrations

IMPORTANCE OF THE IMMUNE SYSTEM IN CARCINOGENESIS

elimination of cells carrying mutations

MECHANISMS OF EFFECT OF GENOTOXIC XENOBIOTICS

Genotoxic compounds

direct-acting

do not require metabolic activation prior to interaction with macromolecules (alkylating agents)

indirect-acting

require metabolic activation (mostly the CYP P450 system) and formation of electrophiles that bind to nucleic acids and cause mutations (polycyclic aromatic hydrocarbons, nitrosamines, mycotoxins)

epigenetic

do not bind to DNA, but transform cells indirectly via enzymatic and hormonal imbalance, inhibition of immune functions and repair systems (estrogens)

mechanism of action – modification of gene expression (methylation), do not cause changes to the structure of DNA, epigenetic changes are reversible

MECHANISMS OF EFFECT OF GENOTOXIC XENOBIOTICS

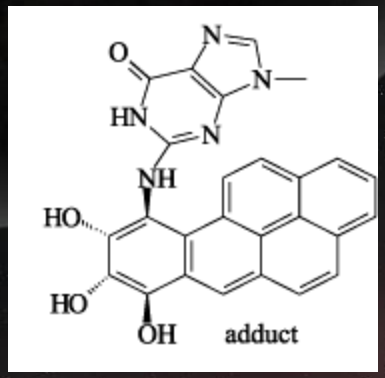
DNA-damaging agents:

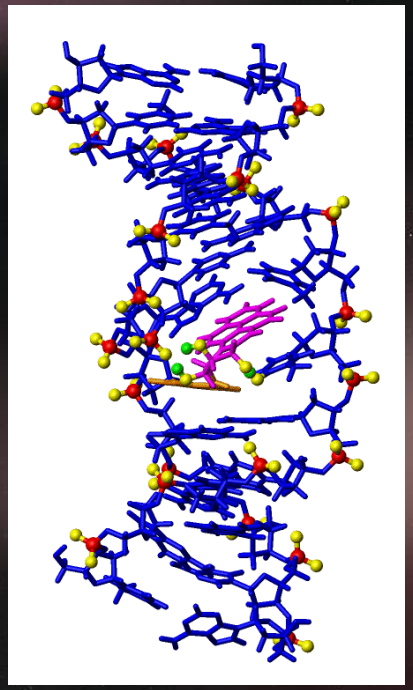
electrophilic compounds bind to macromolecules

↓

DNA adducts

guanine





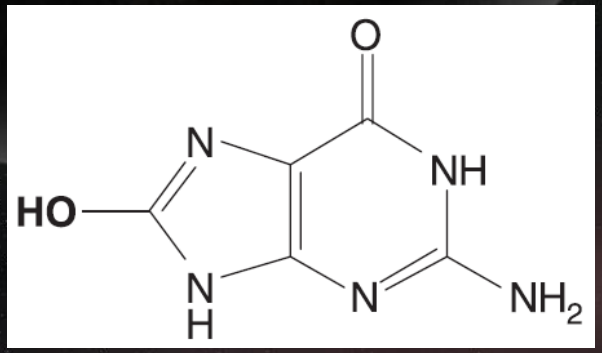
MECHANISMS OF EFFECT OF GENOTOXIC XENOBIOTICS

DNA-damaging agents:

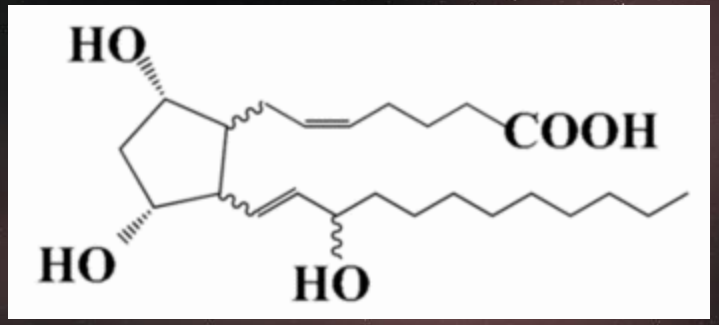
reactive oxygen species (ROS)

↓

oxidative damage to DNA, lipids, proteins



8-hydroxyguanine



15-F2t-Isoprostane

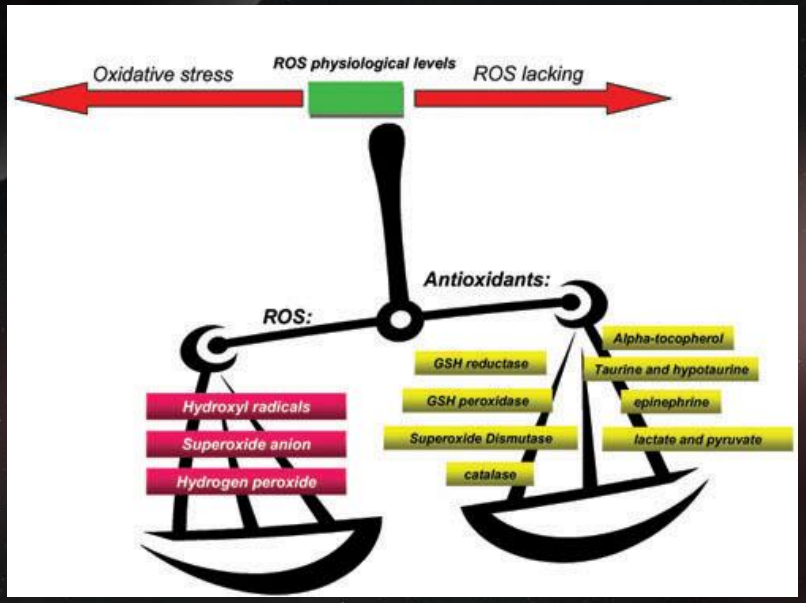
MECHANISMS OF EFFECT OF GENOTOXIC XENOBIOTICS

reactive oxygen species (ROS)

sources: endogenous (metabolism, inflammation)
exogenous (diet, lifestyle, environmental pollution)

oxidants: hydroxyl radical, superoxide, hydrogen peroxide
antioxidants: superoxide dismutase, catalase, glutathione peroxidase, vitamins, glutathione

OXIDATIVE STRESS = imbalance between the levels of oxidants and antioxidants



MECHANISMS OF EFFECT OF GENOTOXIC XENOBIOTICS

Oxidative stress affects all biomolecules: DNA, lipids and proteins

DNA: bases, sugars, sugar-phosphate backbones of DNA
single stranded breaks, mutations

lipids: peroxidation of lipids in cellular membranes (arachidonic acid)
fluidity of membranes, transport, propagation of oxidative stress

proteins: side chains of amino acids
fragmentation of proteins, cross-linking, enzymatic and structural function

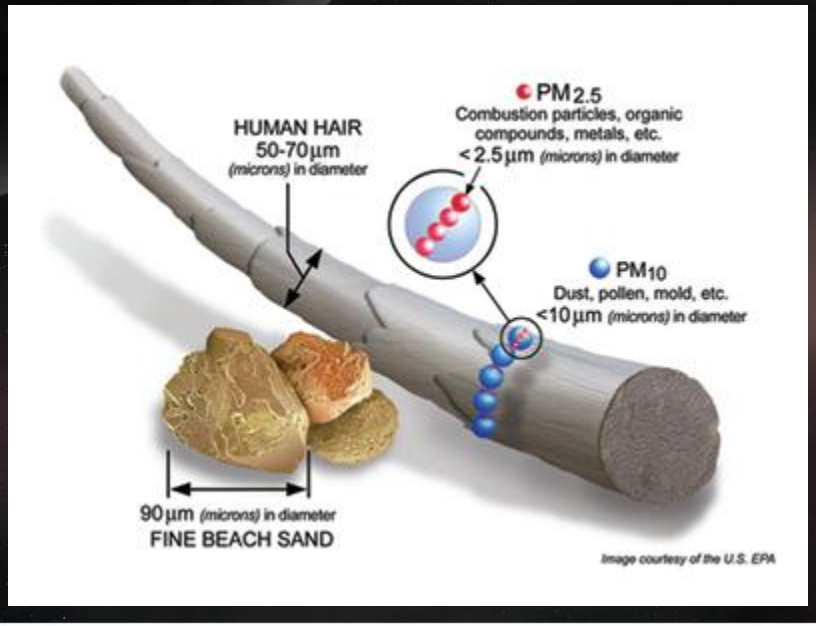
MECHANISMS OF EFFECT OF GENOTOXIC XENOBIOTICS

Oxidative stress mediates the effect of air pollution on the organism

Particulate matter (PM)

< 10 µm (PM10)	affect upper and lower respiratory tract
< 2.5 µm (PM2.5)	penetrate to alveoli
< 100 nm (PM0.1)	nanoparticles; enter cells and may damage macromolecules

Chemical compounds are bound to particulate matter



INDIVIDUAL SUSCEPTIBILITY

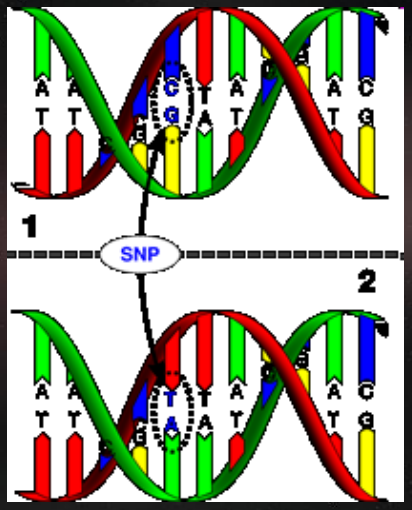
Mutations vs. single nucleotide polymorphism (SNP) - a SNP has a frequency of a minor allele ≥ 1%, mutation < 1%

SNPs are mostly located in non-coding regions of the genome

SNPs are the source of the genetic variability in the population

The presence of SNPs may result in amino acid changes in the protein, SNPs may affect splicing or regulation of gene expression

Genetic polymorphisms in genes encoding enzymes metabolizing carcinogens, in DNA repair genes and genes controlling cell cycle



3. TERATOGENIC EFFECTS OF XENOBIOTICS

Teratogenicity the ability of an exogenous factor to induce congenital malformations by affecting embryonic development

Critical period for induction of malformations – first eight weeks of pregnancy

Teratogenic changes are not hereditary

Carcinogenic ≠ teratogenic

Examples: nitrosamines, aflatoxin, chlorinated hydrocarbons
Toxoplasma gondii, Treponema pallidum
ionizing radiation

4. ALLERGENIC EFFECTS OF XENOBIOTICS

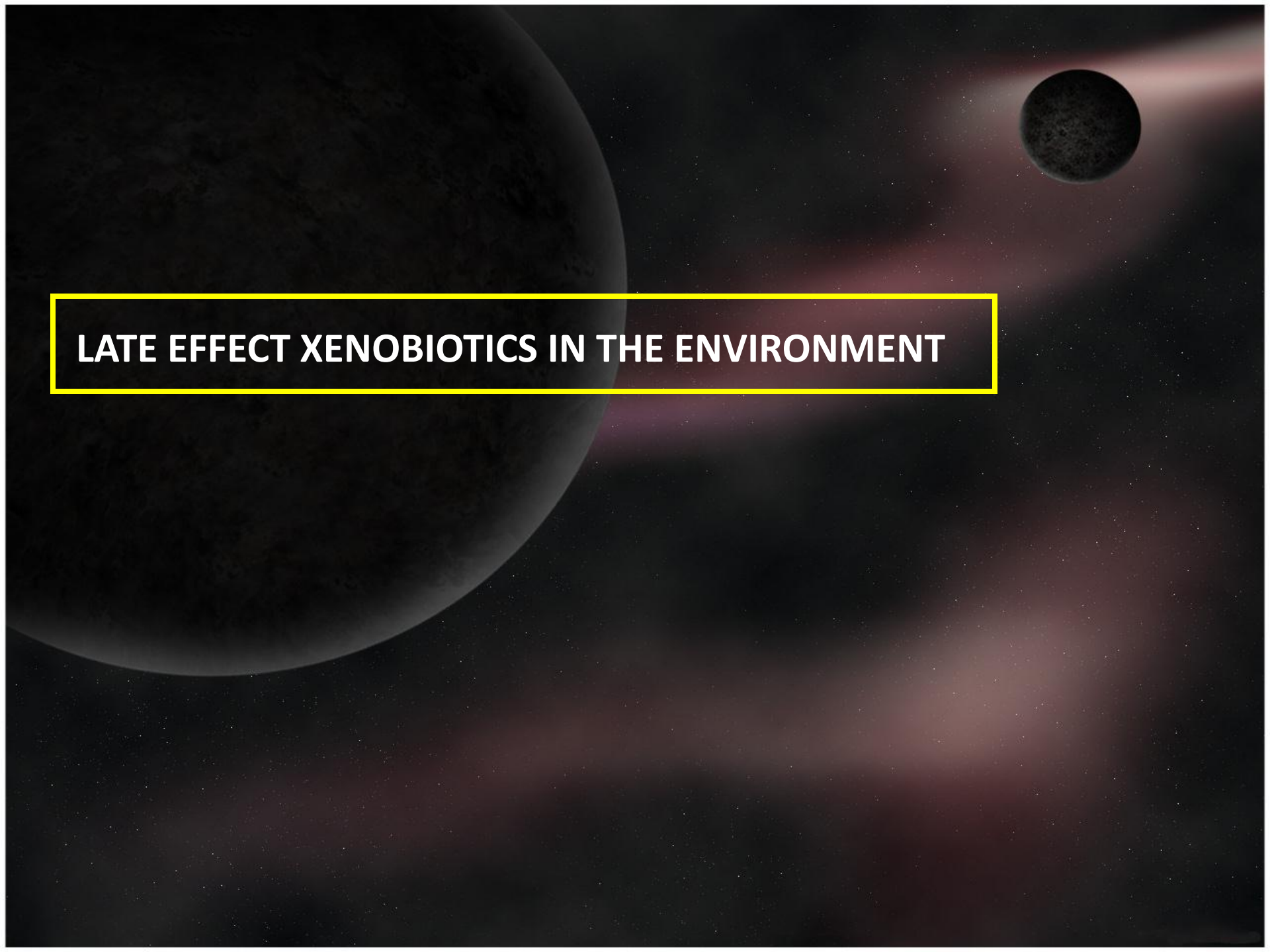
Allergy — excessive reaction of the immune system to antigen

Prevalence — in children up to 30%

Alergen — any compound acting as antigen, usually proteins, lipids, polysacharides, or haptens

Types of allergy

- small children – food allergies
- older childern and adults – respiratory tract allergies (pollen, dust…)
- contact allergies – textile, wool, fur, yeasts, molds…


LATE EFFECT XENOBIOTICS IN THE ENVIRONMENT

LATE EFFECT XENOBIOTICS IN THE ENVIRONMENT

Xenobiotics are ubiquitous – water, food, air, soil…

Food, fresh water

xenobiotics of natural origin – flavonoids, mycotoxins (aflatoxin B1)

artificial compounds – nitrates, nitrites, nitrosamins (meat, smoked fish, sausages, cheese)

polycyclic aromatic hydrocarbons (PAHs), PCB, chlorinated hydrocarbons

mercury, arsenic, chromium, cadmium, nickel, lead

chlorination of fresh water

LATE EFFECT XENOBIOTICS IN THE ENVIRONMENT

Ambient air

- industrial and agricultural activity – metals and their compounds, organic chemicals
- industry, traffic, burning of fossil fuels – particulate matter and compounds bound to it

Household

- furniture, carpets, construction material – formaldehyde, styrene, acrylates, phtalates, vinyl chloride
- pesticides – gardening
- cosmetics

LATE EFFECT XENOBIOTICS IN THE ENVIRONMENT

Occupational environment

Czech Republic – 1.5 mil. workers occupationally exposed to mutagens and/or carcinogens

Xenobiotics

carcinogenic for humans (benzidine, vinyl chloride, benzene) (IARC Group 1)

probably carcinogenic (acrylonitrile, PCB, tetrachloromethane) (IARC Group 2A)

Classification according to IARC

International Agency for Research on Cancer
World Health Organization

Group 1: The agent is *carcinogenic to humans* (1,3-butadiene, benzene, benzo[a]pyrene, ethylene oxide, formaldehyde, vinyl chloride)

Group 2A: The agent is *probably carcinogenic to humans* (acrylamide, androgenic steroids, cisplatin, N-methyl-N-nitrosourea, UV radiation)

Group 2B: The agent is *possibly carcinogenic to humans* (acrylonitrile, acetaldehyde, bleomycin, chloroform, lead)

Group 3: The agent is *not classifiable as to its carcinogenicity to humans*

Group 4: The agent is *probably not carcinogenic to humans*

http://monographs.iarc.fr/ENG/Preamble/index.php

Biological monitoring of exposure to xenobiotics

Biological monitoring of exposure to xenobiotics

Biological monitoring

methods used to detect genotoxic chemicals in water, soil, air, blood, urine, exhaled air

based on the ability of genotoxic chemicals to damage genetic material and induce mutations

Biomarker

a biological parameter reflecting the quality of the environment on the level of the organism

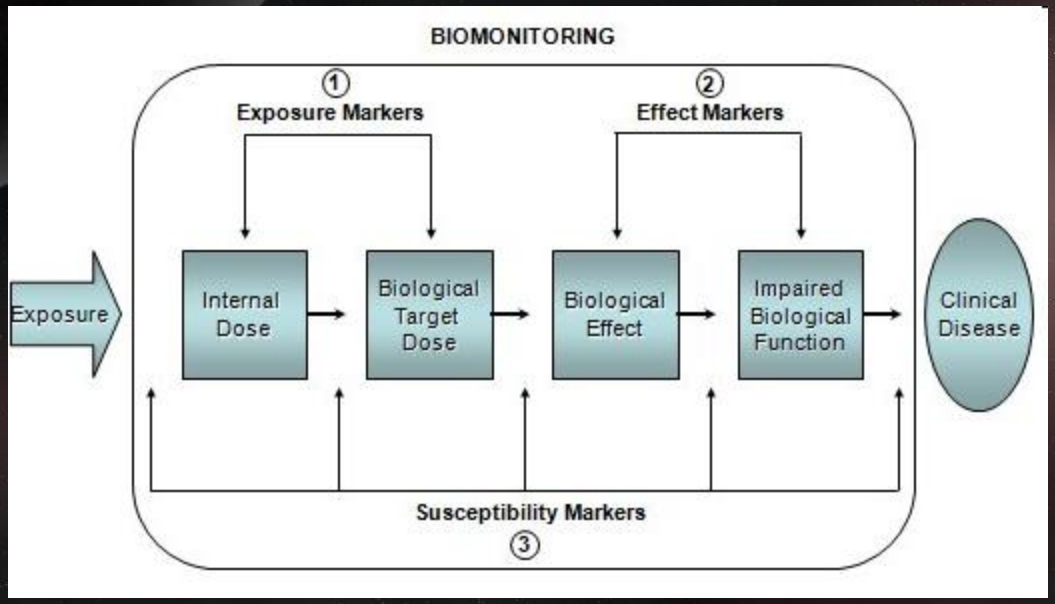
Biological monitoring of exposure to xenobiotics

Classification of biomarkers:

Biomarkers of exposure – contact of the organism with xenobiotics, estimation of exposure; example: DNA and protein adducts; measured in body fluids, cells, tissues

Biomarkers of effect – negative health effects after exposure to xenobiotics, hazard identification, dose-response assessment; example: cytogenetic analyses

Biomarkers of susceptibility – characteristics affecting the response of the organism after exposure to xenobiotics; example: genetic polymorphisms



Biological monitoring of exposure to xenobiotics – methods

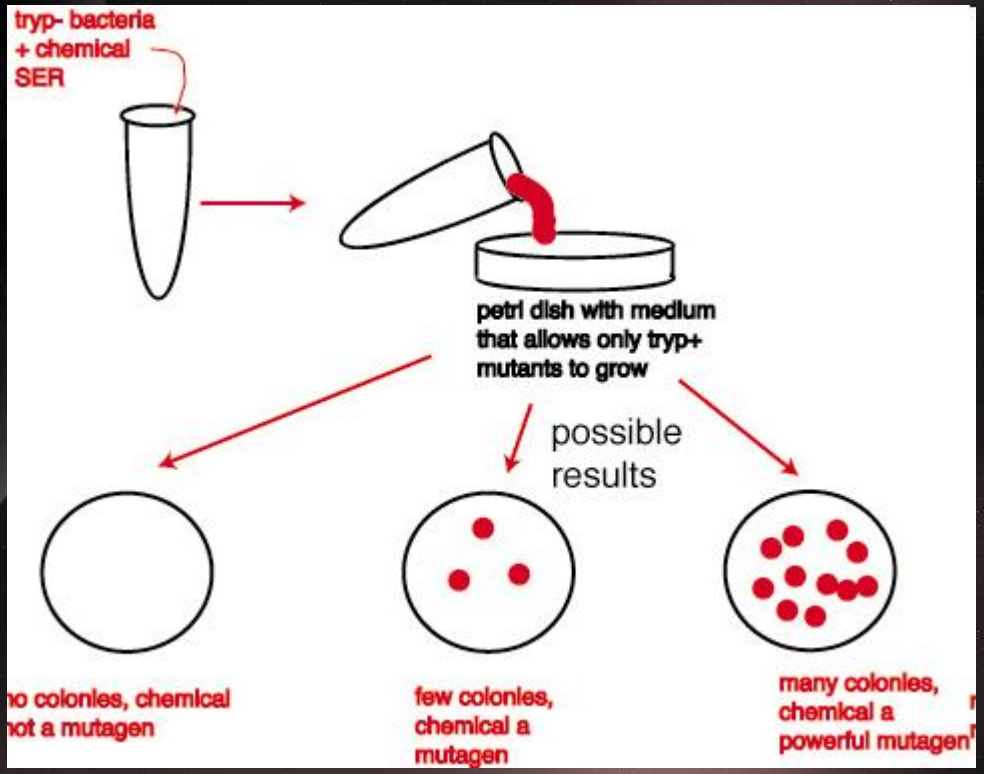
Ames test

Salmonella typhimurium

mutations in genes involved in histidine synthesis

test whether treated bacteria grow on histidine-free media

mutagenicity of the urine of exposed and control subjects



Biological monitoring of exposure to xenobiotics – methods

Cytogenetic analysis of peripheral blood lymphocytes

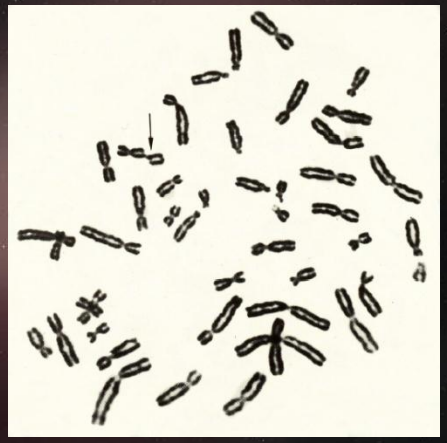
Conventional method

detection of unstable aberrations (chromosomal breaks)

relatively inexpensive

time-consuming (analysis of max. 200 metaphases)

suitable for professional, but not environmental exposures

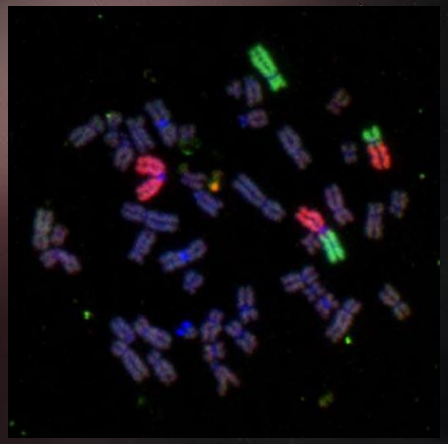


Biological monitoring of exposure to xenobiotics - methods

Cytogenetic analysis of peripheral blood lymphocytes

Fluorescence *in situ* hybridization (FISH)

- detection of both stable and unstable aberrations
- detection is limited to painted chromosomes
- expansive (fluorescent probes)
- more sensitive – suitable even for environmental exposures
- analysis can be automated – faster



Biological monitoring of exposure to xenobiotics - methods

Cytogenetic analysis of peripheral blood lymphocytes

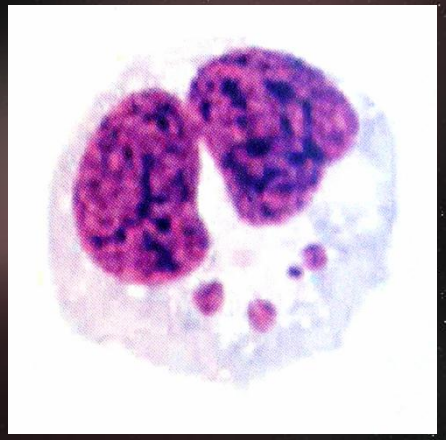
Analysis of micronuclei

detection of unstable aberrations

relatively inexpensive

faster than the conventional method

analysis can be automated



Biological monitoring of exposure to xenobiotics - methods

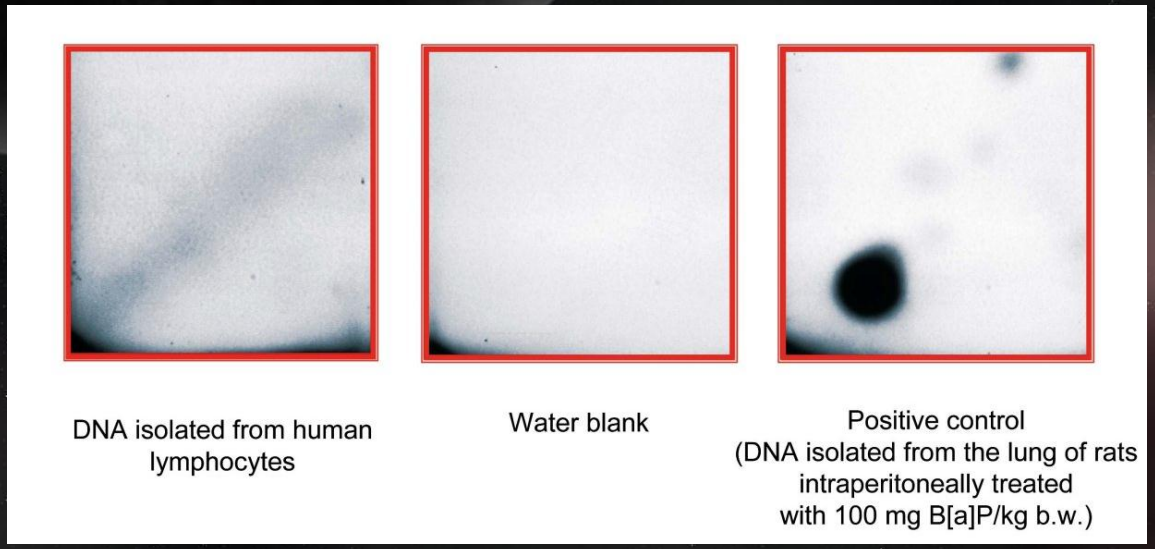
^{32}P postlabeling

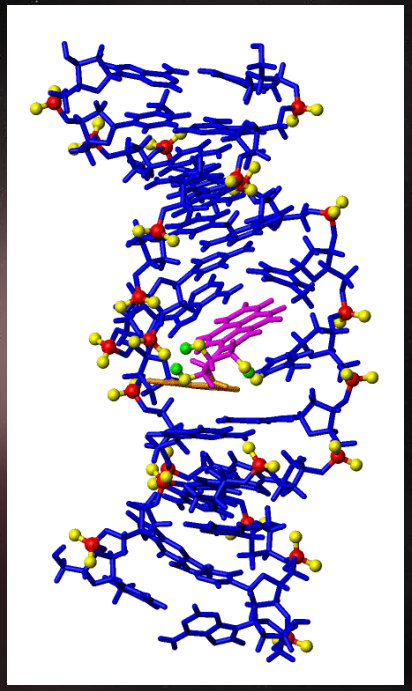
detection of PAH-DNA adducts in DNA from peripheral blood lymphocytes

highly sensitive

relatively slow and time-consuming

expensive





Biological monitoring of exposure to xenobiotics - methods

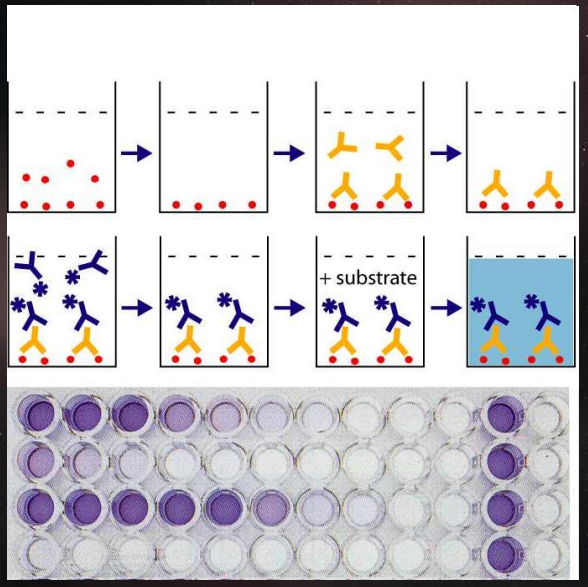
Detection of oxidative stress markers – ELISA

a universal method for the detection of various compounds (antigens)

fast, relatively inexpensive

high-throughput

problems with specificity – depends on the primary antibody



OCCUPATIONAL VS. NON-OCCUPATIONAL EXPOSURE TO XENOBIOTICS

Occupational exposure	Non-occupational exposure
Time of exposure is defined (work shifts)	Time of exposure is often lifelong
Concentrations of xenobiotics measurable	Concentrations of xenobiotics usually very low
Chemical compounds are known and detectable	Indefinable mixtures
Exposed subjects are selected based on specific criteria (age, gender, profession)	All subjects are exposed regardless age, gender
Exposure can be regulated	Exposure cannot be regulated

Biomonitoring of occupational exposure to xenobiotics in Czech Republic

Occupational exposure:

- printing, rubber and shoe industry
- coal pressure gasification, coke-oven workers
- coal and uranium miners
- exposure to wood dust
- soot and tar production
- cytostatics

Biomonitoring of occupational exposure to xenobiotics in Czech Republic

Cytogenetic laboratories

Cytogenetic analysis of peripheral blood lymphocytes by conventional method

Ames test – mutagenicity of urine (non-specific effect)

Increased levels of chromosomal aberrations → preventive measures

EVERY YEAR 60,000 NEW CANCER CASES IN CZECH REPUBLIC

≈ 4% (approx. 2,500) CAUSED BY OCCUPATIONAL EXPOSURE TO XENOBIOTICS

Cancers suspected to be associated with professional exposure to xenobiotics

Bladder cancer	Aromatic amines, benzidine dyes
Liver cancer	Vinyl chloride (monomer)
Cancer of nasal cavity and middle ear	Wood dust, chromium and nickel compounds
Skin cancer	Arsenic, by-products of coal gasification
Lung cancer	Arsenic, berylium, nickel, diesel exhaust
Leukemia	Benzene, ethylene oxide
Mesothelioma	Asbestos

Biomonitoring of non-occupational exposure to xenobiotics in Czech Republic

Exposure to relatively low concentrations of complex mixtures of xenobiotics

Polycyclic aromatic hydrocarbons (benzo[a]pyrene) – incomplete combustion

Biomarkers

conventional cytogenetic analysis

FISH

analysis of micronuclei

analysis of PAH-DNA adducts

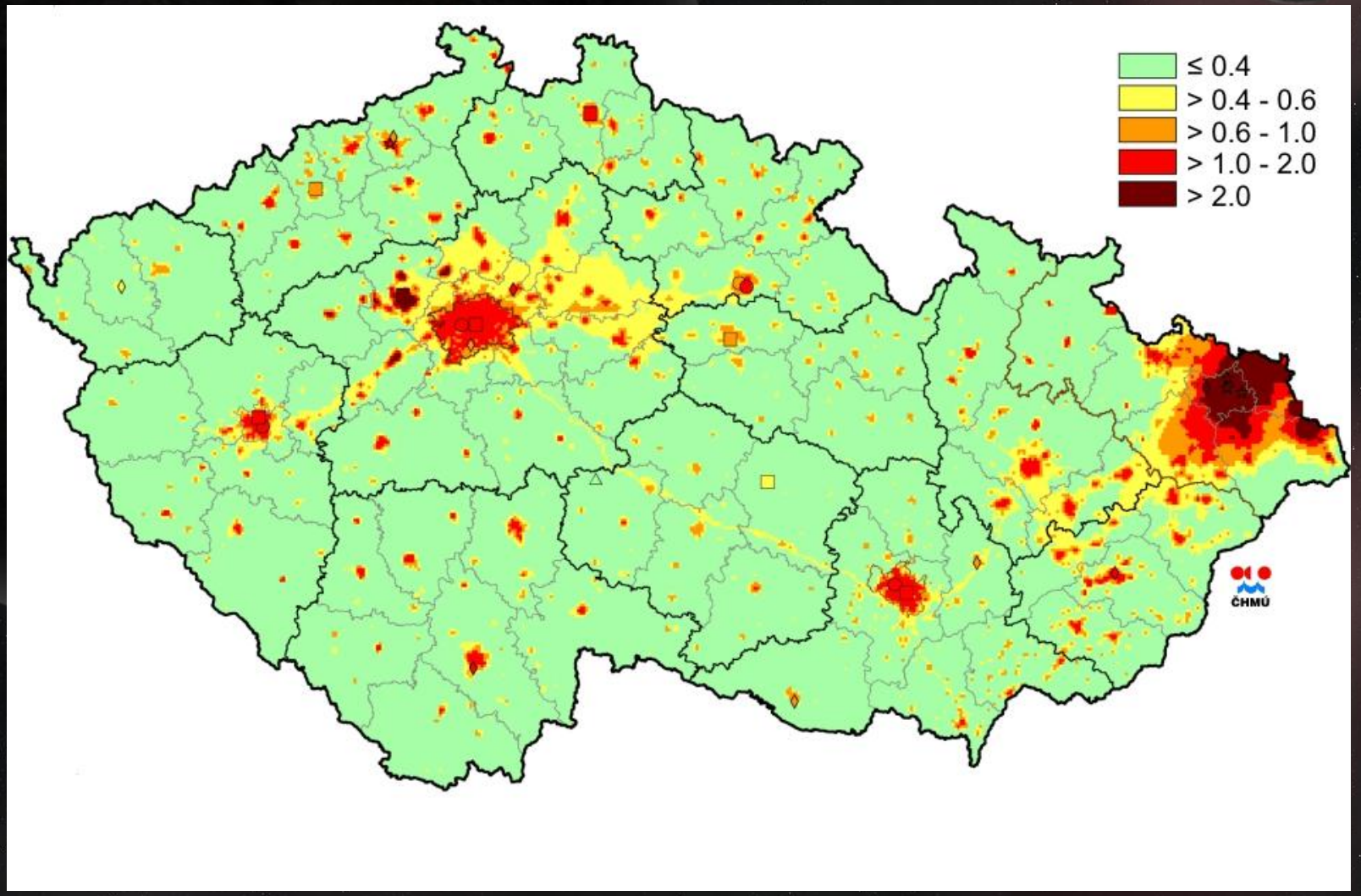
oxidative stress markers

genotyping

gene expression analysis

Biomonitoring of non-occupational exposure to xenobiotics in Czech Republic

Average concentrations of benzo[a]pyrene in 2008 (ng/m^3)



Biomonitoring of non-occupational exposure to xenobiotics in Czech Republic

Biomarkers can be affected by other factors

- smoking
- age
- diet
- individual differences in the capacity of immune system, metabolism of xenobiotics and DNA repair

Environmental exposure to particulate matter and polycyclic aromatic hydrocarbons

Pollutant	Biomarker	Group
PM2.5	8-oxodG	bus drivers + controls
PM10	8-oxodG	bus drivers + controls
B[a]P	15-F_{2t}-IsoP	bus drivers + controls
PM2.5	15-F_{2t}-IsoP	bus drivers + controls
PM2.5	8-oxodG	children
PM10	8-oxodG	children
B[a]P	8-oxodG	children
B[a]P	micronuclei	policemen
B[a]P	FISH	policemen
PM2.5	FISH	policemen

Environmental exposure to particulate matter and polycyclic aromatic hydrocarbons

Pollutant	Biomarker	Group
B[a]P	PAH-DNA adducts	policemen vs. controls

Exposure to environmental pollutants and pregnancy outcomes

Intrauterine growth restriction (IUGR) and low birth weight (LBW)

The risk of IUGR increases 1.22-fold per each 10 ng/m^3 increase of c-PAHs in the first gestational month

Prevention of genotoxic effects

Occupational exposure

decrease the contact of the worker with genotoxic compounds

Non-occupational exposure

sources are not always known, exposure to complex mixtures of chemicals → suitable diet (fruits and vegetables, vitamins – vitamin C, beta-carotene, flavonoids)

vitamins stimulate immunological processes, block ROS, decrease levels of oxidative stress

higher need of vitamins – smokers, alcohol drinking, pregnancy, stress, diseases

TAKE-HOME MESSAGE

- Xenobiotics are everywhere

- Many of them are harmful to human organism

- Their effects on the organism can be monitored using biomarkers

- Their negative impact on the organism can be prevented in both occupationally and non-occupationally exposed populations