Molecular Markers in Toxicology and Epidemiology

Development, Validation, and Application of Biomarkers

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Introduction

Virtually any change produced by an environmental contaminant, whether at a biochemical, cellular, organismal, community or population level, can be regarded as a biomarker. However, for a biomarker to be useful as a monitoring tool, it should meet one or more of the following criteria :

Specificity - is the change a direct result of exposure to the contaminant? An example of an indirect response would be if the contaminant evoked a stress response which in turn brought about changes in various biomarkers .

Sensitivity - is the change the first to be produced by the contaminant?

Practicality - are there cheaper ways to get the same answer ?

ADVANTAGES OF BIOCHEMICAL AND CELLULAR BIOMARKERS

- Biochemical and cellular events tend to be more sensitive, less variable, more highly conserved between species, and often easier to measure than stress indices commonly examined at the organismic level.
- Biochemical and molecular alterations are the first detectable quantifiable responses to environmental changes.
- Biochemical markers can serve as markers for both exposure and effect in organisms.

DISADVANTAGES OF BIOCHEMICAL AND CELLULAR BIOMARKERS

Age, diet, environmental factors, seasonal variation, and reproductive cycle may alter a number of structural states representing normality and could be potentially confounding issues in attempts to use morphological criteria as biomarkers of effect .

- **Overlap between anticipated toxic state and some aspects of the range of normal morphology may exist .**
- **It is difficult to relate biochemical responses to the health of the organism and to adverse effects on the population, the type of information which is often the bottom line in environmental monitoring. This problem can be overcome, however, by selecting biomarkers which detect cellular and biochemical events which are intimately involved in protecting and defending the cell from environmental insults .**

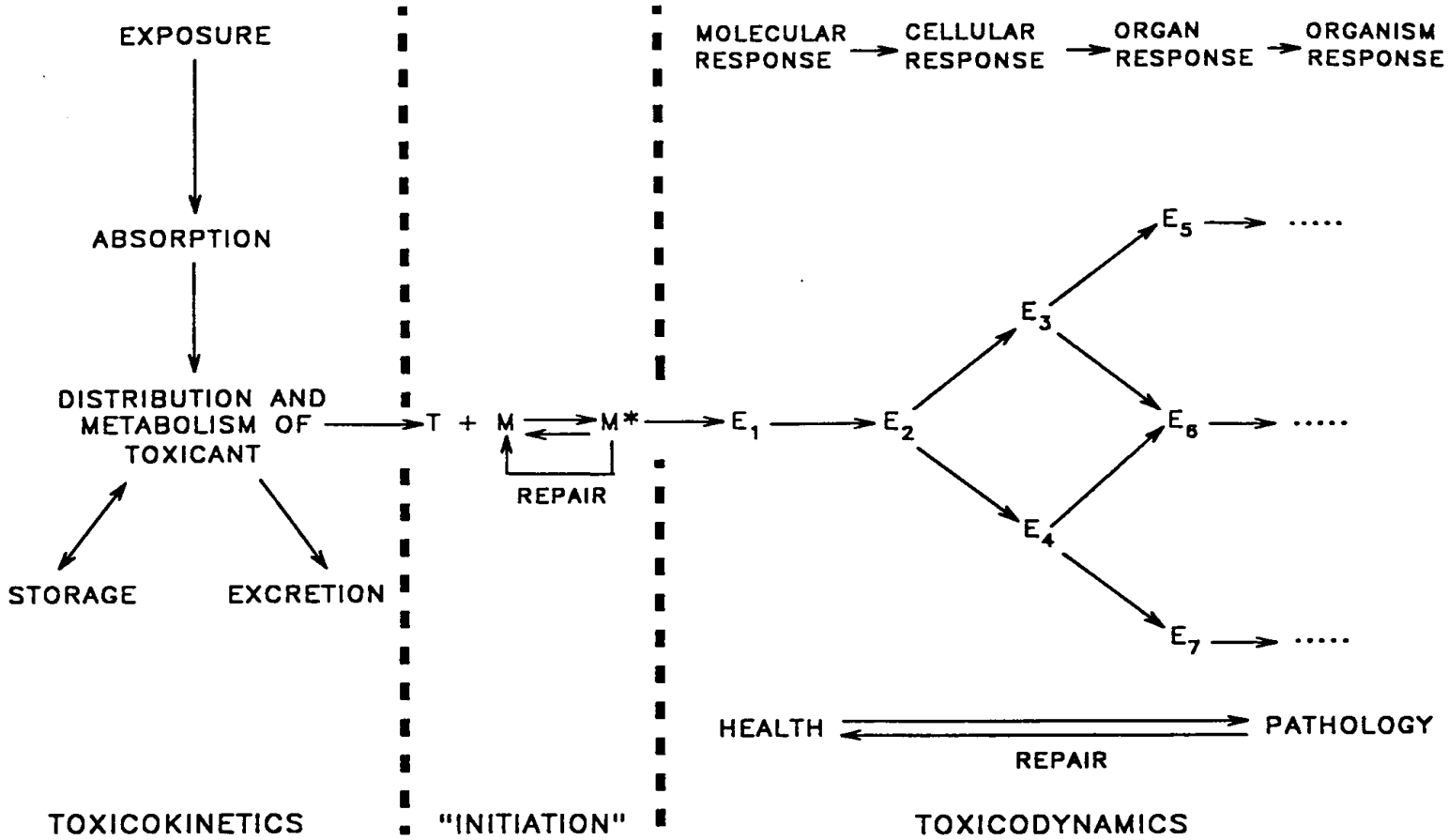
Definitions

- Biomarkers: Molecular, biochemical, or cellular alterations that are measurable in biological media, such as human tissues, cells, or fluids
- Molecular epidemiology: Incorporation of biomarkers into analytical epidemiological research

Fundamental principles of toxicology

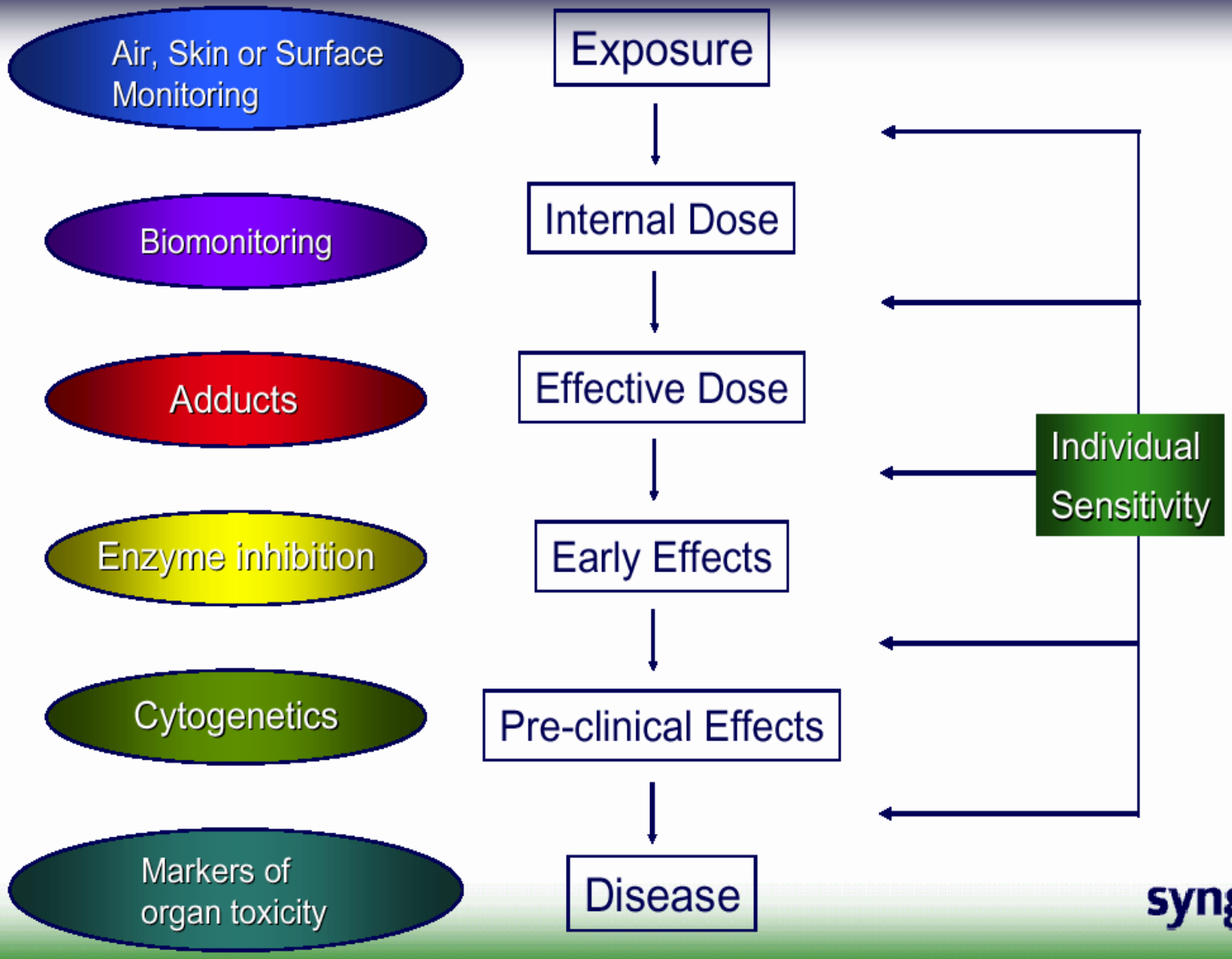
- **Principle I:** The toxic action of a substance is a consequence of the physical/chemical interaction of the active form of that substance with a molecular target within the living organism
- **Principle II:** The magnitude of the toxic effect will be a function of the concentration of altered molecular targets which in turn is related to the concentration of the active form of the toxicant at the site where the molecular targets are located

THE TOXICOLOGICAL PROCESS



Continuum between exposure and disease outcome

(adapted from Talaska et al., 2002)



syngenta

Markers of internal dose

- A direct measure of toxic chemicals or its metabolites in cells, tissues, or body fluids (e.g., blood, urine, feces, milk, amniotic fluid, sweat, hair, nails, saliva(
  - integrate multiple portals of entry
  - integrate fluctuating exposures
  - relate time of exposure to internal dose

Examples of biomarkers of internal dose

- Exhaled breath
  - volatile organic compounds (ethanol(
- Blood levels
  - styrene, lead,cadmium, arsenic
- Fat concentration
  - PCBs and PBBs, DDT, and TCDD
- Metabolites in urine
  - Aflatoxin, benzene, arsenic
- Mutagens in urine
  - Chemotherapeutic agents, carcinogens
- Hair samples
  - arsenic
- Blood
  - carboxyhemoglobin
  - carbon monoxide
- Blood
  - methemoglobinemia
  - organic nitrates

Markers of Biologically effective dose: Assessment of the interactions of toxicants with their molecular targets

- DNA adducts
  - cellular DNA
    - Benzo(a)pyrene DNA adducts in peripheral lymphocytes of coke oven workers
    - cislatinum DNA adducts in WBCs of chemotherapy patients
    - O6 methyldeoxyguanosine in GI mucosa from nitrosamine ingestion
- Protein adducts
  - Albumin adducts
    - PAHs, aflatoxins
  - Hemoglobin adducts
    - ethylene oxide, aromatic amines, PAHs, nitrosamines

Markers of early biological effect: assessment of molecular sequelae of toxicant cell interactions

- Genetic alterations in target and reporter genes
  - mutated oncogenes
  - hprt
  - thymidine kinase
  - glycophorin A
  - loss of tumor supressor genes
  - gene rearrangements
- Nuclear aberrations
  - single strand breaks
  - unscheduled DNA synthesis
  - DNA hyperploidy
  - micronuclei
  - sister chromatid exchanges
  - chromosomal gaps and breaks
- Altered Enzymatic activities
  - elevated protoporphyrin (lead(
  - decreased acetycholinesterase (organophosphates(
  - elevated xenobiotic metabolizing enzymes (TCDD, PAHs(

Markers of altered structure and function: assessment of morphological and/or functional changes following toxicant cell interactions

- Serum markers of disease
  - elevated serum GSTs, ALA, SDH (liver toxicity(
  - creatinine kinase (myocardial toxicity(
- Proliferation markers
  - mitotic frequency
  - thymidine labeling index
  - nuclear antigens
  - ornithine decarboxylase
  - polyamine levels
- Differentiation markers
  - cytokeratins
  - involucrin
  - transglutamase
- Differentially expressed genes
  - EGF, TGF-B, serum a-fetoprotein
- Cellular/tissue changes
  - metaplastic lesions
  - changes in sperm counts and mobility
  - macrophage activity
  - red cell counts

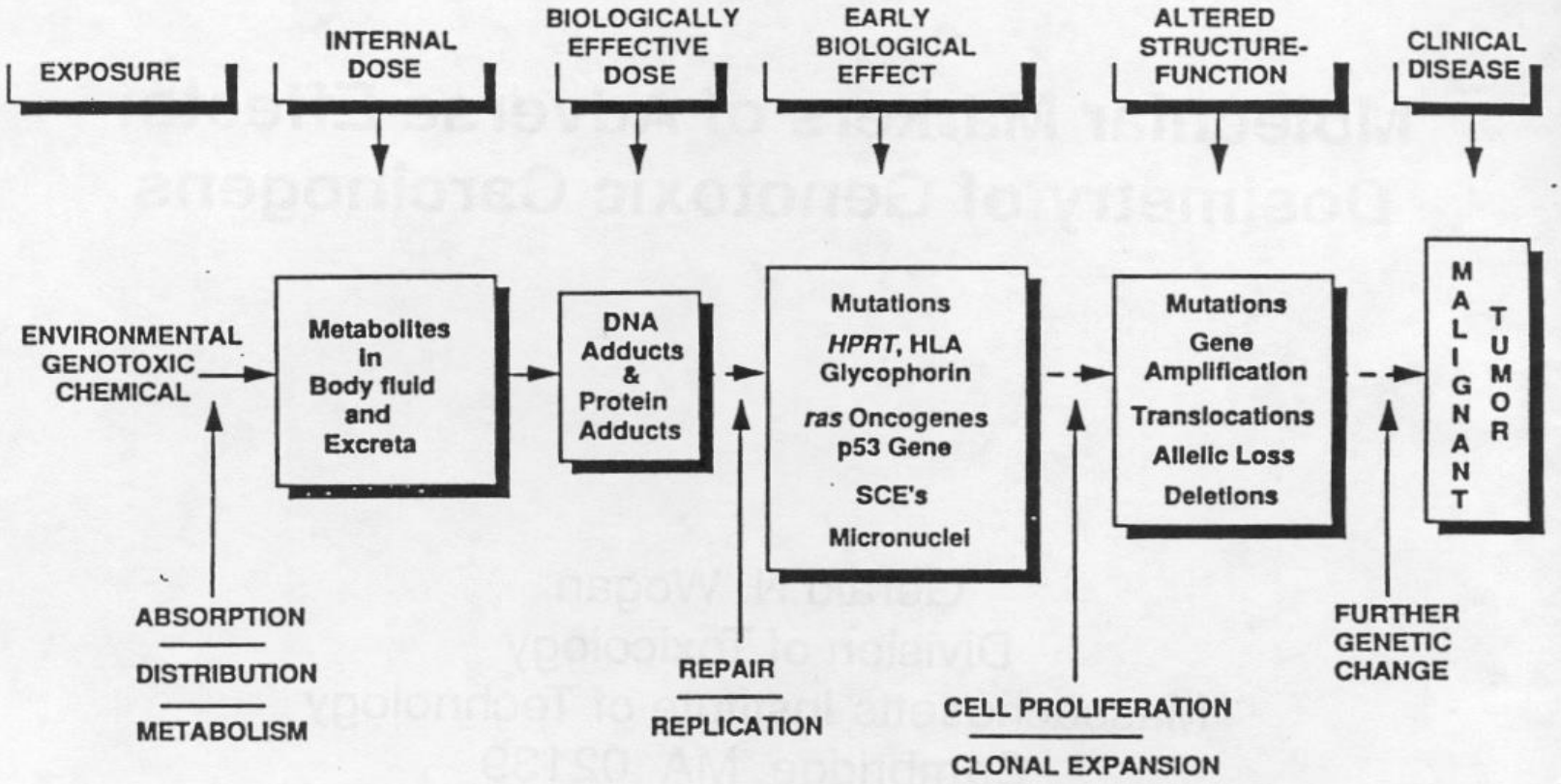
Criteria for selecting intermediate biomarkers

- Is there a causal relationship between the biomarkers and disease ؟
- Does the biomarker appear at a defined stage of the disease process؟
- Can the biomarker be modulated by eliminating exposure ؟
- Can the biomarker be obtained by non-invasive techniques؟
- Do the biomarker and its assay provide acceptable sensitivity, specificity, and accuracy؟
- Is the biomarker stable and easy to measure؟

The validation process: confirming the biomarker disease link

- What is the intra- and interindividual variability؟
- What is the background؟
- What are the optimal sampling conditions ? (e.g. timing, seasonality, repetitive or serial sampling(
- Is there agreement of mutually confirmatory methods for measuring the same biomarker؟
- Is there a relationship between the biomarker and disease؟
- Can the biomarker levels be modulated during intervention؟
- Is there a dose response؟

MOLECULAR BIOMARKERS OF GENOTOXIC EXPOSURE



Markers of Biologically effective dose DNA and protein adducts

- Tobacco smoking
- dietary exposure
- medicinal exposure
- Occupational exposure
- Oxidative damage

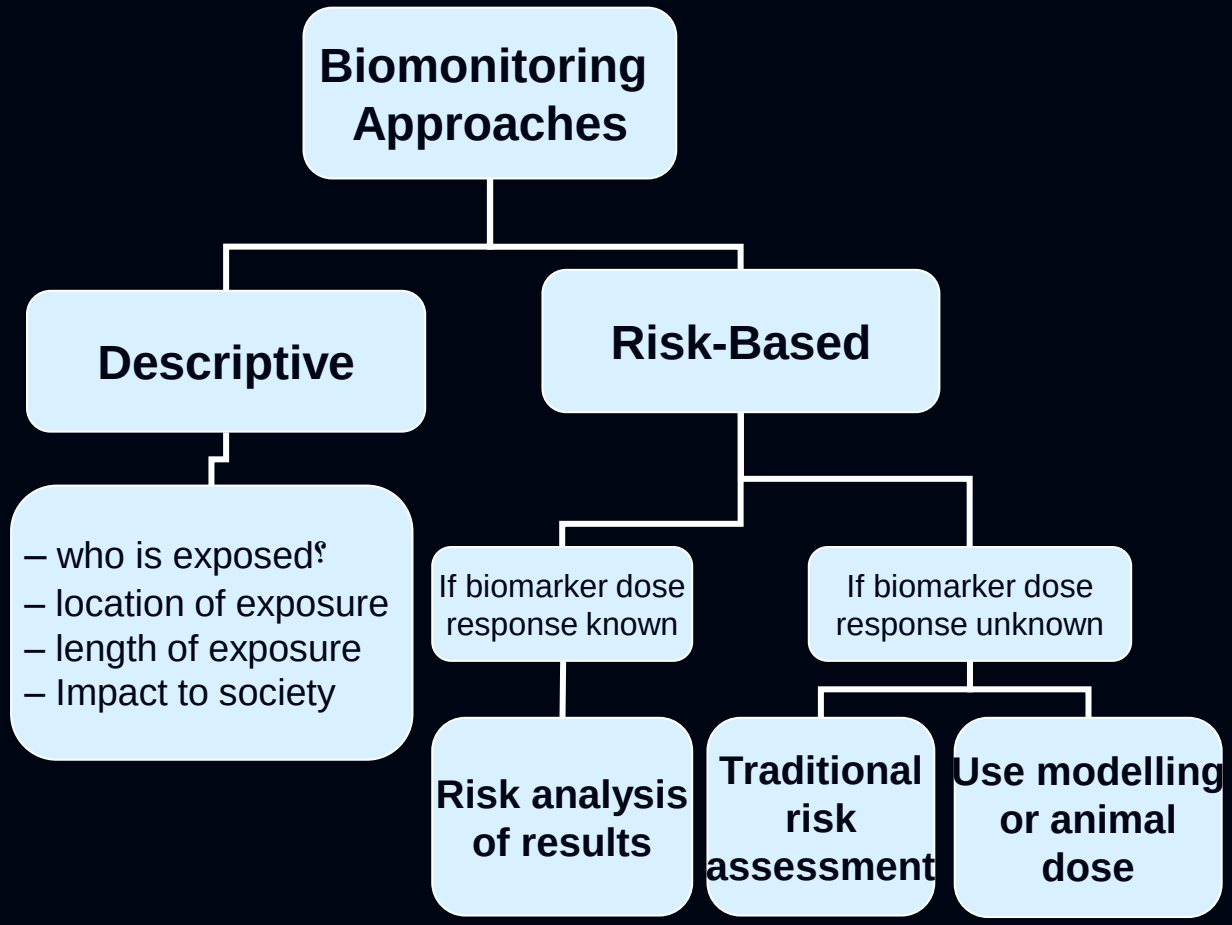
- The choice of biomarker is dependent upon the unique conditions under which it is to be applied. Some important factors to consider in order to use a biochemical biomarker effectively include the following :

- the environmental question being addressed
- the nature of the chemicals of interest
- the species appropriate to the situation
- the types of metabolites anticipated
- the nature of the biological sample
- possible modifying factors specific to the situation
- limits of detection for the analytical procedures
- quality assurance/quality control considerations
- cost

Application of Biomarkers in Epidemiology

WHY USE BIOMARKERS?

- Clinical uses
- Research uses
- Public health uses
- Policy uses



Chemical	Biomarker	Some interpretive options
Polybrominated diphenyls (PBDE(	PBDE in blood and breast milk	Identify exposed population, key information gaps, need for new toxicity and exposure data
Lead	Blood lead	Follow population exposures over time
Organo-phosphates	Parent compound, primary & secondary metabolites, blood and urine	Develop reference ranges, evaluate exposed subpopulations, evaluate public health interventions
Phthalates	Primary & secondary urinary metabolites	Develop reference ranges, identify and follow exposed subpopulations ‹
Dioxin	Dioxin in blood or lipid	Use of pharmacokinetic modeling to estimate body burden

Chemical	Biomarker	Relative utility in clinical medicine
Lead	Blood Lead	Identify and manage lead poisoning in individual patients – very useful clinically‹
Arsenic	Urinary Arsenic	Identify recent arsenic exposure – somewhat useful clinically
Organo-phosphates	Serum or red blood cell cholinesterase level	High intra- and inter-individual variability, overlap with toxic levels, results not available in a timely fashion, lab errors common, not useful clinically
Nitrate/ nitrite	Methemoglobin	Nonspecific and expressed as % of total hemoglobin, must be interpreted within the context of full exposure history and physical exam – somewhat useful clinically
Benzene	Benzene in blood or expelled breath	Short half-life so only useful within a few hours of high exposure – not useful clinically outside of occupational setting

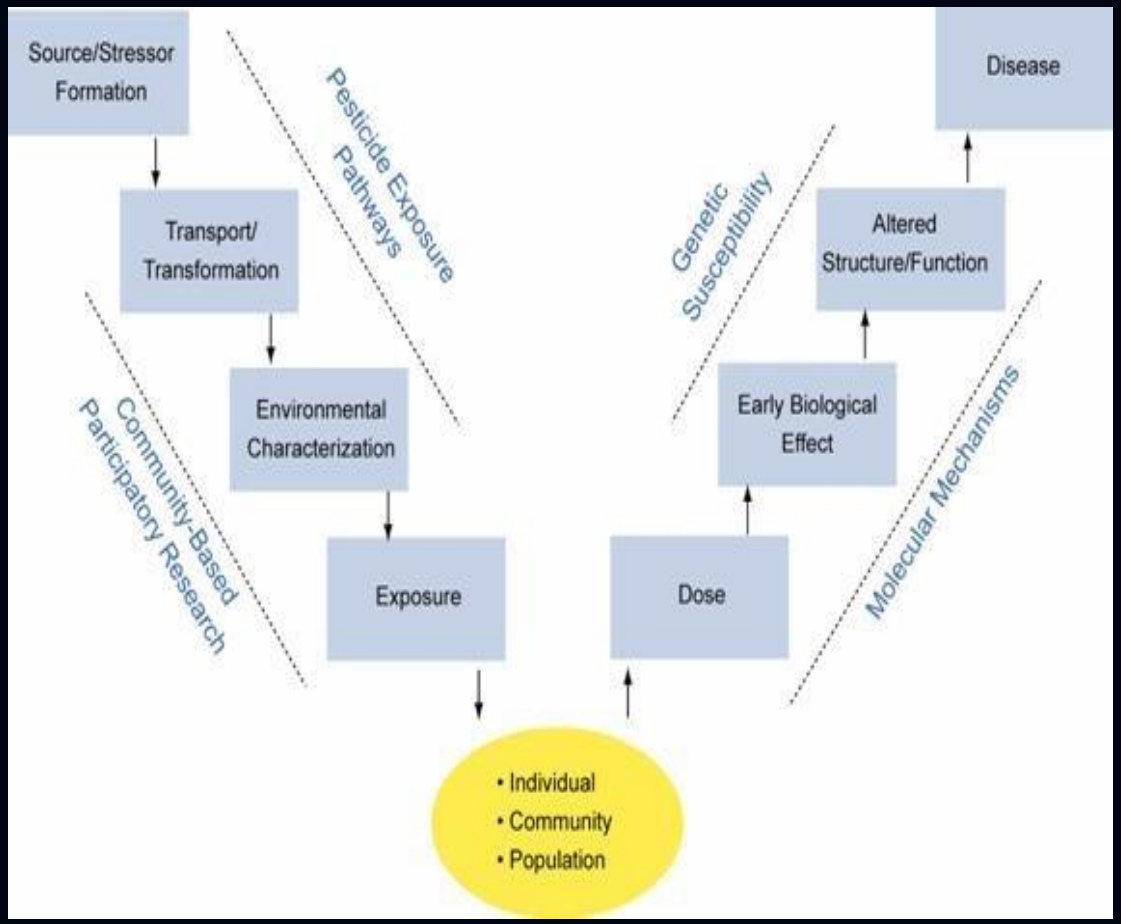
Examples of order of magnitudes for levels of detection for some biomarkers

Marker	Matrix	Units
Polycyclic aromatic hydrocarbon	Urine	ng/L
Cotinine	Serum	ng/mL
Benzene	Blood	ng/mL
Organophosphate metabolites	Urine	⌈g/L
Arsenic	Urine	⌈g/L
Bisphenol A	Urine	⌈g/L
Lead	Blood	⌈g/dL
Polybrominated diphenyl ethers	Serum	ng/g lipid
Dioxin	Serum	pg/g lipid

Biomarkers are most useful when both "up stream" and "down stream" knowledge is complete

- Primary sources of environmental contaminant understood
- Pathways/routes of exposure understood
- Human exposure is related to animal toxicology studies
- Exposure-dose relationship understood
- Timing and duration of exposure known

The Environmental Public Health Continuum (EPHC(



Issues Facing Biomarker Development

METHODOLOGICAL ISSUES

- Analytical technique
- Environmental contaminants and controls
- Laboratory contamination and quality assurance
- Correct choice of biomarker for study design and question
- Rationale for selecting environmental chemicals of interest
- Coordination with related research – epidemiology, toxicology, pharmacokinetic modeling, exposure assessment

RISK COMMUNICATION ISSUES

Who gets the results and why?

- Exposures need context
  - Source and route
  - Bioavailability
  - Toxicity

At risk communities may have unrealistic expectations

- Lag between research and intervention

Why biomarkers are not always useful

- Incomplete knowledge of toxicity
- Inappropriate clinical use of research tools

ETHICAL ISSUES

- Informed consent
- Ability to inform dangerously exposed/at risk individuals
- Biobanking of genetic materials
- Ethical standards differ between researchers and community
  - Individual value versus community value
- 

MANY NATIONAL & REGIONAL BIOMONITORING PROGRAMS EXIST

- In the U.S., decades of biomonitoring
- Increasing number of environmental chemicals monitored
- Increasing number of programs and agencies involved

EXAMPLES OF BIOMONITORING PROGRAMS

In the U.S.

- **HEI**: Human Exposure Initiative
- **HHANES**: Hispanic Health & Nutrition Examination Survey
- **NHATS**: National Human Adipose Tissue Survey
- **NHANES**: National Health & Nutrition Examination Survey
- **NHEXAS**: National Human Exposure Assessment Survey

In Europe & Canada:

- **Canada**: Health Canada's biomonitoring initiatives
- **European Union**: European Human Biomonitoring
- **Germany**: Human Biomonitoring Commission
- **Sweden**: Swedish Environmental Protection Agency on Environmental Pollutants

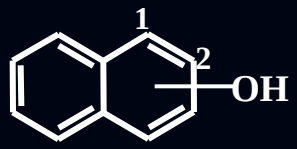
Specific Examples of Chemicals Causing Harm to Humans and Animals

Polycyclic aromatic Hydrocarbons

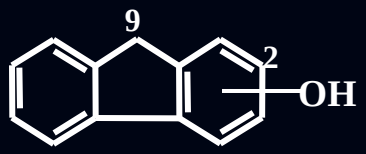
What are polycyclic aromatic hydrocarbons?

- **Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or charbroiled meat. PAHs are usually found as a mixture containing two or more of these compounds, such as soot .**

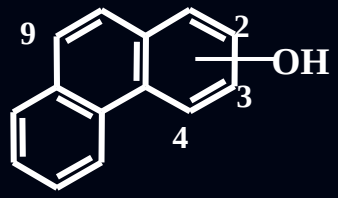
- **Some PAHs are manufactured. These pure PAHs usually exist as colorless, white, or pale yellow-green solids. PAHs are found in coal tar, crude oil, creosote, and roofing tar, but a few are used in medicines or to make dyes, plastics, and pesticides**



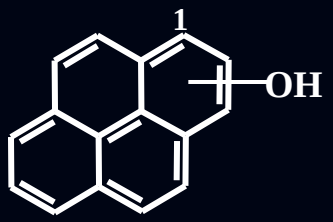
Hydroxynaphthalene (OHNap(



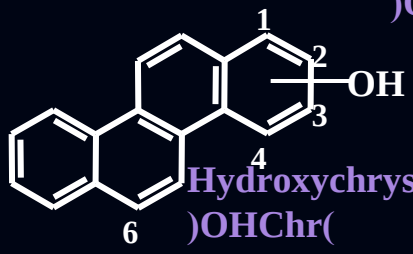
Hydroxyfluorene (OHFle(



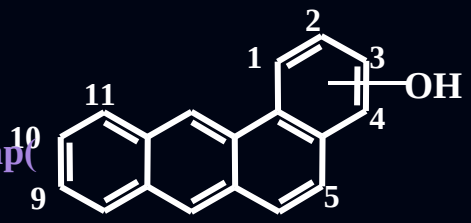
Hydroxyphenanthrene (OHPhe(



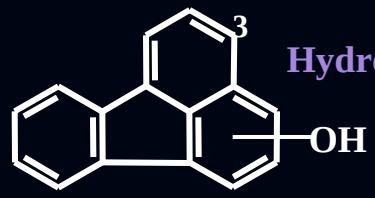
Hydroxypyrene (OHPyr(



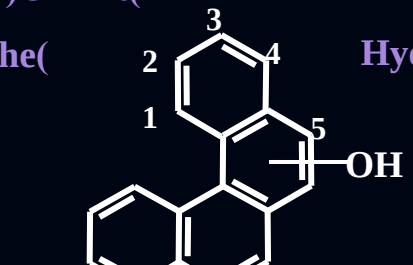
Hydroxychrysene
)OHChr(



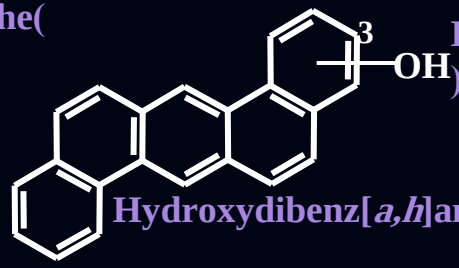
Hydroxybenz[*a*]anthracene
)OHBaA(



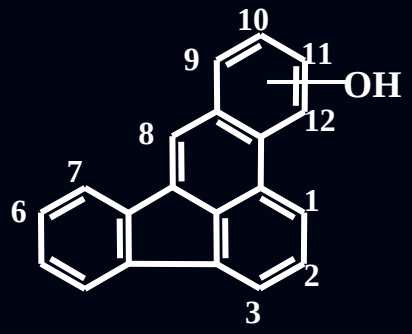
Hydroxyfluoranthene
)OHFrt(



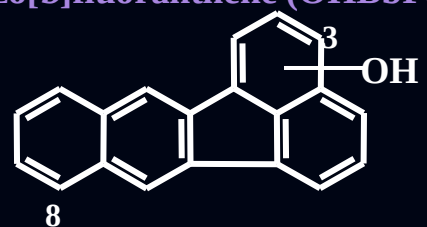
Hydroxybenzo[*c*]phenanthrene
)OHBcPhe(



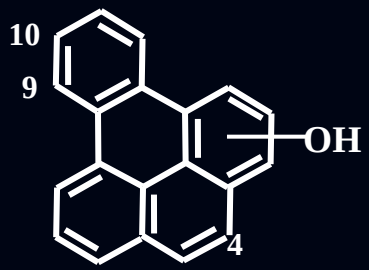
Hydroxydibenz[*a,h*]anthracene (OHDBA(



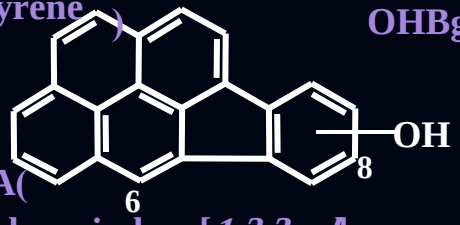
Hydroxybenzo[*j*]fluoranthene
)OHBjF(



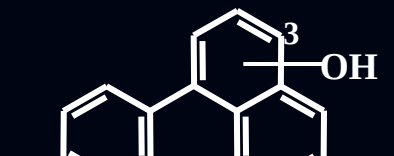
Hydroxybenzo[*k*]fluoranthene (OHBkF(

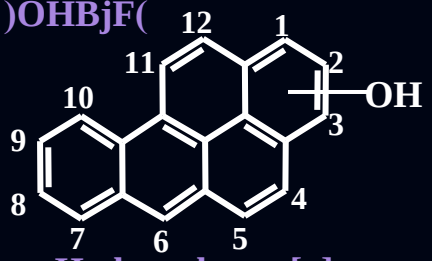


Hydroxybenzo[*e*]pyrene
)OHBeP(

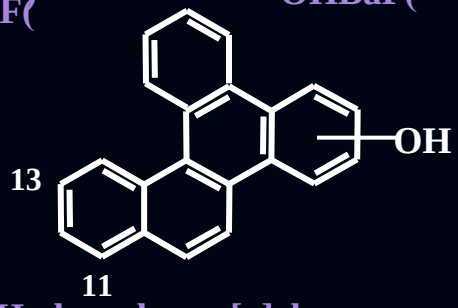


Hydroxyindeno[*1,2,3-cd*]pyrene (OHID





Hydroxybenzo[*a*]pyrene
OHBaP(



Hydroxybenzo[*g*]chrysene
OHBgChr(

Structures of monohydroxy derivatives of polycyclic aromatic hydrocarbons (PAHs(
Position numbers indicate the positions of hydroxy group for the tested monohydroxy derivatives of PAHs.

How are we exposed to PAHs today؟ ؟ ؟

- Exposure to polycyclic aromatic hydrocarbons usually occurs by breathing air contaminated by wild fires or coal tar, or by eating foods that have been grilled .
- PAHs have been found in at least 600 of the 1,430 National Priorities List sites identified by the Environmental Protection Agency (EPA.(
- Breathing air containing PAHs in the workplace of coking, coal-tar, and asphalt production plants; smokehouses; and municipal trash incineration facilities .
- Breathing air containing PAHs from cigarette smoke, wood smoke, vehicle exhausts, asphalt roads, or agricultural burn smoke.
- Coming in contact with air, water, or soil near hazardous waste sites.
- Eating grilled or charred meats; contaminated cereals, flour, bread, vegetables, fruits, meats; and processed or pickled foods.
- Drinking contaminated water or cow's milk .
- Nursing infants of mothers living near hazardous waste sites may be exposed to PAHs through their mother's milk.

Is Exposure to PAHs Associated with an Increase in Cancer Risk?

- Epidemiologic studies have reported an increase in lung cancer in humans exposed to coke oven emission, roofing tar emissions, and cigarette smoke. Each of these mixtures contains a number of PAHs .

- Animal studies have reported respiratory tract tumors from inhalation exposure to benzo(a)pyrene and forestomach tumors, leukemia, and lung tumors from oral exposure to benzo(a)pyrene .

- EPA has classified benzo(a)pyrene as a Group B2, probable human carcinogen .

PAH Exposure

Metabolism

$T_{1/2}$ = hours

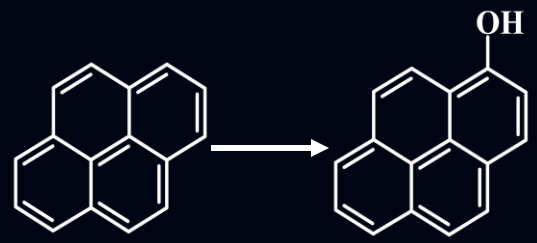
PAH Adducts
PAH Metabolites

Urinary/biliary Excretion

Carcinogenic / Genotoxic

.1Typical biomonitoring method

Hydroxylated metabolites of PAHs in urine



Pyrene 1-Hydroxypyrene

Glucuronides
Sulfates

Sample

Urine

Analytical method

HPLC-FL

GC-MS

.2Technical issue

Analysis of just one metabolite sometimes seems to be an indicator of the absorption of only the parent PAH .

.3Our strategy

The simultaneous determination of several PAH metabolites in urine will provide more comprehensive information to estimate the exposure of an individual to PAHs.

Urine (10 mL(

↓

Adjusted to pH 5.0 with 1.0 M HCl
+ 0.1 M Acetate buffer, pH 5.0
Hydrolyzed with ®-glucuronidase/aryl sulfatase
Incubated at 37 °C for 4 h

Hydrolyzed urine

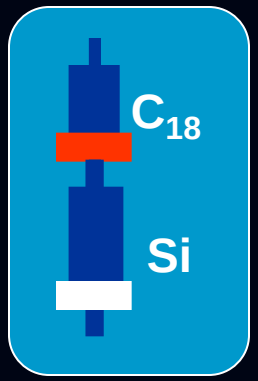
↓

Extracted with Sep-Pak C_{18} cartridge (primed with methanol and water(
Washed with water and methanol/water (1/4, v/v(
Dried with air for 10 min

The SPE cartridge

↓

Connected to a Sep-Pak Silica cartridge
Washed with hexane
Eluted with hexane/ethyl acetate (9/1, v/v) (20 mL(



Eluate

↓

Residue

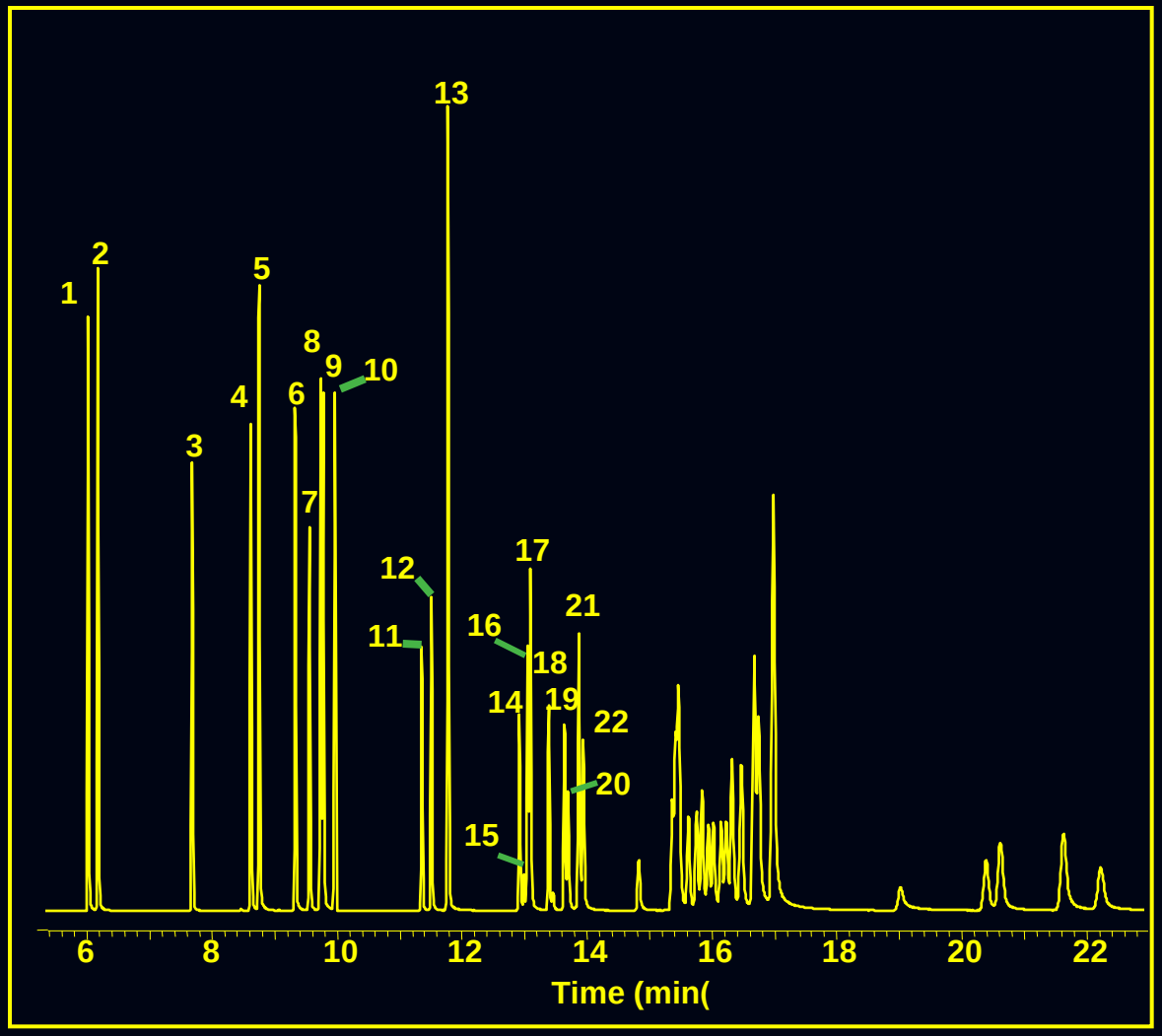
↓

+Methanol (200 uL(

HPLC injection (20 uL(

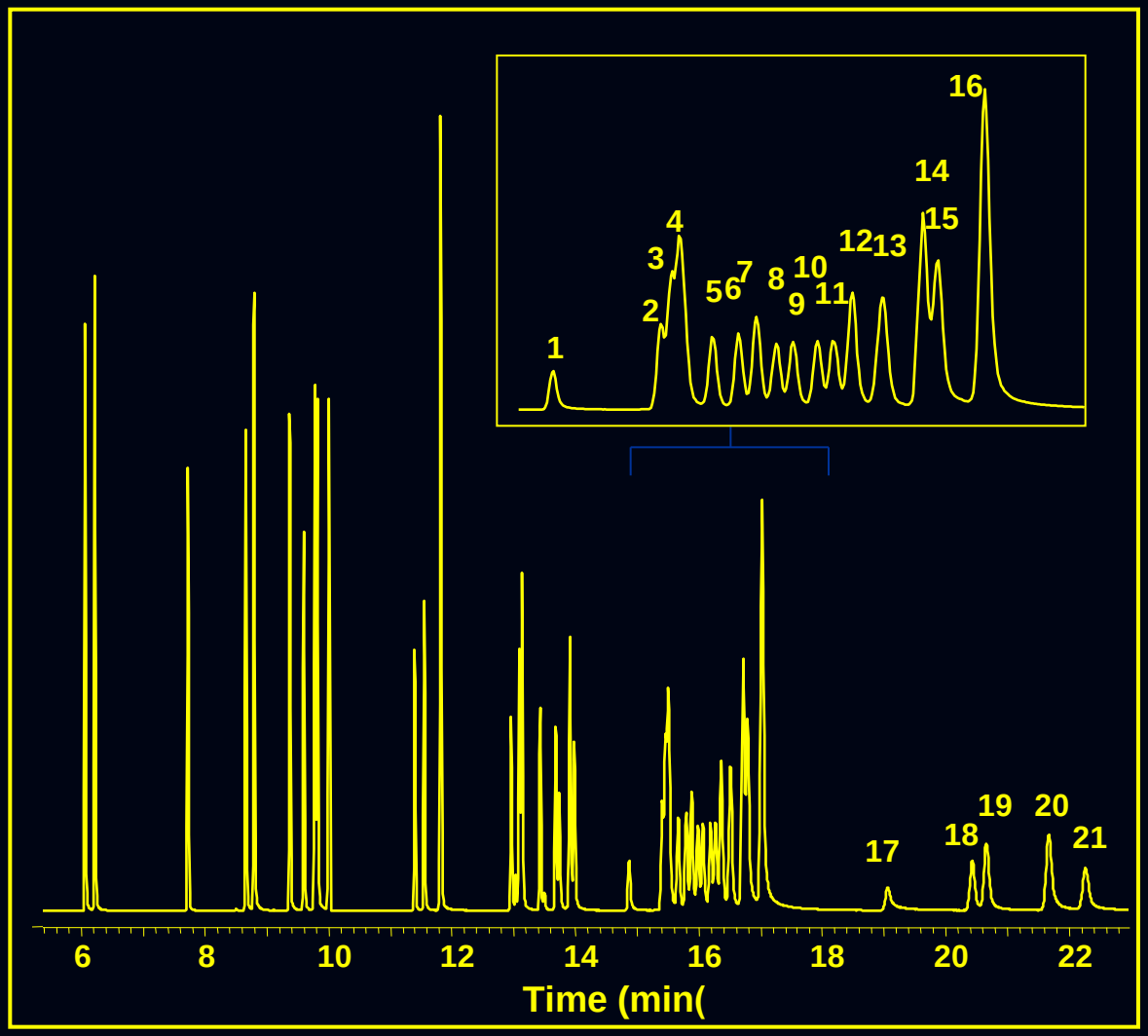
Treatment of human urine using solid phase extraction for the determination of OHPAHs

HPLC analysis of urinary hydroxy PAHs

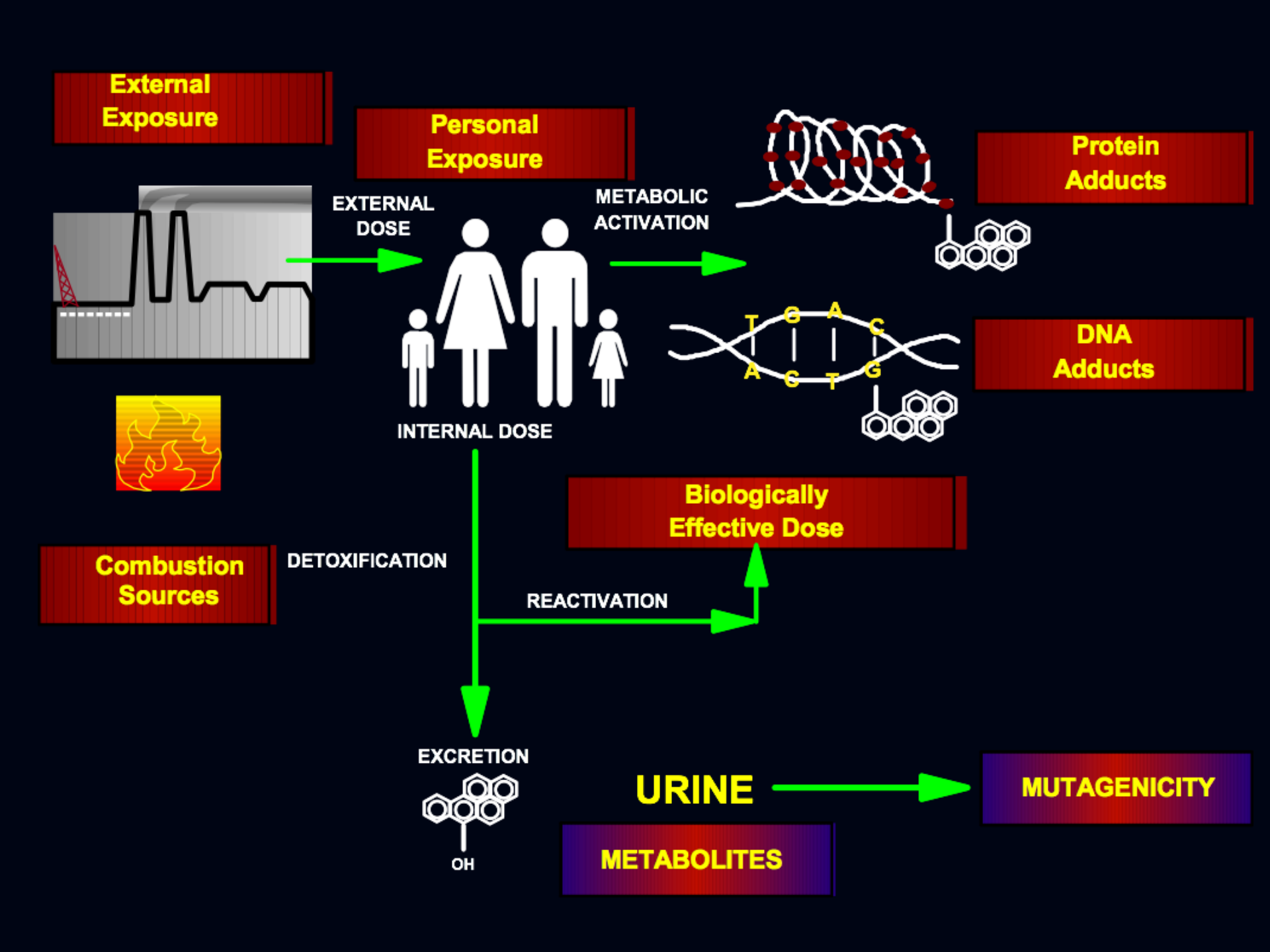


1. -1OH-naphthalene
2. -2OH-naphthalene
3. -3OH-fluorene
4. -2OH-fluorene
5. -9OH-fluorene
6. -9OH-phenanthrene
7. -3OH-phenanthrene
8. -2OH-phenanthrene
9. -1OH-phenanthrene
10. -4OH-phenanthrene
11. -1OH-benzo(c)phenanthrene
12. -3OH-fluoranthene
13. -1OH-pyrene
14. -2OH-benzo(c)phenanthrene
15. -1OH-benz(a)anthracene
16. -4OH-chrysene
17. -6OH-chrysene
18. -3OH-benzo(c)phenanthrene
19. -3OH-chrysene
20. -1OH-chrysene
21. -3OH-benz(a)anthracene
    -9OH-benz(a)anthracene
22. -2OH-chrysene

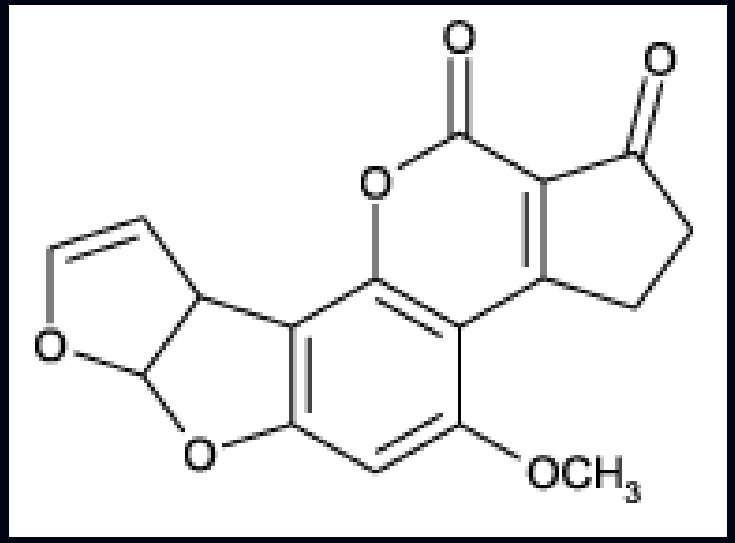
HPLC analysis of urinary hydroxy PAHs



1. -8OH-benzo(b)fluoranthene
2. -7OH-benzo(b)fluoranthene
3. -1OH-benzo(b)fluoranthene
   -9OH-benzo(b)fluoranthene
4. -2OH-benzo(b)fluoranthene
   -12OH-benzo(b)fluoranthene
   -8OH-benzo(b)fluoranthene
5. -9OH-benzo(e)pyrene
6. -3OH-benzo(b)fluoranthene
7. -12OH-benzo(a)pyrene
8. -5OH-benzo(a)pyrene
9. -11OH-benzo(b)fluoranthene
10. -6OH-benzo(b)fluoranthene
11. -3OH-benzo(k)fluoranthene
12. -4OH-benzo(e)pyrene
    -10OH-benzo(b)fluoranthene
13. -9OH-benzo(k)fluoranthene
    -7OH-benzo(a)pyrene
14. -10OH-benzo(e)pyrene
15. -3OH-benzo(e)pyrene
16. -3OH-benzo(a)pyrene
    -2OH-benzo(e)pyrene
17. -1OH-indeno-[1,2,3-c,d]-pyrene
18. -2OH-indeno-[1,2,3-c,d]-pyrene
19. -6OH-indeno-[1,2,3-c,d]-pyrene
20. -8OH-indeno-[1,2,3-c,d]-pyrene
21. -3OH-dibenzo[a,h]anthracene


External Exposure
Personal Exposure
Protein Adducts
EXTERNAL DOSE
METABOLIC ACTIVATION
DNA Adducts
INTERNAL DOSE
Biologically Effective Dose
Combustion Sources
DETOXIFICATION
REACTIVATION
EXCRETION
OH
URINE
MUTAGENICITY
METABOLITES

Aflatoxin B1



Aflatoxin B1: Background

- Hepatotoxic mycotoxin (toxin from a fungus) produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*

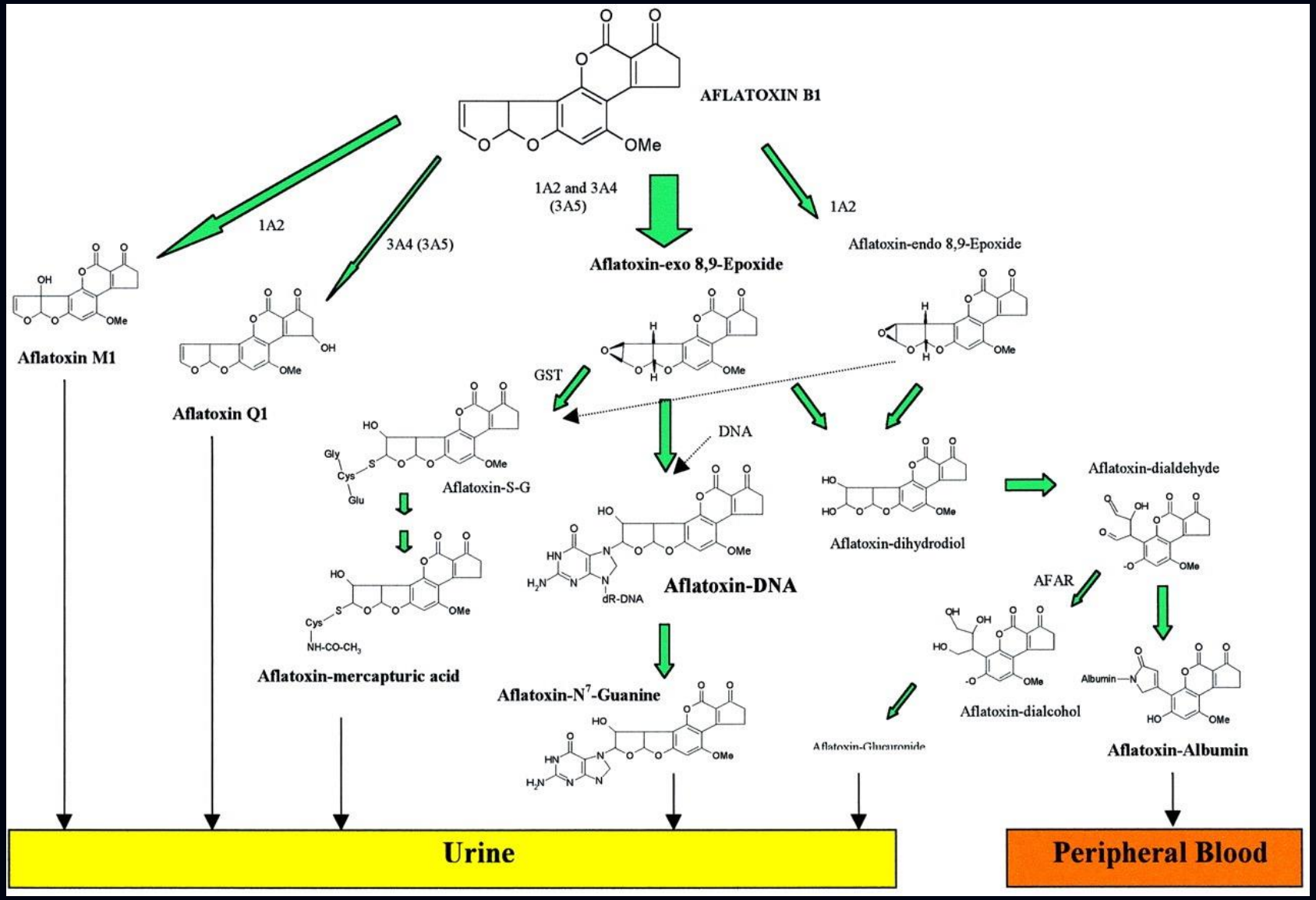
- Causes aflatoxicosis and liver disease

Aspergillus spp. grow ubiquitously on plants and crops from tropical and subtropical areas: peanuts, figs, spices, corn, maize, Brazil nuts, pecans, walnuts, soybeans, pistachios, wheat and grains

Background

- The carcinogenic metabolite of Aflatoxin B1 is found in the milk of mammals who consume contaminated crops

- *Aspergillus* growth and Aflatoxin B1 production is dependant upon the temperature, humidity, host plant type, and the strain of fungus; high humidity usually required for growth


AFLATOXIN B1
1A2
3A4 (3A5)
1A2 and 3A4 (3A5)
1A2
Aflatoxin M1
Aflatoxin Q1
Aflatoxin-exo 8,9-Epoxide
Aflatoxin-endo 8,9-Epoxide
GST
DNA
Aflatoxin-S-G
Aflatoxin-DNA
Aflatoxin-dihydrodiol
Aflatoxin-dialdehyde
AFAR
Aflatoxin-mercapturic acid
Aflatoxin-N7-Guanine
Aflatoxin-dialcohol
Aflatoxin-Albumin
Urine
Peripheral Blood

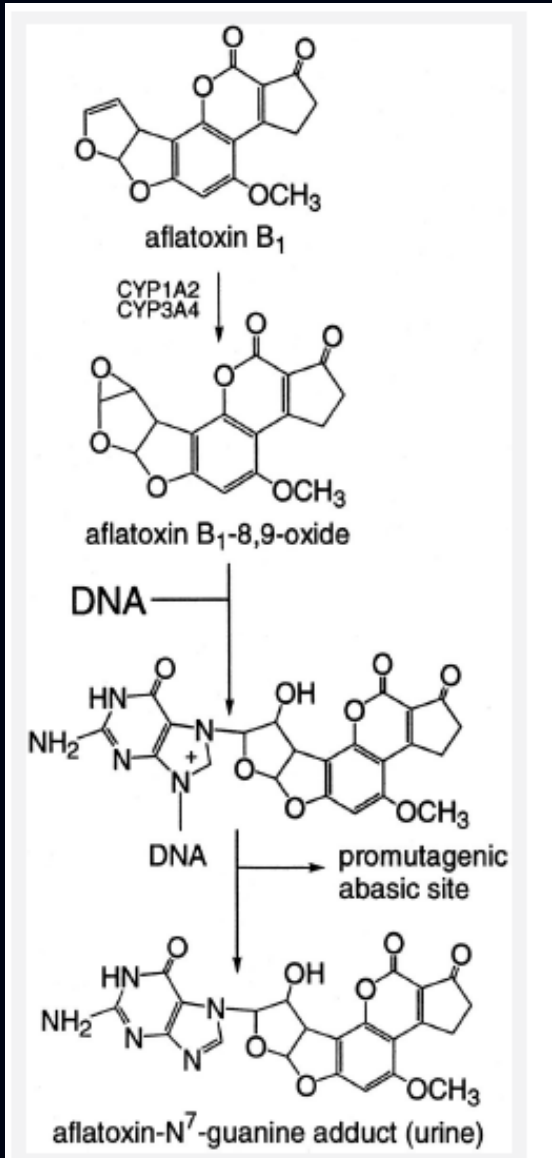


Figura 6. *Formación aducto aflatoxina –DNA y compuesto utilizado como biomarcador de exposición en orina. Fuente: Referencia 14.*

AFB1-8,9-epoxide Is Cancer-causing!!

- One of the most serious effects of the AFB1-8,9-epoxide metabolite is it reacts with DNA and proteins to form an adduct .

Other Adverse Effects of AFB1-8,9-epoxide

- Lipid accumulation in the liver due to decreased lipid transport and reduced oxidation

-Symptoms of liver failure occur with acute aflatoxicosis:

-Jaundice

-ascites (fluid build up (

-portal hypertension

-necrosis of the liver

Other Chronic Effects of Aflatoxin

- Immunological Suppression
  - Using animal models, AFB1 has been shown to impair normal immune function either by reducing phagocytic activity or reduce T cell number and function .
- Nutritional Interference
  - Aflatoxin is shown to have a dose response relationship between exposure to aflatoxin and rate of growth among small children. In addition, it also interferes in nutrient modification such as Vitamin A or D in animal models .

Aflatoxins and Food Production

Major crops affected by aflatoxins include maize (corn) and groundnuts (peanuts). Agricultural practices can be modified to reduce aflatoxin production / contamination.

Farming practices

- irrigation
- pesticide use
- time of harvest

Storage practices

- drying techniques
- processing, such as shelling peanuts

Aspergillus on maize

Drying maize

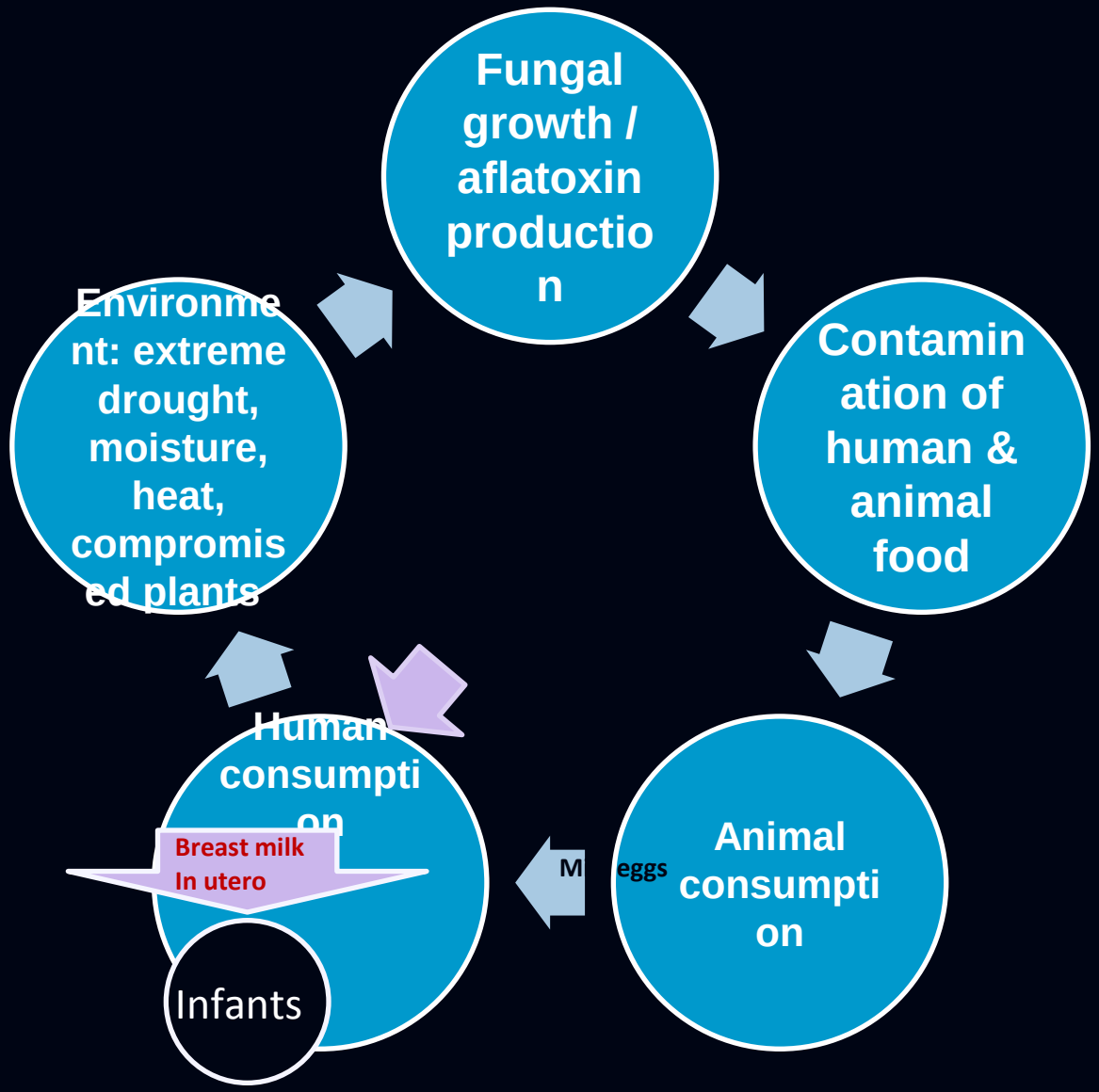
Aflatoxins in Farmed Animals

- Poultry
  - Highly sensitive
  - Aflatoxin toxicity impairs uptake of essential nutrients as well as causing tissue damage

- Ruminants
  - Ruminants are relatively insensitive; however, aflatoxin exposure can cause growth impairment in young or lactating animals.
  - Metabolites in milk and related dairy products
    - Aflatoxin consumed by cows is excreted in milk as the M1 metabolite.
    - The M1 metabolite can be absorbed by calves or humans causing growth failure.
    - The M1 metabolite also remains present in milk-based products such as cheese and yogurt.
- Fish
  - When farmed fish are accidentally fed contaminated grains, large die-offs may occur.
    - Rainbow trout are highly sensitive

Animal deaths and reduced productivity from aflatoxin exposure can have significant negative 'economic' impact in addition to the negative health outcomes for those who consume contaminated animal products.

Aflatoxins: Human, Animal, and Environmental Interactions

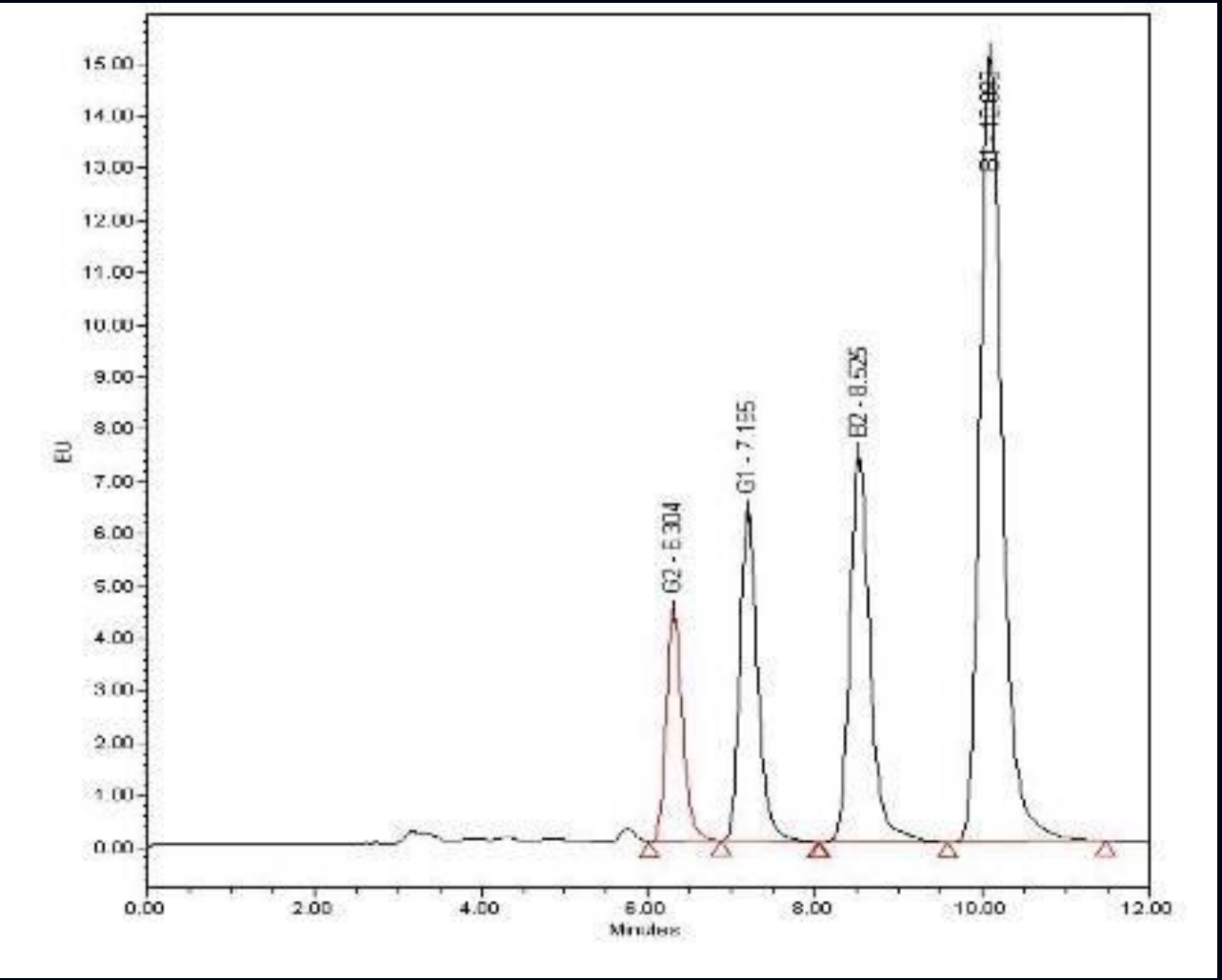


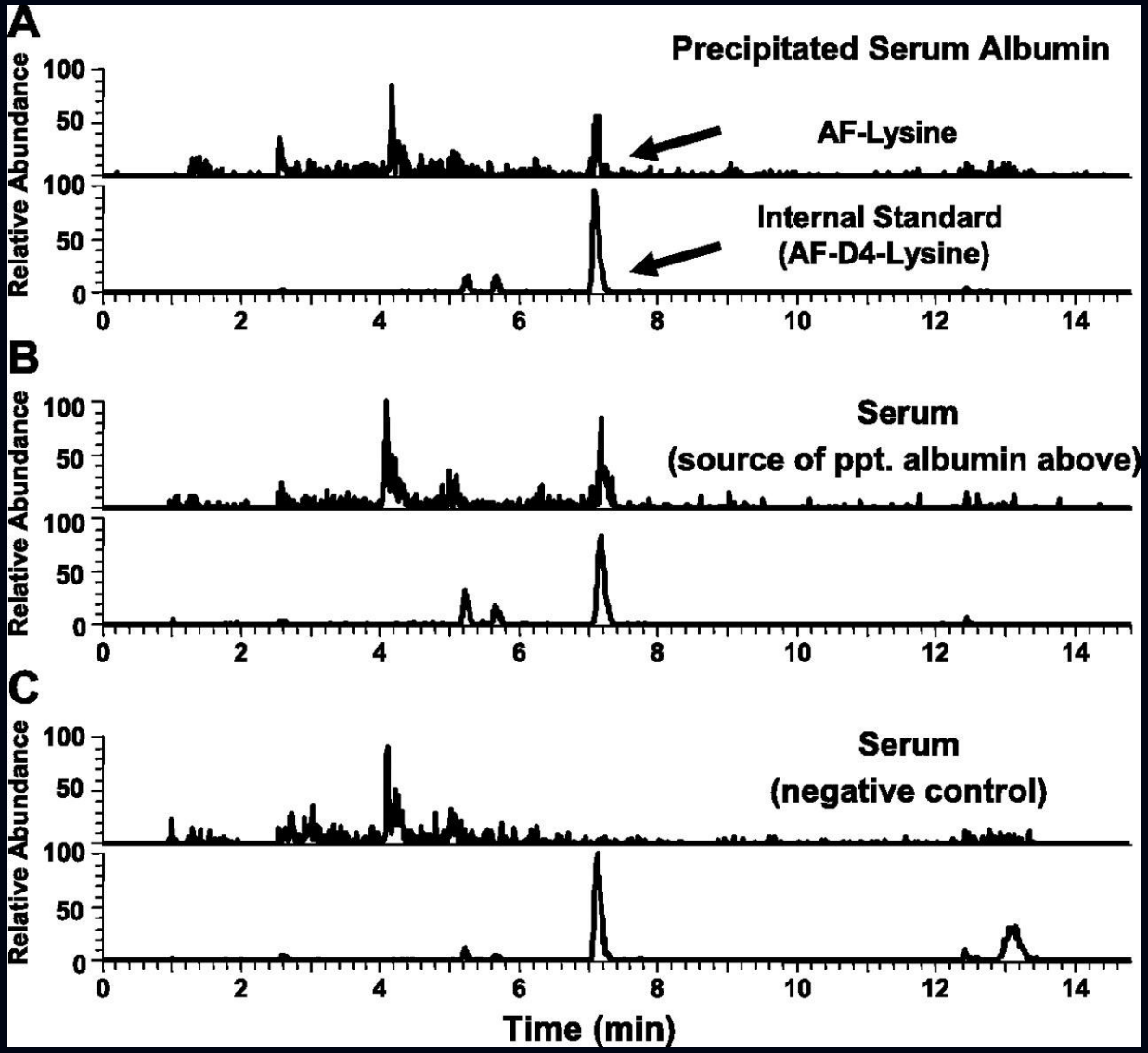
Allowable Aflatoxin Levels in Human Foods

Amount	Food type
20ppb	Foods in general
0.5ppb (aflatoxin M(1	Milk
20ppb	Peanuts and peanut products
20ppb	Pistachio nuts
20ppb	Brazil nuts

Allowable Aflatoxin Levels in Animal Feeds

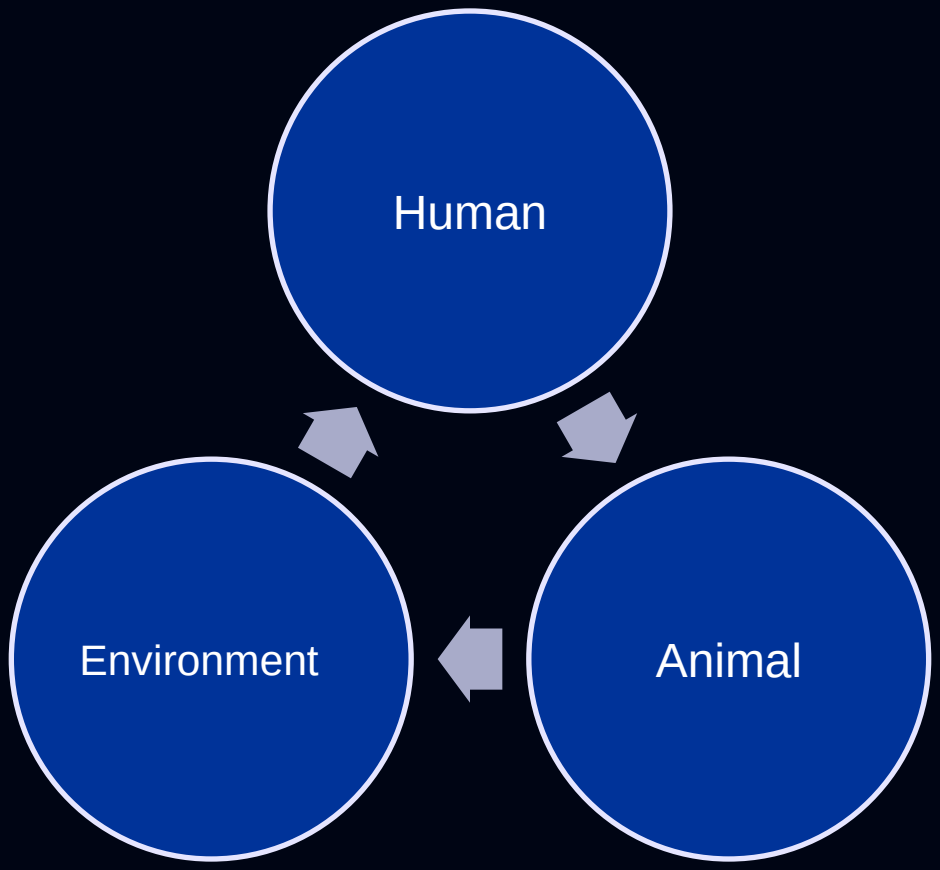
Amount	Feed Type
20ppb	For corn and other grains intended for immature animals (including immature poultry) and for dairy animals, or when its destination is not known
20ppb	For animal feeds, other than corn or cottonseed meal;
100ppb	For corn and other grains intended for breeding beef cattle, breeding swine, or mature poultry
200ppb	For corn and other grains intended for finishing swine of 100 pounds or greater
300ppb	For corn and other grains intended for finishing (i.e., feedlot) beef cattle and for cottonseed meal intended for beef cattle, swine or poultry


EU
Minutes
G2 - 6.304
G1 - 7.155
B2 - 8.525
B1 - 15.087
0.00
2.00
4.00
6.00
8.00
10.00
12.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00


A
Precipitated Serum Albumin
AF-Lysine
Internal Standard
(AF-D4-Lysine)
Relative Abundance
100
50
0
0
2
4
6
8
10
12
14
B
Serum
(source of ppt. albumin above)
C
Serum
(negative control)
Time (min)

The Benefits of an Interdisciplinary Health Approach

- Educating stakeholders on the interconnectedness of humans, animals and the environment is the first step in preventing aflatoxin-related health issues



Effective Ways to Implement Biomarkers

- 1. Develop Biomarkers of Environmental Contaminations (crops, feed, livestock)

- 2. Develop Biomarkers of Human Exposure (urine, blood, saliva, breast milk)

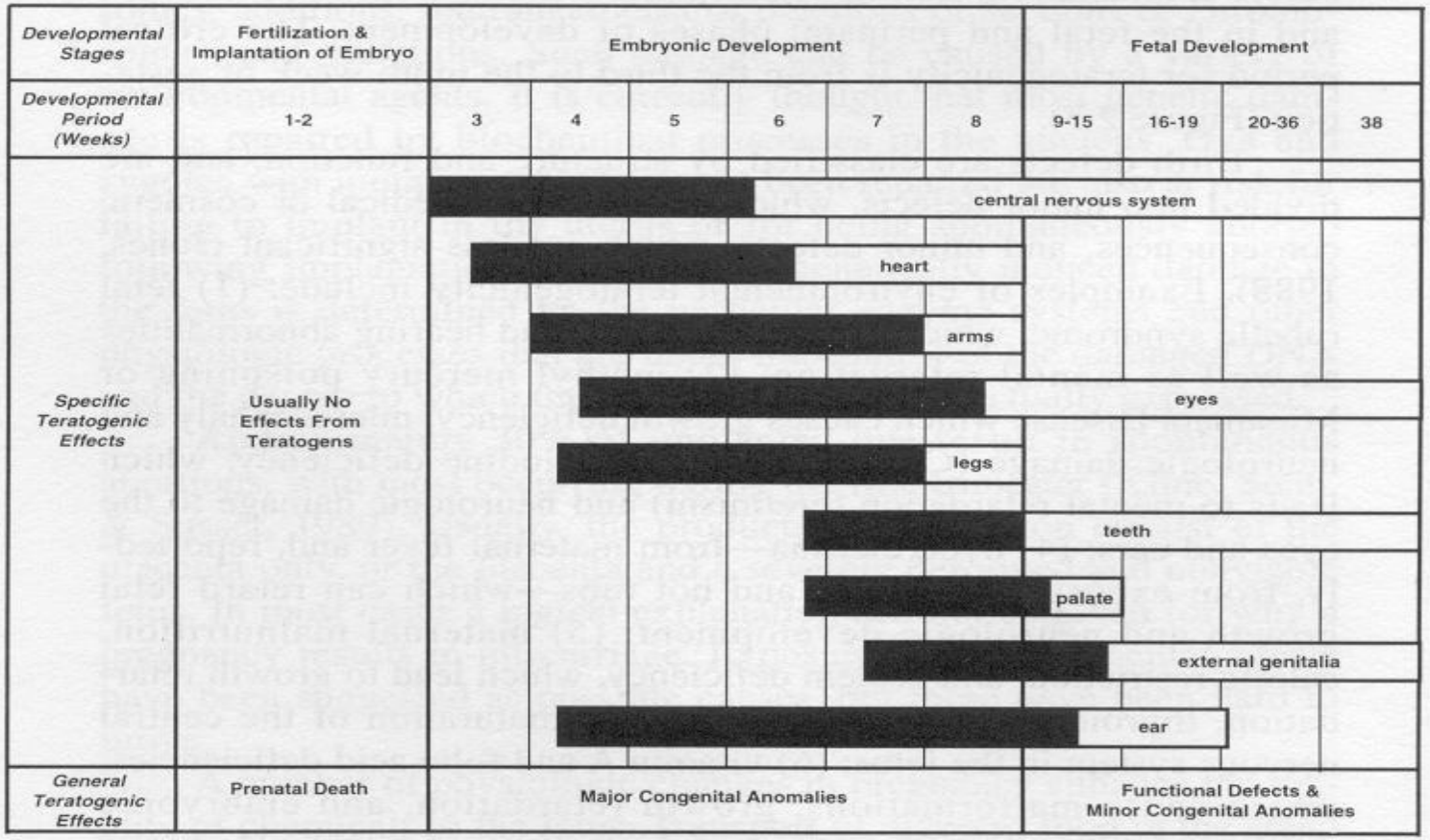
Aromatic Amine Hemoglobin Adducts in Women Smokers and Nonsmokers During Pregnancy : Correlations with Gestational Age, Neonatal Birth Weight , Ethnicity, and Pharmacogenetics

Chemical Constituents of Tobacco Smoke That Have Been Classified or Identifi their Carcinogenicity, Reproductive Toxicity, or Other Health Hazard

COMPOUND	IARC Classification[a]	U.S. EPA Classification[b]	CAL/EPA Prop 65[c]//TAC[d]
Organic Compounds			
Acetaldehyde	2B	B2	yes//yes
Acetamide	2B		yes//yes
Acrolein	3	C	--- //yes
Acrylonitrile	2A	B1	yes//yes
4-Aminobiphenyl	1		yes//yes
Aniline	3	B2	yes//yes
o-Anisidine	2B		yes//yes
Benz[a]anthracene	2A	B2	yes//yes
Benzene	1	A	yes//yes
Benzo[b]fluoranthene	2B	B2	yes//yes
Benzo[j]fluoranthene	2B		yes//yes
Benzo[k]fluoranthene	2B	B2	yes//yes
Benzo[a]pyrene	2A	B2	yes//yes
1,3-Butadiene		B2	yes//yes
Captan	3		yes//yes
Carbon disulfide[e]			yes//yes
Carbon monoxide[e]			yes//---
Chrysene	3	B2	yes//yes
DDT	2B		yes//---
Dibenz[a,h]acridine	2B		yes//yes
Dibenz[a,j]acridine	2B		yes//yes
Dibenz[a,h]anthracene	2A	B2	yes//yes
7H-Dibenzo[c,g]carbazole	2B		yes//yes
Dibenzo[a,e]pyrene	2B		yes//yes
Dibenzo[a,h]pyrene	2B		yes//yes
Dibenzo[a,i]pyrene	2B		yes//yes
Dibenzo[a,l]pyrene	2B		yes//yes
1,1-Dimethylhydrazine	2B		yes//yes
1-Naphthylamine	3		yes//---
2-Naphthylamine	1		yes//---
Nicotine[e]			yes//---
2-Nitropropane	2B		yes//yes
N-Nitrosodi-n-butylamine	2B	B2	yes//---
N-Nitrosodiethanolamine	2B	B2	yes//---
N-Nitrosodiethylamine	2A	B2	yes//---
N-Nitroso-n-methylethylamine	2B	B2	yes//---
N'-Nitrosonornicotine	2B		yes//---
N-Nitrosopiperidine	2B		yes//---
N-Nitrosopyrrolidine	2B		---//yes
Styrene	2B		---//yes
Toluene[e]			yes//yes
2-Toluidine	2B		yes//yes
Urethane	2B		yes//---
Vinyl chloride	1		yes//yes

Relationship between Gestational Age, Neonatal Birth Weight, and Hemoglobin Adducts to Benzo[*a*]pyrene in Maternal Smokers and Nonsmokers

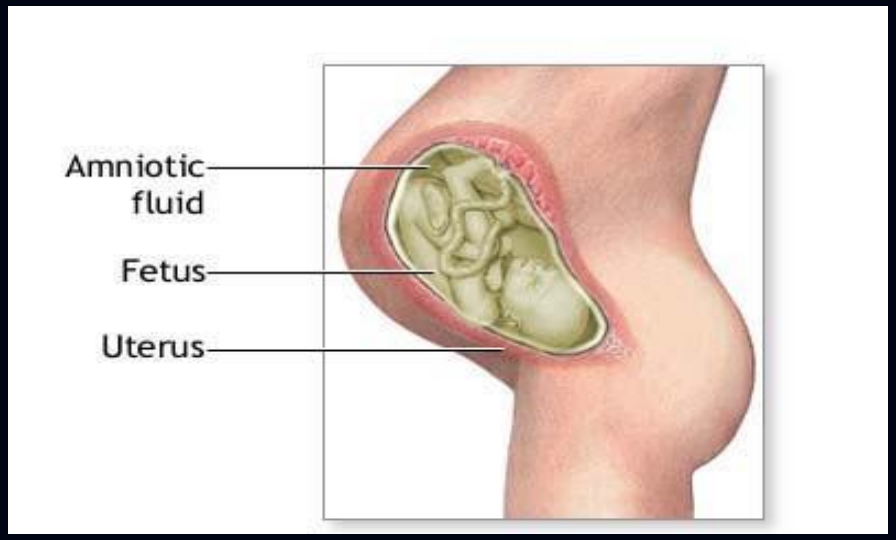
Compounds	Processed tobacco (per gram)	Mainstream (per cigarette)	Evidence for IARC evaluation of carcinogenicity	
			In lab animals	In humans
PAH				
Benz(a)anthracene		20-70 ng	Sufficient	NA
Benzo(b)fluoranthene		4-22 ng	Sufficient	NA
Benzo(j)fluoranthene		6-21 ng	Sufficient	NA
Benzo(k)fluoranthene		6-12 ng	Sufficient	NA
Benzo(a)pyrene	0.1-90 ng	20-40 ng	Sufficient	Probable
Chrysene		40-60 ng	Sufficient	NA
Dibenz(a,h)anthracene		4 ng	Sufficient	NA
Dibenzo(a,l)pyrene		1.7-3.2 ng	Sufficient	NA
Dibenzo(a,l)pyrene		Present	Sufficient	NA
Indeno(1,2,3-c,d)pyrene		4-20 ng	Sufficient	NA
5-Methylchrysene		0.6 ng	Sufficient	NA

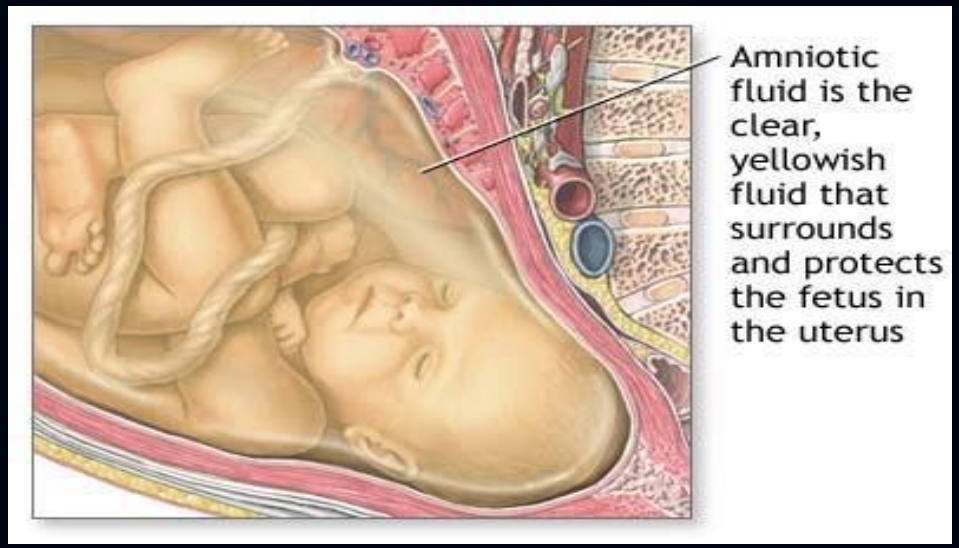

Developmental Stages
Fertilization & Implantation of Embryo
Embryonic Development
Fetal Development
Developmental Period (Weeks)
1-2
3
4
5
6
7
8
9-15
16-19
20-36
38
Specific Teratogenic Effects
Usually No Effects From Teratogens
central nervous system
heart
arms
eyes
legs
teeth
palate
external genitalia
ear
General Teratogenic Effects
Prenatal Death
Major Congenital Anomalies
Functional Defects & Minor Congenital Anomalies

Assessing Tobacco Exposure During Pregnancy

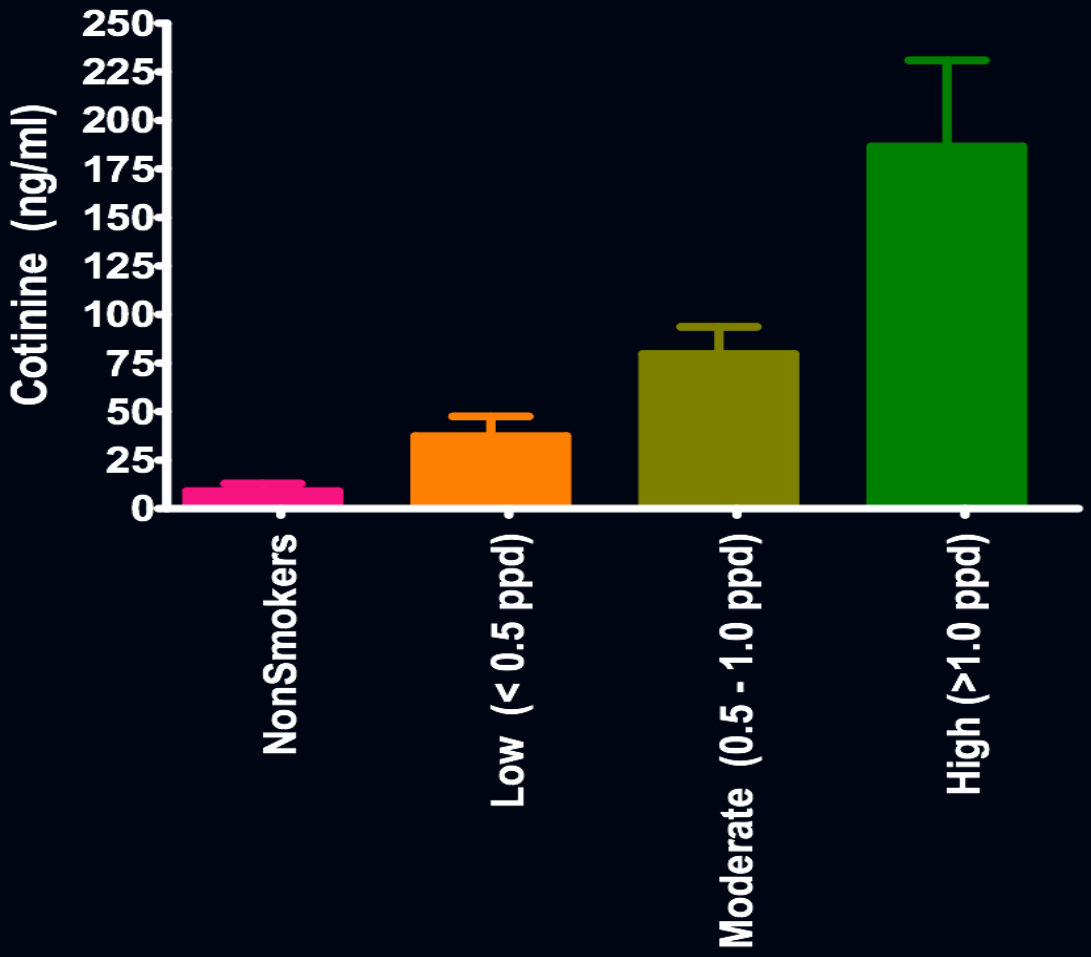
Amniotic Fluid PAH

Biomarkers of PAH exposure during pregnancy (1st trimester exposure assessment(

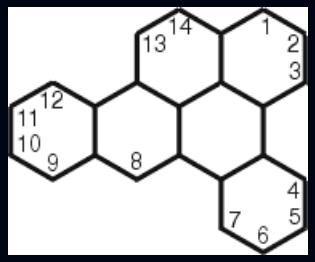


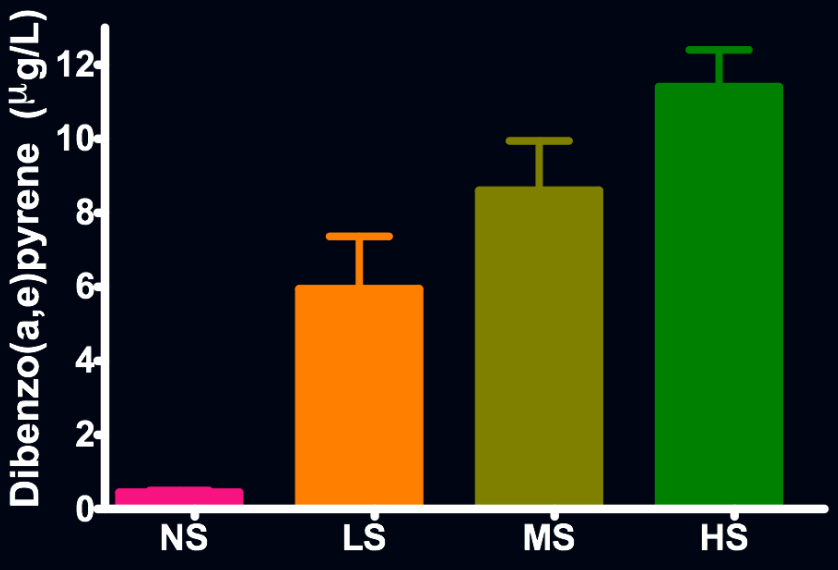


Amniotic Fluid Cotinine

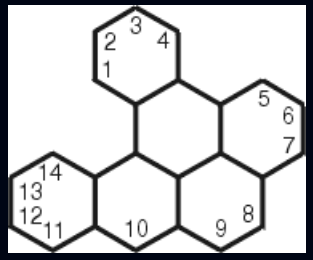


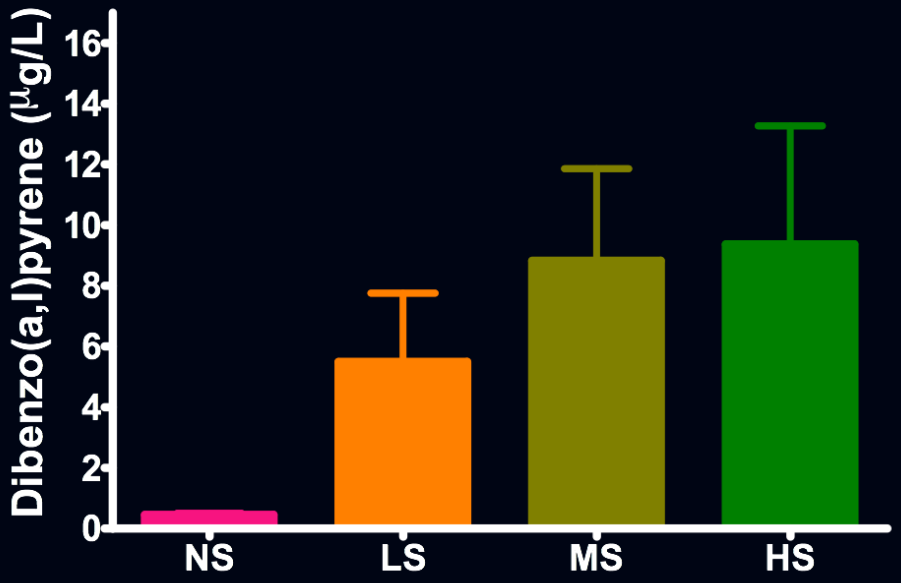
Dibenzo(a,e)pyrenes



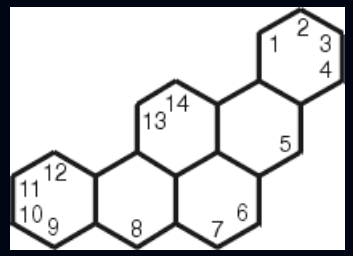


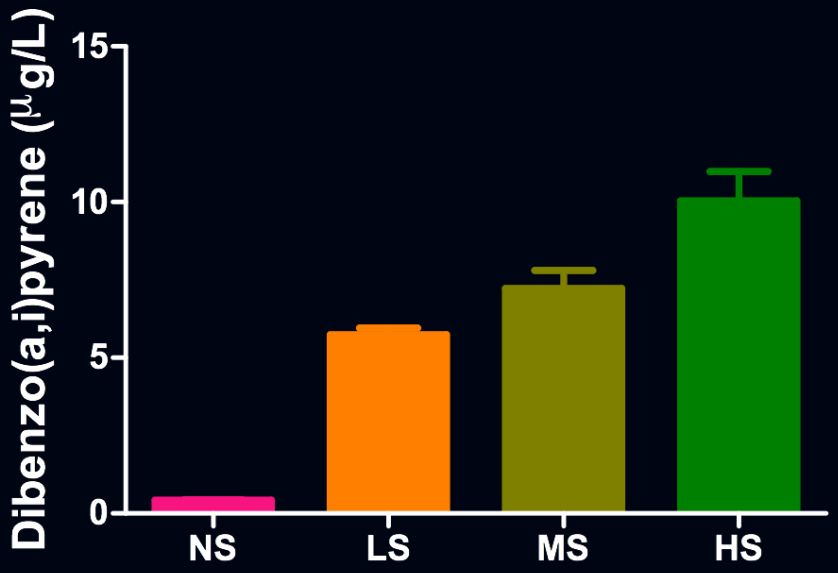
Dibenzo(a,l)pyrenes



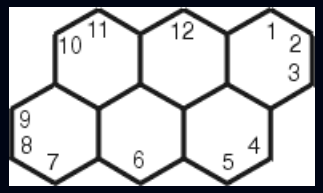


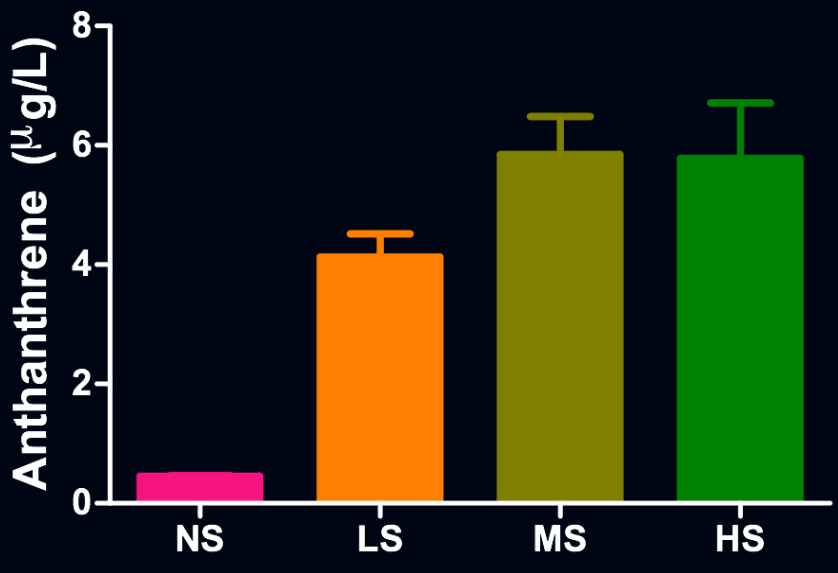
Dibenzo(a,i)pyrenes

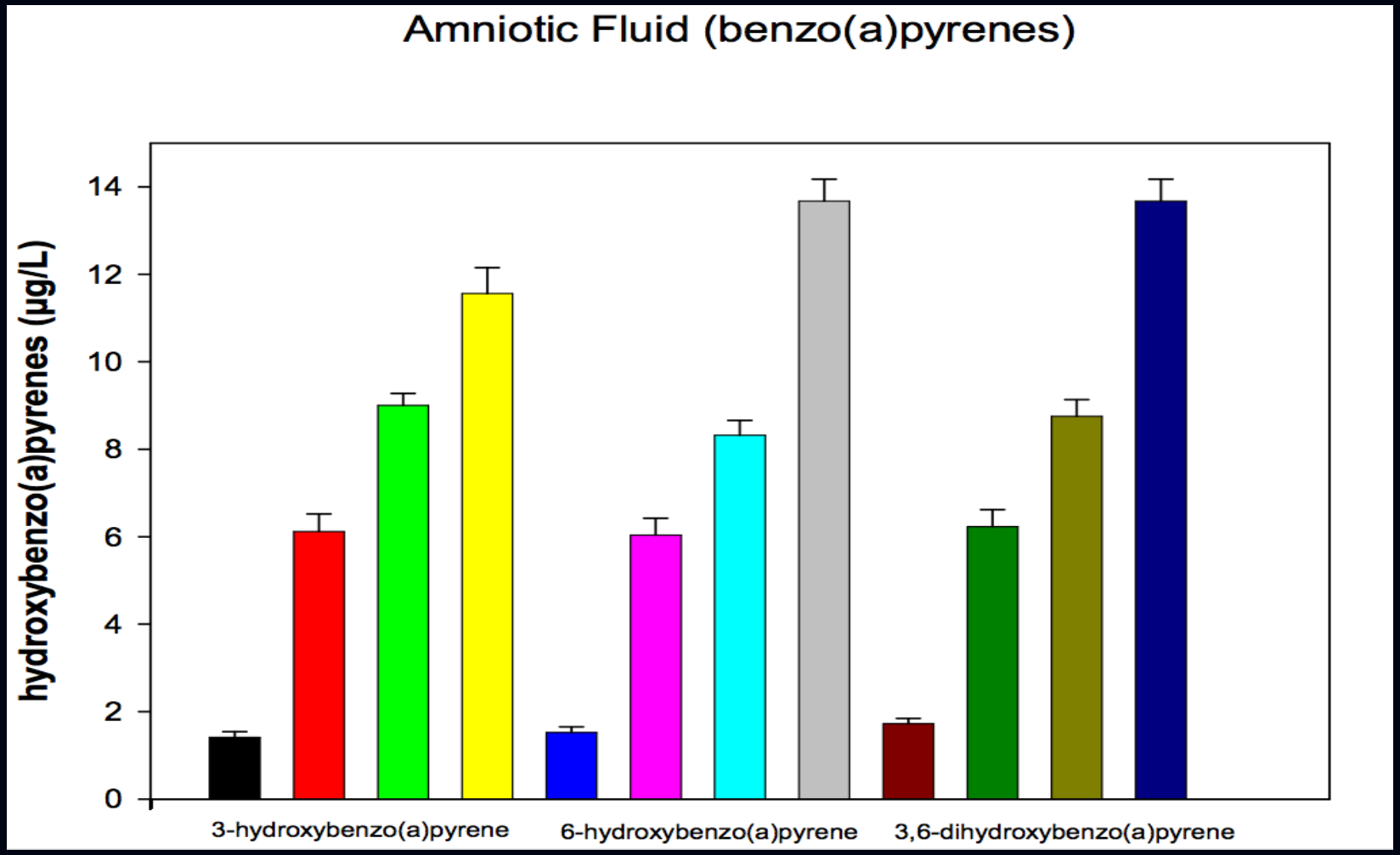


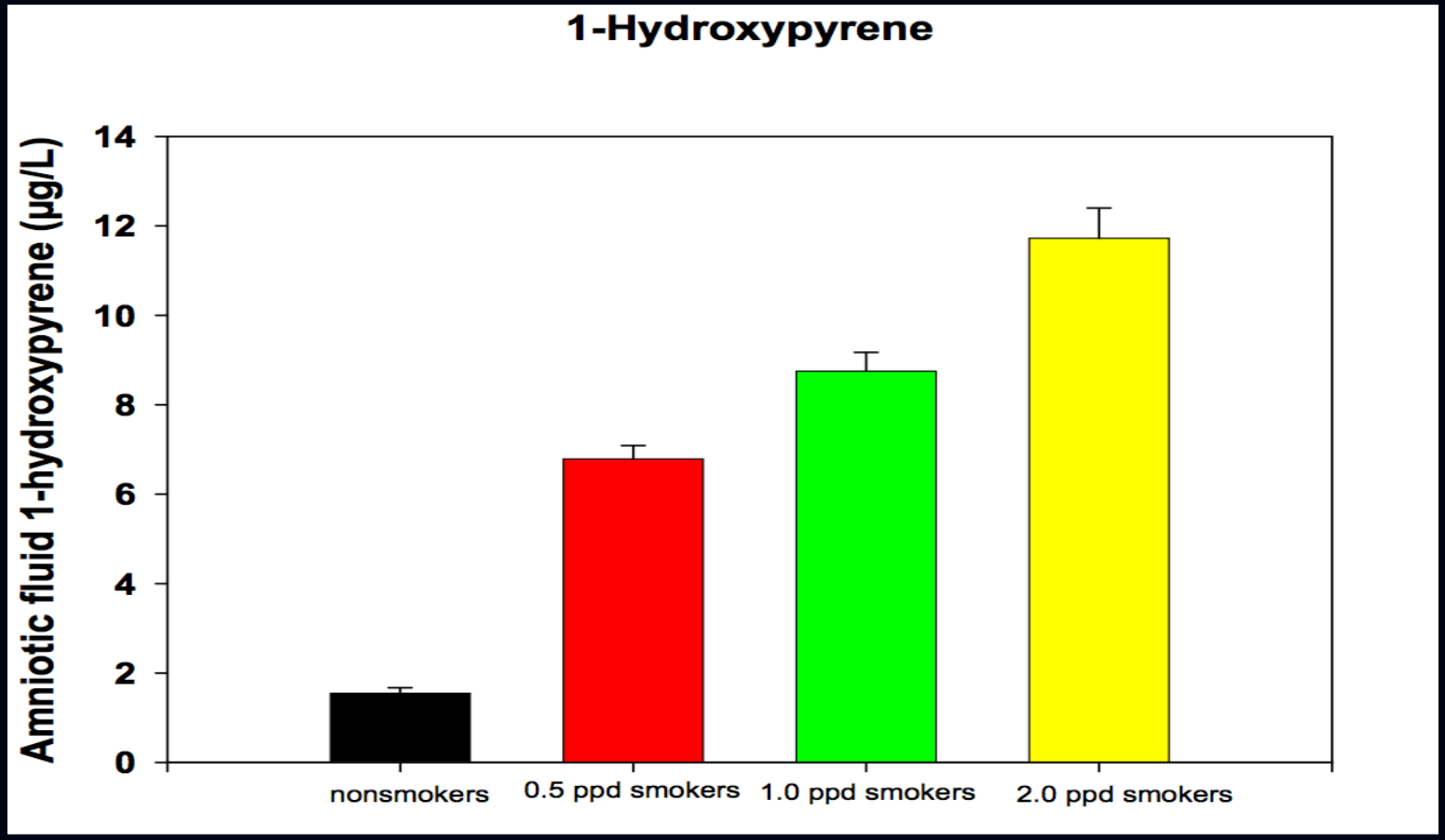


Anthanthrene

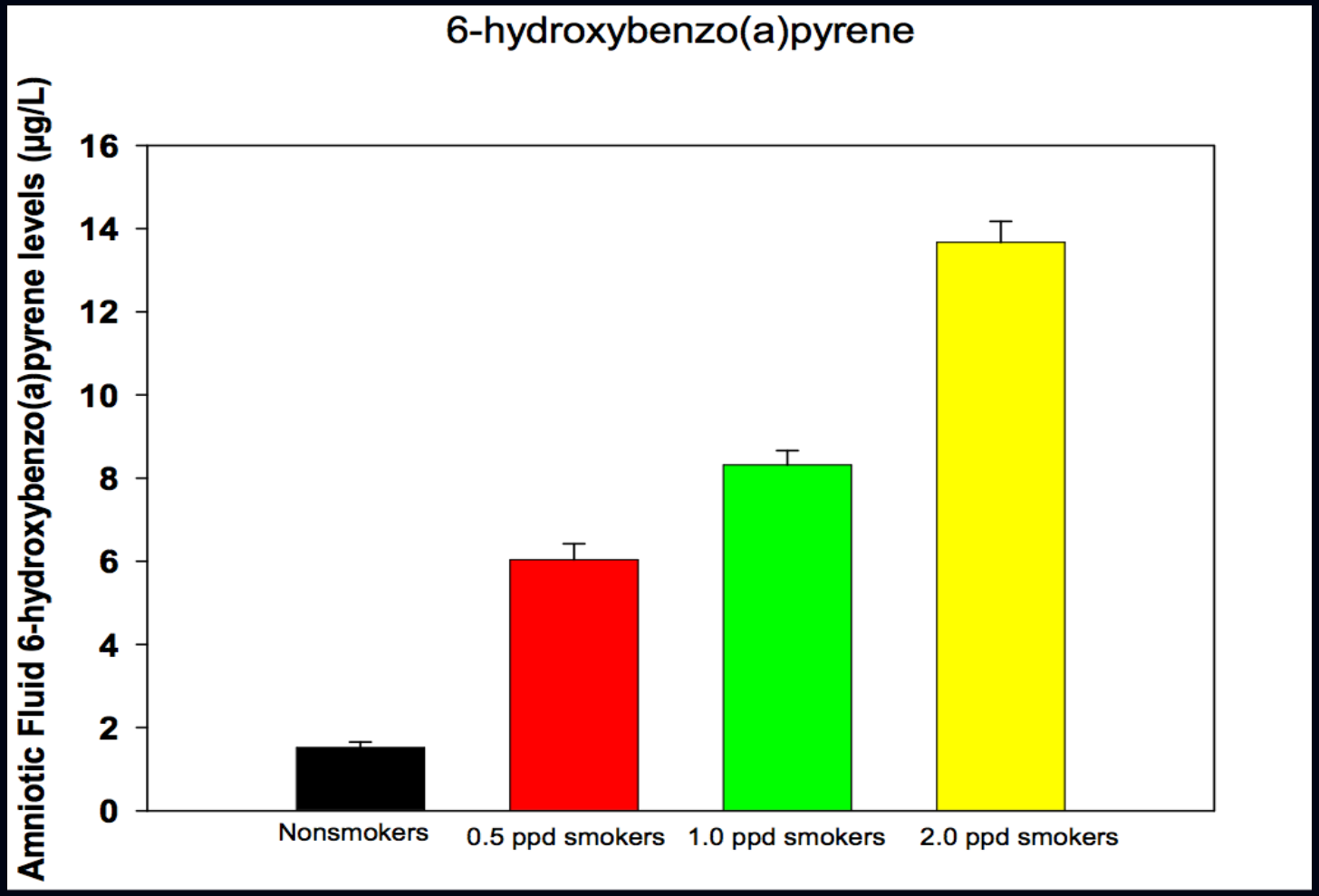




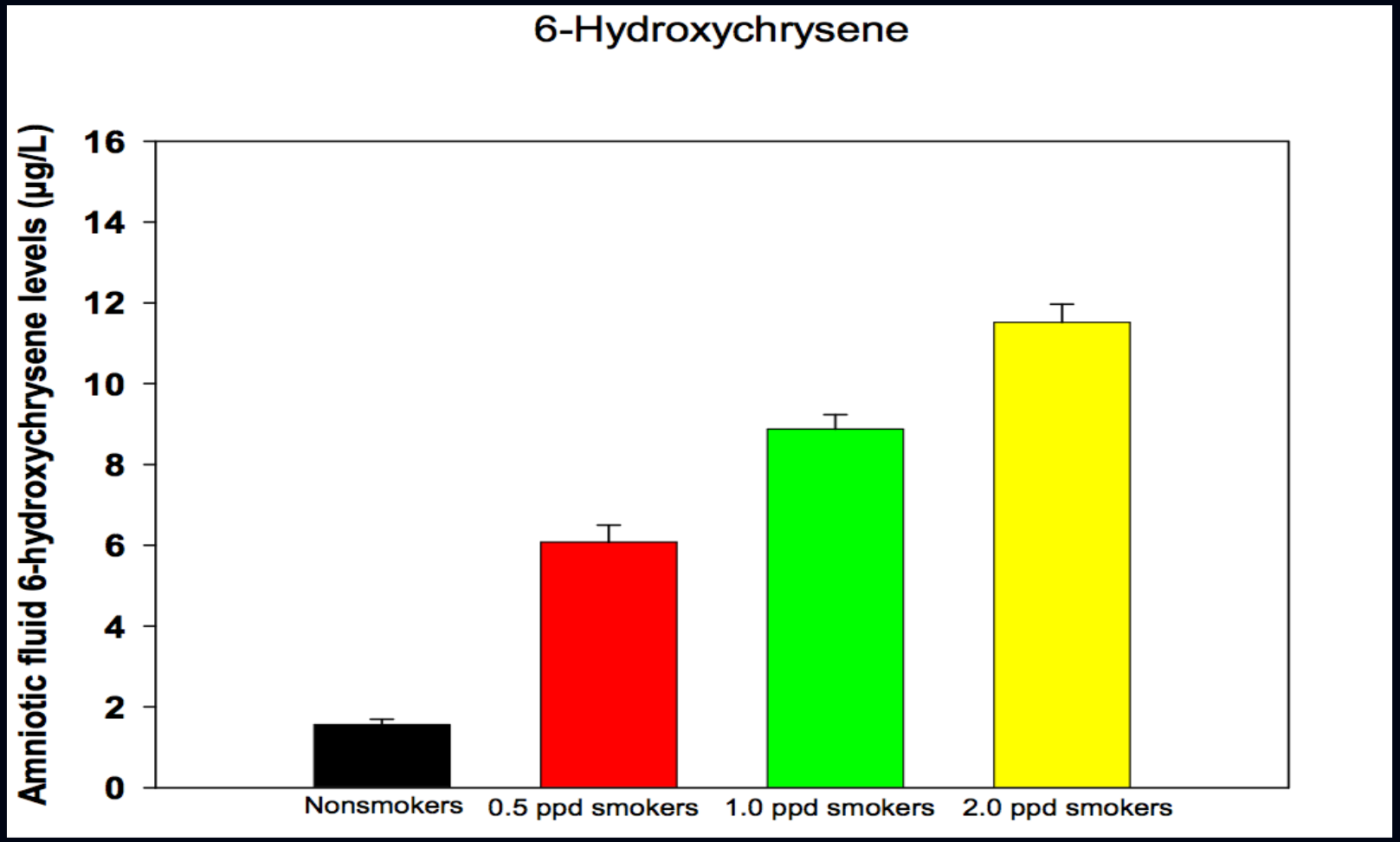

Amniotic Fluid (benzo(a)pyrenes)
hydroxybenzo(a)pyrenes (µg/L)
0
2
4
6
8
10
12
14
3-hydroxybenzo(a)pyrene
6-hydroxybenzo(a)pyrene
3,6-dihydroxybenzo(a)pyrene



Levels of 1-hydroxypyrene detected in amniotic fluid samples from nonsmokers and smokers



Levels of 6-hydroxybenzo(a)pyrene detected in amniotic fluid samples from nonsmokers and smokers

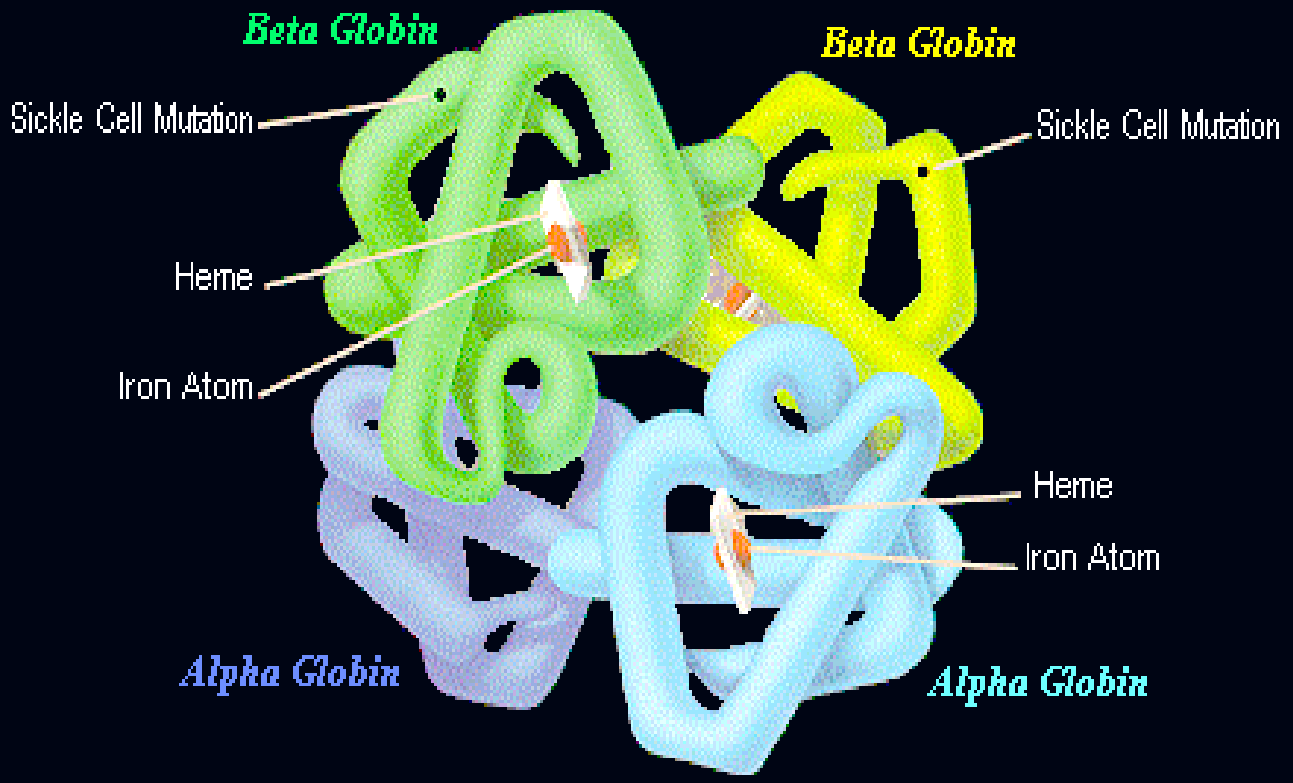


Levels of 6-hydroxychrysene detected in amniotic fluid samples from nonsmokers and smokers

Application of Hemoglobin Adducts in Maternal and Fetal Blood as Biomarkers to Tobacco Carcinogens

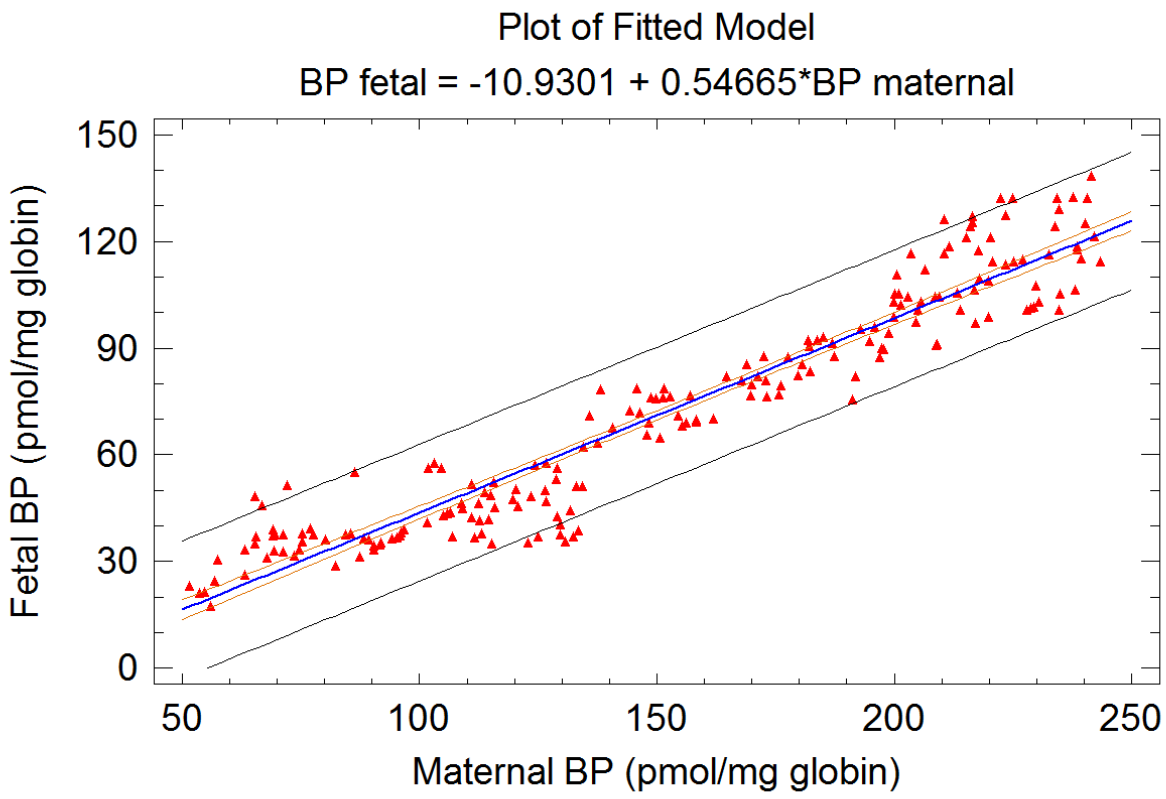
A Molecule To Breathe With

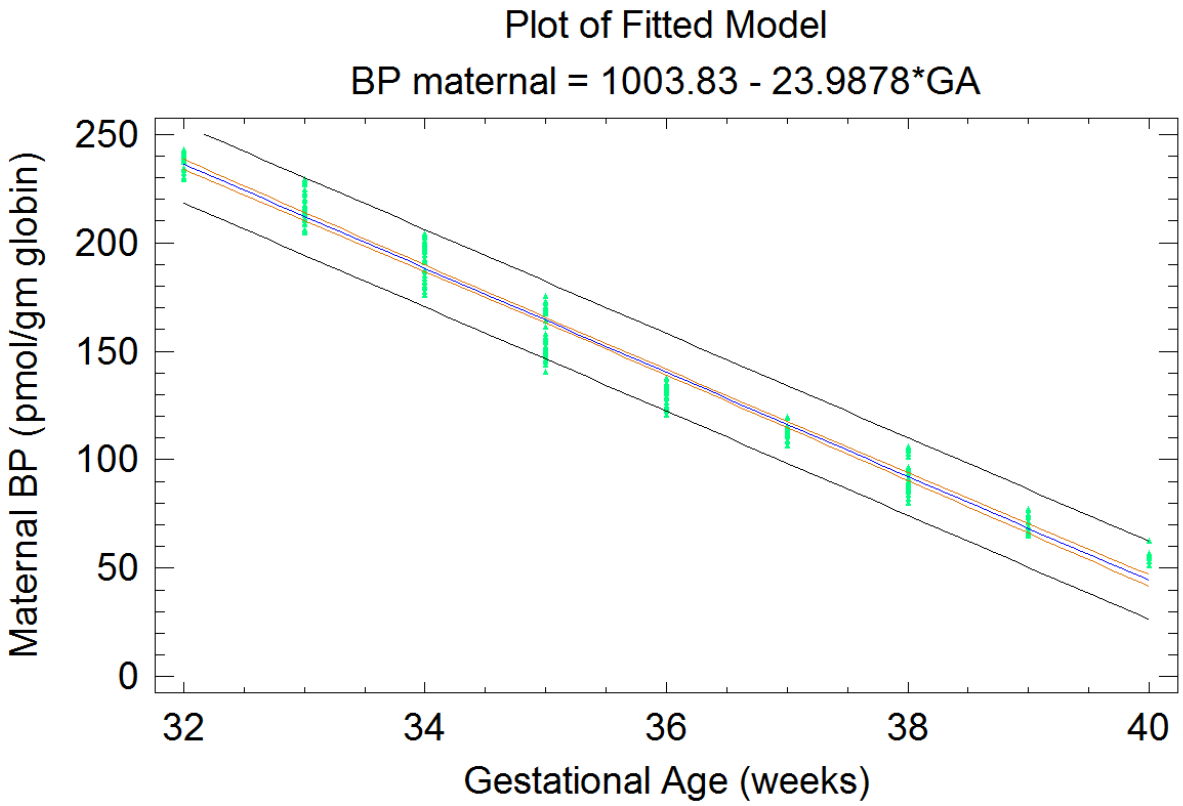
HEMOGLOBIN

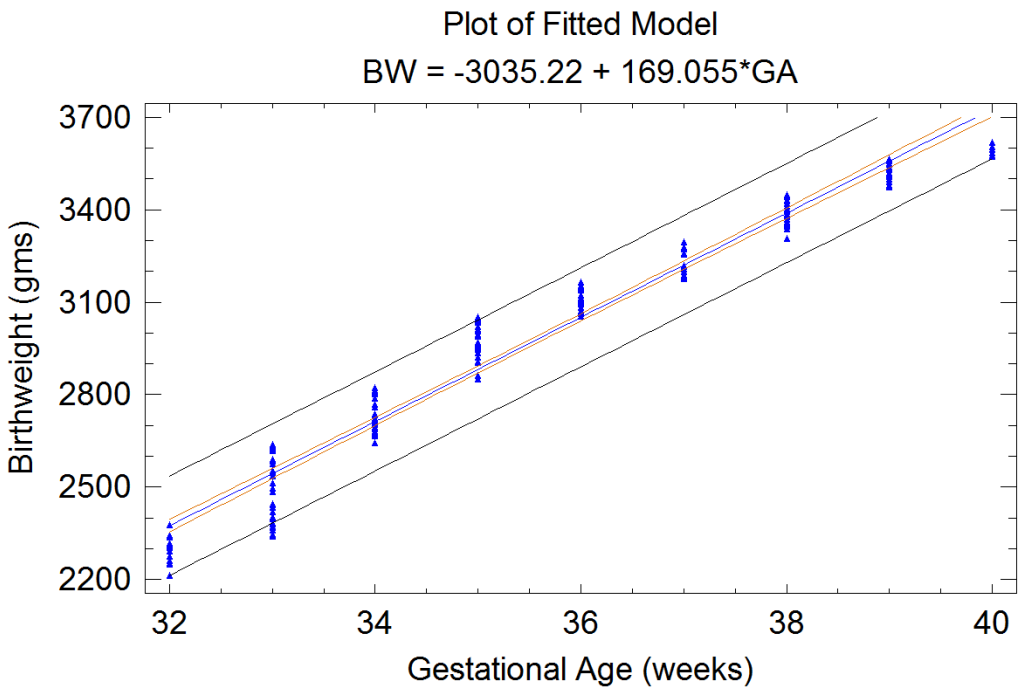


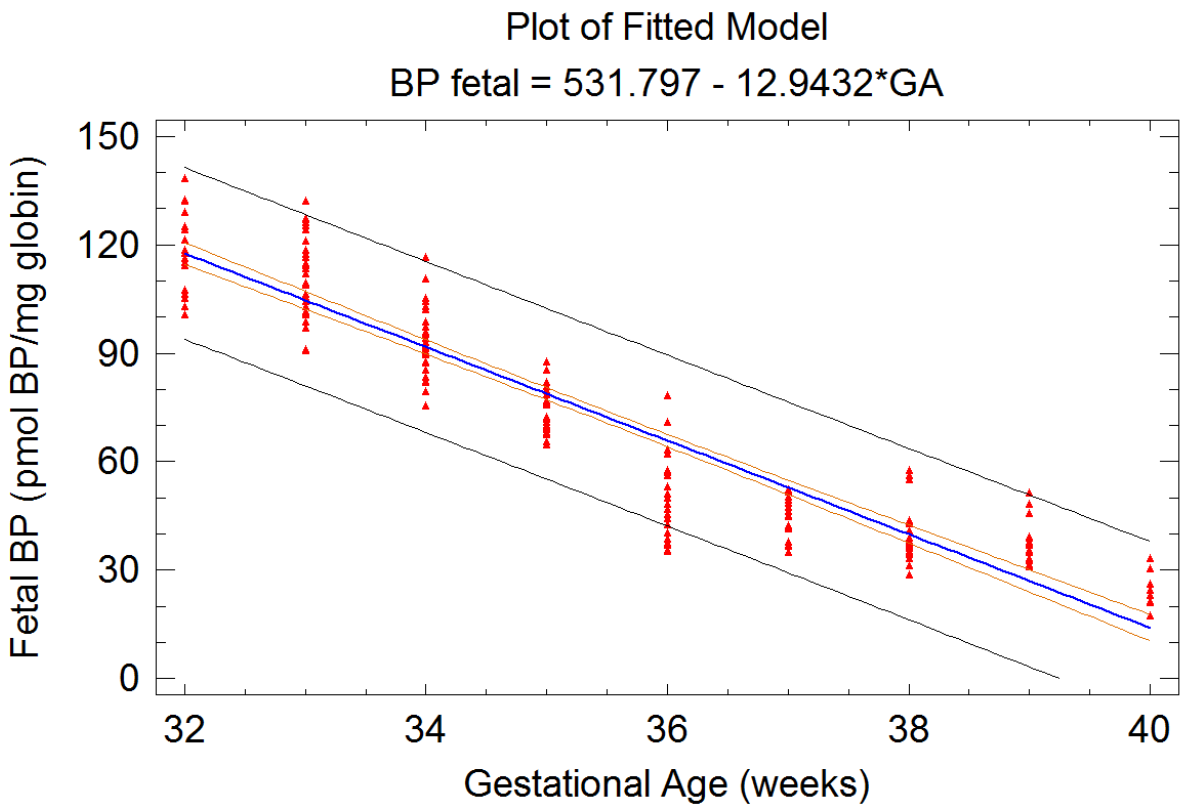
Aromatic Amine Hemoglobin Adducts in Women Smokers and Nonsmokers During Pregnancy : Correlations with Gestational Age, Neonatal Birth Weight , Ethnicity, and Pharmacogenetics

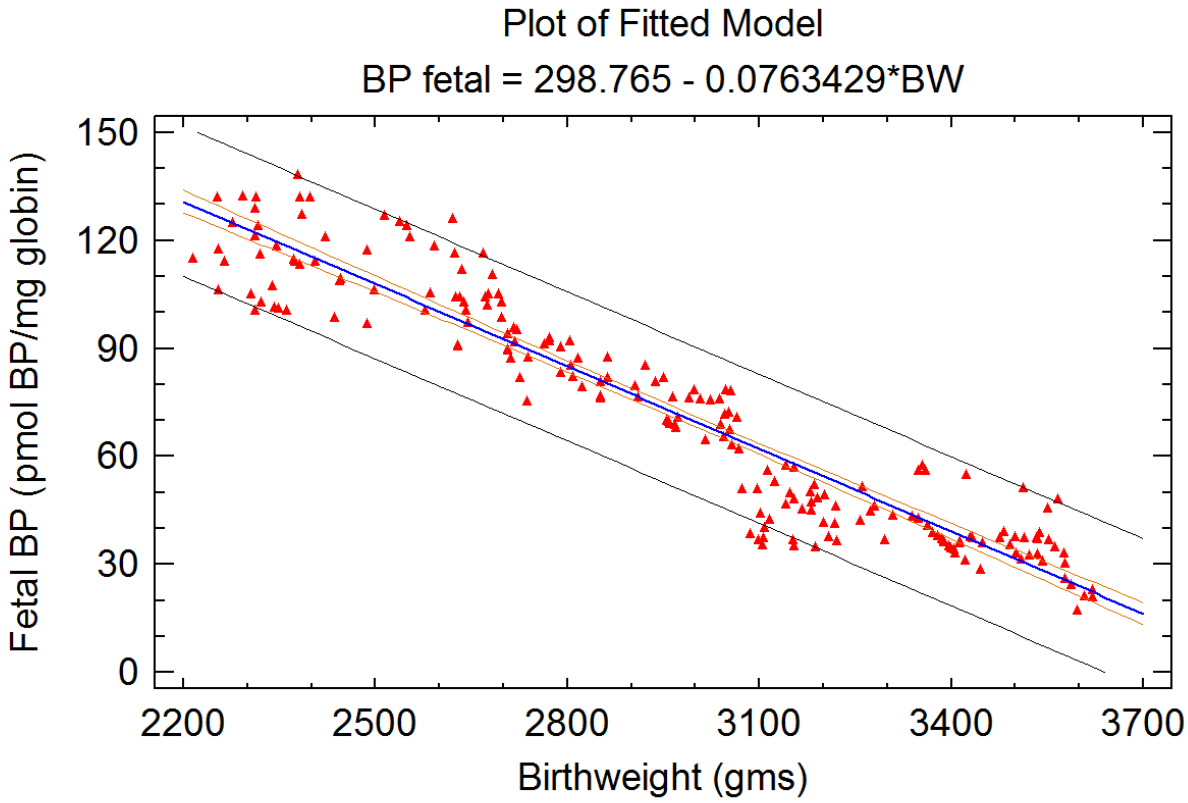
- **Matched Maternal and Cord Blood Samples obtained from Norton's Hospital Downtown and Norton's Suburban Hospital**
- **Ethnicities**
  - Caucasian
  - African American
  - Hispanic
- **Sample Characteristics**
  - Women (18 - 35 years of age(
  - Single pregnancy (no multiples(
  - No pre-existing health problems
  - Women that have significant health related effects during pregnancy are eliminated from study
  - Smokers (> 1 pack per day smokers(
  - 32 >week gestational age infants not included


Plot of Fitted Model
BP fetal = -10.9301 + 0.54665*BP maternal
Fetal BP (pmol/mg globin)
150
120
90
60
30
0
50
100
150
200
250
Maternal BP (pmol/mg globin)


Plot of Fitted Model
BP maternal = 1003.83 - 23.9878*GA
Maternal BP (pmol/gm globin)
250
200
150
100
50
0
32
34
36
38
40
Gestational Age (weeks)


Plot of Fitted Model
BW = -3035.22 + 169.055*GA
Birthweight (gms)
3700
3400
3100
2800
2500
2200
32
34
36
38
40
Gestational Age (weeks)


Plot of Fitted Model
BP fetal = 531.797 - 12.9432*GA
Fetal BP (pmol BP/mg globin)
150
120
90
60
30
0
32
34
36
38
40
Gestational Age (weeks)


Plot of Fitted Model
BP fetal = 298.765 - 0.0763429*BW
Fetal BP (pmol BP/mg globin)
150
120
90
60
30
0
2200
2500
2800
3100
3400
3700
Birthweight (gms)

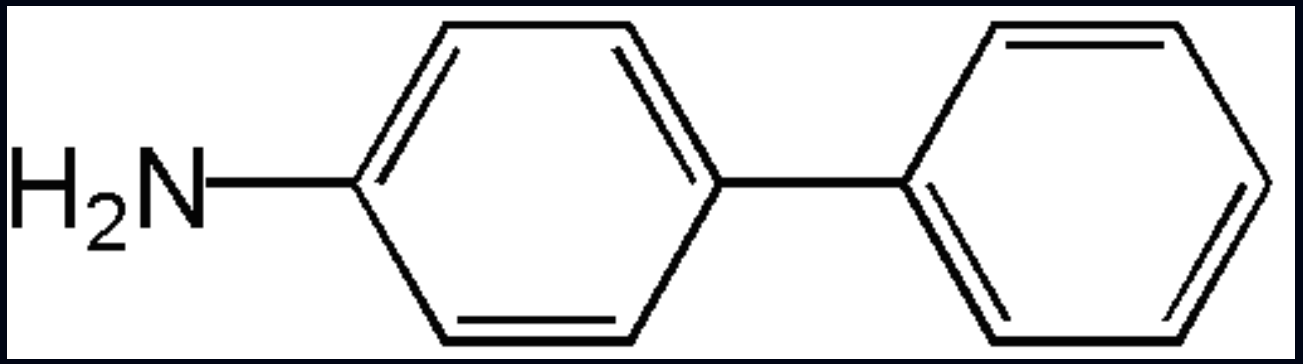
Aromatic Amine Hemoglobin Adducts in Women Smokers and Nonsmokers During Pregnancy : Correlations with Gestational Age, Neonatal Birth Weight , Ethnicity, and Pharmacogenetics

Aromatic Amines and related carcinogens in Tobacco

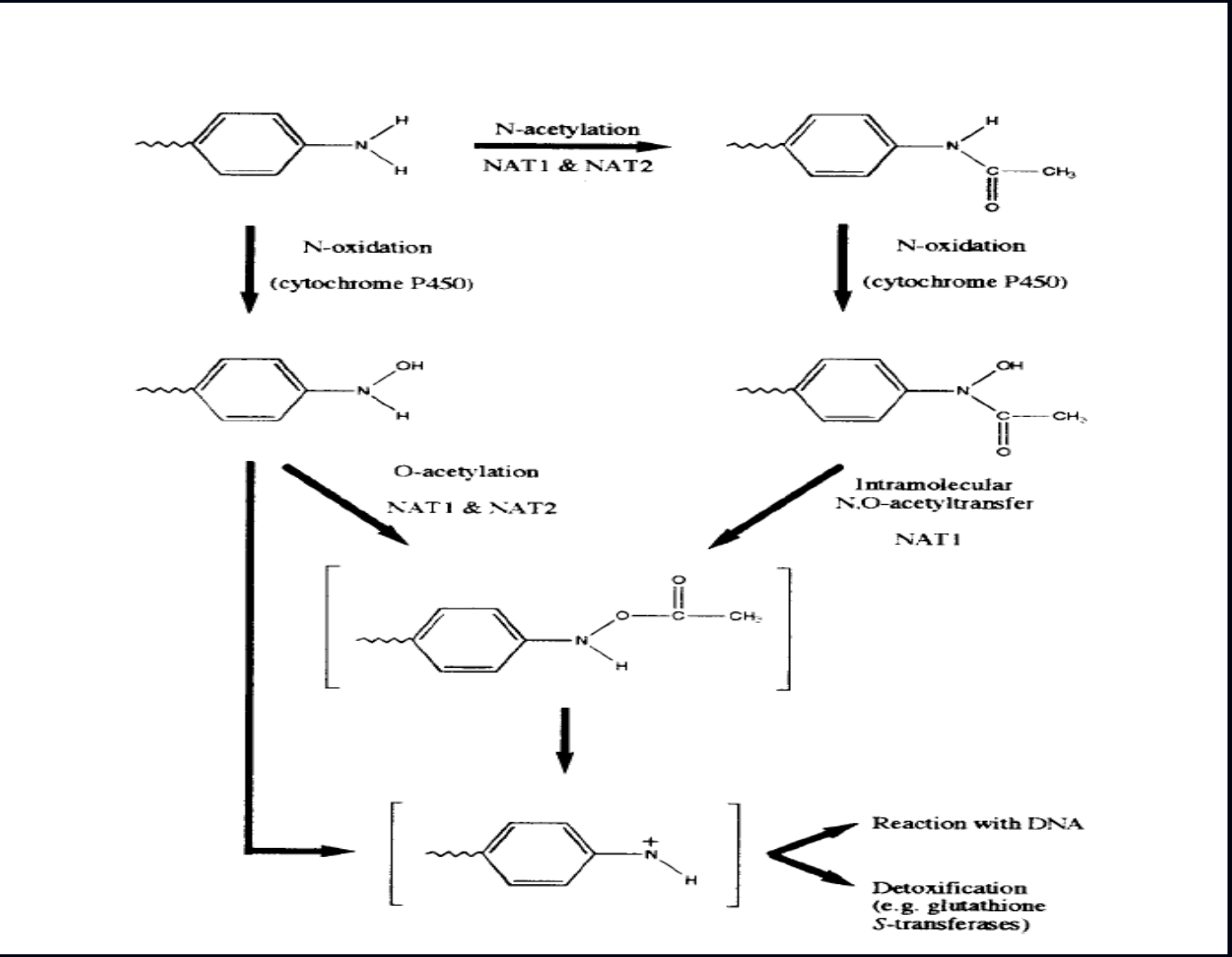
- aniline
- -2toluidine
- -3toluidine
- -4toluidine
- -2ethylaniline
- -3ethylaniline
- -4ethylaniline
- -2,3dimethylaniline
- -2,4dimethylaniline
- -2,5dimethylaniline
- -2,6dimethylaniline
- -1naphthylamine
- -2naphthylamine
- -2methyl-1-naphthylamine
- -2aminobiphenyl
- -3aminobiphenyl
- -4aminobiphenyl
- -1nitropyrene
- -4nitropyrene
- -1,3dinitropyrene
- -1,6dinitropyrene
- -1,8dinitropyrene
- -6nitrochrysene
- -2nitrofluorene
- -5nitroacenaphthylene
- -3,7dinitrofluoranthene
- -3,9dinitrofluoranthene

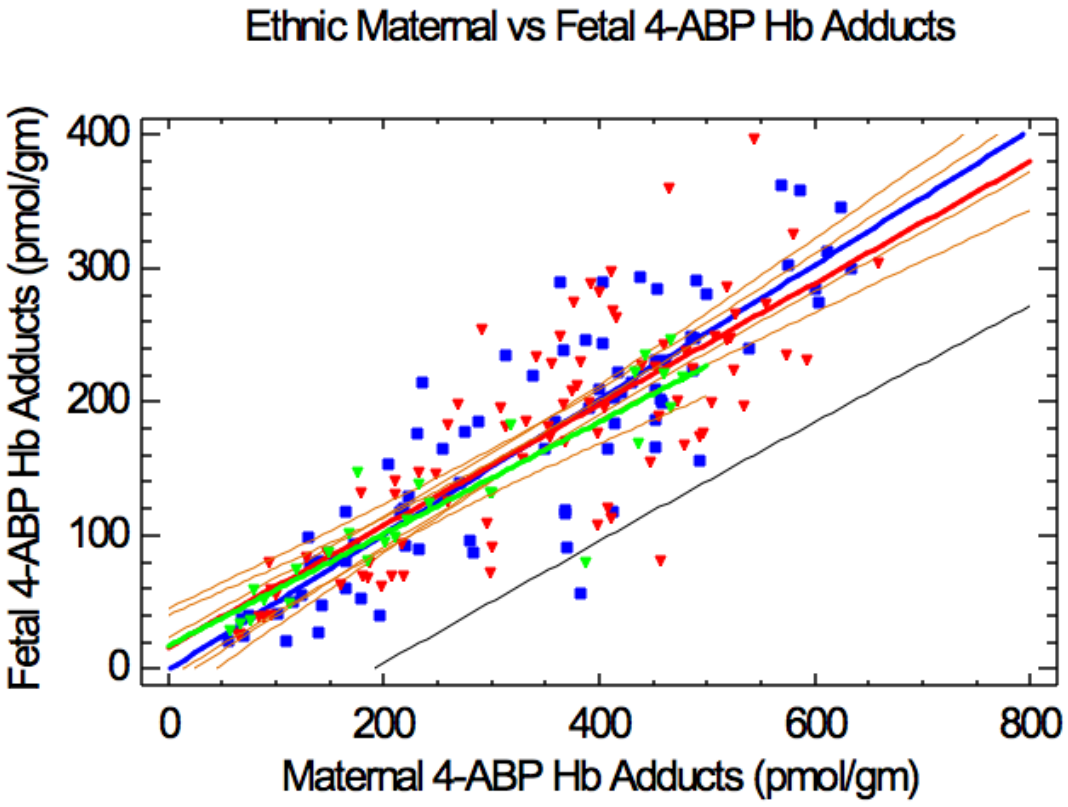
-4Aminobiphenyl Concentration in Tobacco

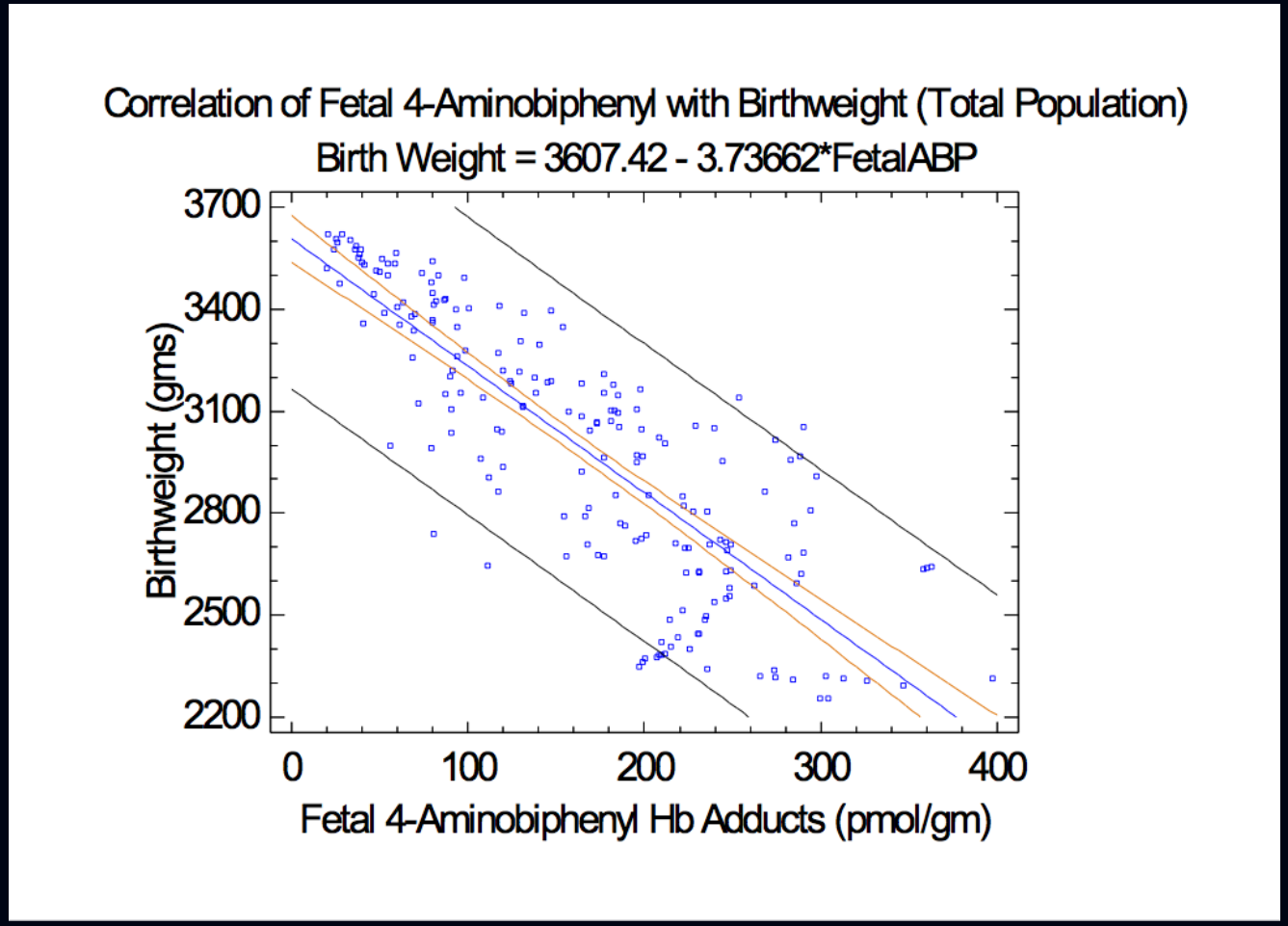
Mainstream cigarette smoke contains 4-aminobiphenyl at levels of 2.4 to 4.6 ng per cigarette (unfiltered) and 0.2 to 23 ng per cigarette (filtered), and sidestream smoke contains up to 140 ng per cigarette



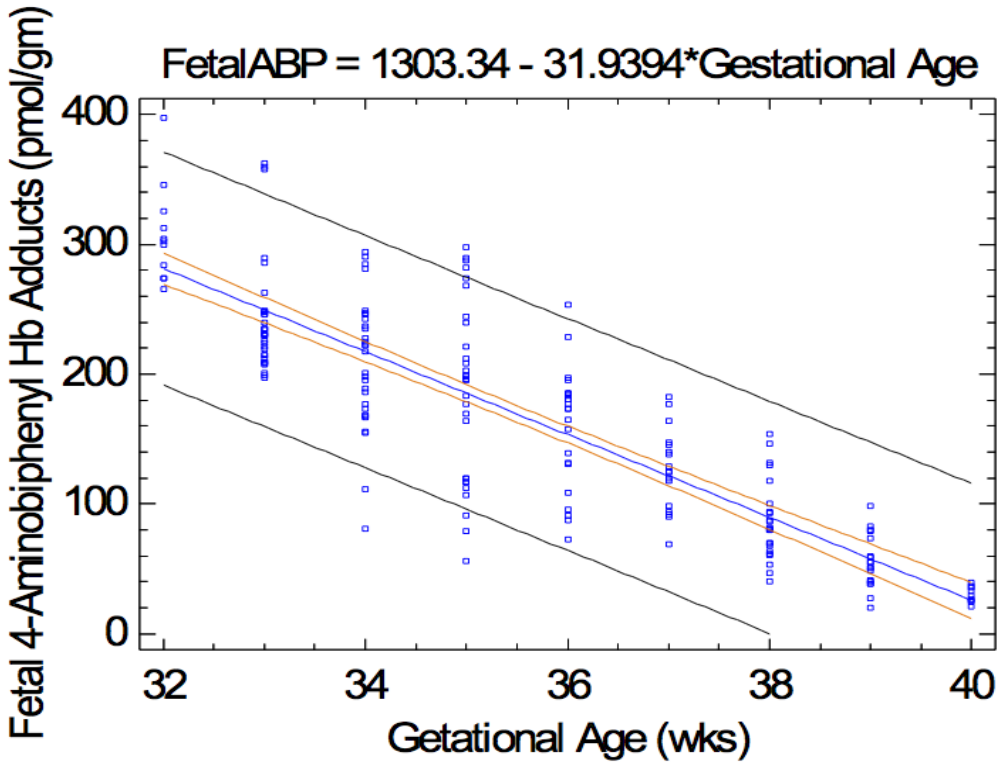
Aromatic Amine Hemoglobin Adducts in Women Smokers and Nonsmokers During Pregnancy : Correlations with Gestational Age, Neonatal Birth Weight , Ethnicity, and Pharmacogenetics



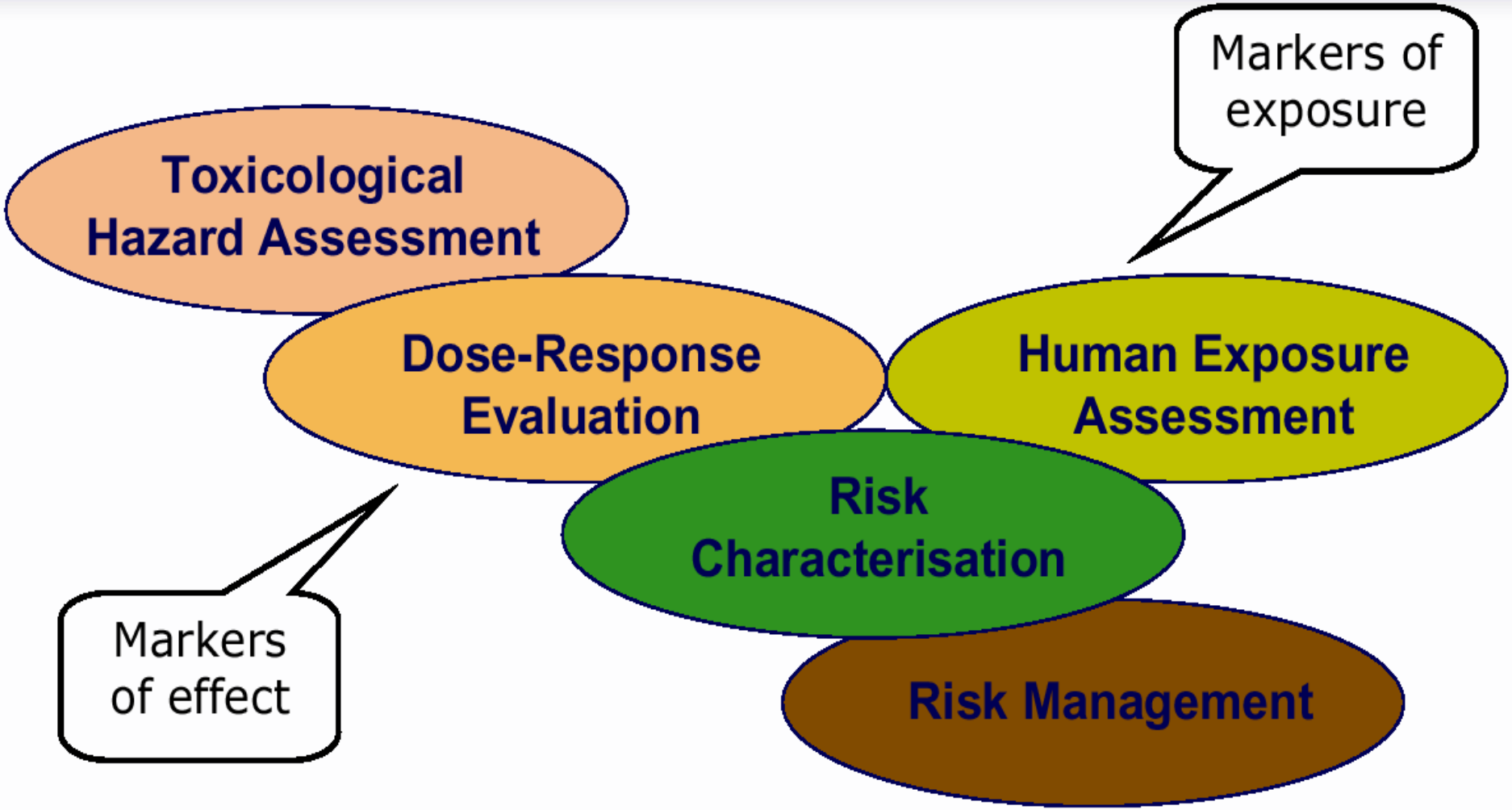

Ethnic Maternal vs Fetal 4-ABP Hb Adducts
Fetal 4-ABP Hb Adducts (pmol/gm)
400
300
200
100
0
0
200
400
600
800
Maternal 4-ABP Hb Adducts (pmol/gm)


Correlation of Fetal 4-Aminobiphenyl with Birthweight (Total Population)
Birth Weight = 3607.42 - 3.73662*FetalABP
Birthweight (gms)
3700
3400
3100
2800
2500
2200
0
100
200
300
400
Fetal 4-Aminobiphenyl Hb Adducts (pmol/gm)

Correlation of Gestational Age (wks) vs Fetal 4-Aminobiphenyl Hb Adducts



Use of biomarkers in risk assessment



syngenta

Conclusions