Biomarkers of exposure and effect for environmental carcinogens, and their applicability to human molecular epidemiological studies

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Exposure to an environmental carcinogen

Exposure source → Target site interaction → Adverse outcome

Biomarkers of susceptibility

Biomarkers of exposure ↔ Biomarkers of effects

EXPOSURE ASSESSMENT

RISK ASSESSMENT
Environmental cancer risk, nutrition and individual susceptibility

**Coordinator:** Konrad Rydzynski, Nofer Institute of Occupational Medicine, Lodz, Poland

**Duration:** 2005-2010

**Budget:** 11.0 million euro

**Network:** 25 partners, 13 countries

- The main focus of this virtual research centre of excellence will be on the use of biomarkers of exposure and bioindicators of disease in molecular epidemiology of cancer
ECNIS: 25 participants (including SMEs) working in fields related to carcinogenesis such as: diet, environment, occupation, lifestyle, exposure assessment

NIOM (Poland)
VUB (Belgium)
UCL (Belgium)
UC (Denmark)
FIOH (Finland)
DKFZ (Germany)
UM (Germany)
BIU (Germany)
NHRF (Greece)
FJOKK (Hungary)
ISI (Italy)
IRCCS (Italy)
Collegium Med. (Poland)
ICO (Spain)
KI (Sweden)
ULUND (Sweden)
UNIMAS (The Netherlands)
IRAS-UU (The Netherlands)
ULEIC (UK)
ICR (UK)
UNIVDUN (UK)
IARC (France)
NETIX (Poland)
Leocordia AB (Sweden)
ICL (UK)
The overall objectives of ECNIS

1. To overcome the fragmented nature of research in areas related to carcinogenesis caused by the environment, diet, occupation, or lifestyle, within Europe
2. To integrate joint training and mobility programs in area of environmental cancer molecular epidemiology
3. To develop and validate novel biomarkers of exposure, effect and susceptibility for environmental and occupational cancer risk assessment
4. To identify factors that modulate the environmental and occupational cancer risk resulting from nutrition and lifestyle factors
5. To develop hazard and risk assessment strategies based on mechanism of action of carcinogens
6. To disseminate of acquired knowledge to the scientific community and to external stakeholders
14 workpackages

- **Integrating Activities:**
  Co-ordinated research planning, personnel mobility and sharing infrastructures and data

- **Joint Research Activities:**
  Multidisciplinary investigations in the fields of molecular cancer epidemiology, environmental carcinogenesis and its modulation by nutrition and genetics

- **Spreading of Excellence Activities:**
  Training and mobility programmes and sharing of new scientific knowledge with researchers, the general public, regulators, health care specialists, industry, etc.
Inventory of available resources

Reviews/reports
• biomarkers of carcinogen exposure and early effects
• state of validation of biomarkers of carcinogen exposure and early effects and their applicability to molecular epidemiology
• epidemiological concepts of validation of biomarkers for the identification/quantification of environmental carcinogen exposures

Research projects mostly on validation including: urinary DNA oxidation products; acetaldehyde DNA adducts; Comet assay for DNA damage, $^{32}$P-postlabeling; genotype methodology; long lived adducts
Major aspects in interpretation of biomarker data: ECETOC

The analytical integrity of data
• *Is the answer right?*

The data's ability to describe exposure
• *Is the answer specific and selective?*

The relationship between the biomarker and effects
• *What is the biological relevance of the answer?*

An overall evaluation and weight of evidence
• *What is the risk assessment and what do we tell the subject?*

Guidance for the interpretation of biomonitoring data. Document No 44, 2005

http://www.ecetoc.org
Carcinogen biomarkers

The analytical integrity of data. *Is the answer right?*

Analytical validation

- standard operating procedures for
  - pre-analytical (sample collection, storage)
  - analytical (procedures, quality controls)
  - post-analytical (statistics, reporting)

- recoveries, reproducibilities and accuracy, limit of detection/quantitation, etc

- interlaboratory comparison/ comparison of equipment/platforms
Exposure to an environmental carcinogen: analytical integrity of data

Exposure source → Absorption metabolism → Site of toxicological action → Early biological effects → Altered structure/function

Biomarkers

- Exposure determination
- Plasma and urine concentrations
- Target site interaction e.g. DNA adducts
- Early molecular events
  - Gene expression
  - Proteomics
  - Metabonomics
  - Mutation
  - Cytogenetic alterations

Analytical validation

+  +  +/−  +/−
Analytical Validation: interlaboratory, comparison of methods

Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus
ESCODD (European Standards Committee On Oxidative DNA Damage)

### Analytical Validation: Interlaboratory, comparison of methods

Comparison of the DNA adduct levels obtained by different methods for the samples of the second interlaboratory trial

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method of analysis of DNA adducts</th>
<th>${^3}H$ incorporation</th>
<th>Mass spectrometry</th>
<th>${^{32}}P$-postlabelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>High BaP</td>
<td>137.6</td>
<td>20.5</td>
<td></td>
<td>22.2 ± 9.4 (n=33)</td>
</tr>
<tr>
<td>Low BaP</td>
<td>86.4</td>
<td>8.5</td>
<td></td>
<td>11.4 ± 4.1 (n=33)</td>
</tr>
</tbody>
</table>

Phillips et al, Mutagenesis, 1999, 14, 301-315
**Analytical Validation: Comparison of methods**

**Benzo(a)pyrene-N²-dG adducts - LC-MS/MS - mouse liver**

**A**
- Control liver DNA 1d
- B[a]PDE-N²dG SRM m/z 570 to 454
- [¹⁵N₅]B[a]PDE-N²dG SRM m/z 575 to 459

**B**
- Liver DNA 50mg/kg B[a]P 28d
- B[a]PDE-N²dG SRM m/z 570 to 454
- [¹⁵N₅]B[a]PDE-N²dG SRM m/z 575 to 459

**C**
- Liver DNA 200mg/kg B[a]P 1d
- B[a]PDE-N²dG SRM m/z 570 to 454
- [¹⁵N₅]B[a]PDE-N²dG SRM m/z 575 to 459

Acknowledgements: S Kyrtopoulos

3.7 fold difference between methods

Carcinogen biomarkers

The data's ability to describe exposure.  
*Is the answer specific and selective?*

Interpretation may be affected by

- lack of pharmacokinetic data/models
- alternative sources of the biomarker
  e.g. benzene, acrylamide
- endogenous production of the biomarker
  e.g. formaldehyde, ethylene oxide
Benzene metabolism

Trans,trans-muconaldehyde → Trans,trans-muconic acid

Epoxide hydrolase

Benzene → Benzene oxide

S-phenylmercapturic acid → Hydroquinone

P450

Catechol → 1,2-benzoquinone

Phenol → 1,2,4-trihydroxybenzene

1,4-benzoquinone

P450
Using urinary biomarkers to elucidate dose-related patterns of human benzene metabolism

250 benzene-exposed workers (median 1.2ppm)
139 controls (median 0.004ppm)

Median concentration of each metabolite was elevated when the group’s benzene exposure was at or above:
- Catechol 2.0ppm
- Phenol 0.5ppm
- Hydroquinone 0.5ppm
- t,t-muconic acid 0.2ppm
- S-phenylmercapturic acid 0.2ppm

Sources of background benzene metabolite levels:
- Smoke, gasoline, diet, gut flora, medicines (phenol), sorbic acid (t,t-MA)
Acrylamide: globin adducts as biomarker of exposure

\[ CH_2=CHCONH_2 \rightarrow CH_2-CHCONH_2 \]

acrylamide  \( \xrightarrow{\text{P450}} \)  glycidamide

globin-valine-NH\(_2\)

\[ \text{---Val-NH-CH}_2\text{CHCONH}_2 \rightarrow \text{---Val-NH-CH}_2\text{CH}_2\text{CONH}_2 \]

Analysed by \textit{GC-MS} after modified Edman degradation

Background adduct levels from acrylamide to N-terminal valine in hemoglobin measured in non-smokers without occupational exposure to acrylamide.

<table>
<thead>
<tr>
<th>Adduct level (nmol/g)</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 0.031; Range: 0.024 - 0.049</td>
<td>8</td>
<td>Bergmark, 1997</td>
</tr>
<tr>
<td>Mean: 0.033; Range: 0.020 - 0.047</td>
<td>6</td>
<td>Kjuus et al., 2003</td>
</tr>
<tr>
<td>Mean: ~ 0.04; Range: 0.02 - 0.07</td>
<td>18</td>
<td>Hagmar et al., 2001</td>
</tr>
<tr>
<td>Median: 0.021; Range: 0.012 - 0.050</td>
<td>25</td>
<td>Schettgen et al., 2003</td>
</tr>
<tr>
<td>Mean: 0.027 (SD: ± 0.006)</td>
<td>5</td>
<td>Paulsson et al., 2003a</td>
</tr>
</tbody>
</table>

Acrylamide is produced by cooking food - Tareke et al, 2000, 2002
Source of acrylamide adduct background levels: baked and fried carbohydrate-rich foods

Acrylamide in foods (Ahn et al, 2002)

- potatoes
- frying chips
- chipped, fried
- cooked, 12 min
- over-cooked, 17 min
- raw boiled
- as sold

Acrylamide adduct background levels:
- baked and fried carbohydrate-rich foods
- over-cooked
- cooking time
- ppb

Acrylamide concentration in foods (Ahn et al, 2002)
Formaldehyde

Causes nasopharyngeal cancer in humans
IARC: “strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde”.
Direct acting *in vitro* mutagen

Normal intermediary metabolite in humans.
Endogenous blood concentrations estimated as ca 0.1mM
Exposure of animals to 6ppm formaldehyde did not increase blood levels
Modelling indicates that human exposure at 2ppm (OES) would yield ≤0.1% of endogenous levels, i.e. negligible increase

*Unlikely to be a direct systemic effect.*

*Sources of formaldehyde background: normal endogenous metabolism*
Ethylene oxide: DNA adducts as biomarkers of exposure

N-7-(2-hydroxyethyl) guanine

ESI LC-MS/MS, LOD 6 adducts/10⁹ nucleotides

Similar levels of N7-HEG in control heart, colon, lung, kidney, spleen, stomach
Exposure to a single ip dose of 0.01 mg/kg did not increase
liver N7-HEG levels over control

Source of EO adducts: endogenous formation of ethylene oxide from
? lipid peroxidation, methionine oxidation, intestinal bacteria
Summary

• Endogenous/background levels of some carcinogens and/or metabolites and many DNA adducts and oxidative DNA damage products (total at least 1/10^6 nucleotides) have been detected

• May hinder detection of exposure from low doses of exogenous compounds

• Also may result in a lack of observable effect at low dose, (i.e. practical thresholds) for some exogenous compounds
The relationship between the biomarker and effects

What is the biological relevance of the answer?

Interpretation may be affected by

- the mechanism (e.g. DNA reactive or non-DNA reactive)
- the dose response relationship,
  (e.g. is there a biological or practical threshold or a saturable effect)
- background levels
- mixture effects (e.g. synergies, antagonisms)
Examples of thresholds

- It is well established that non-DNA reactive carcinogens may show thresholds
  - However thresholds for effects are now being shown for some genotoxic compounds

E.g. MMS Jenkins et al, Mutagenesis, 2005, 20, 389) (micronuclei, mutation)
  MMS/EMS Doak et al Cancer Res., 2007, 67, 3904 (micronuclei, mutation)
Dose-response relationships: threshold or no threshold?

Possible mechanisms for thresholds for effects of DNA-reacting compounds:

Exposure monitoring → Internal dose → Biologically effective dose → Early biological effects → Disease

- Toxin or active metabolite concentrations
- Target site interaction (e.g. adducts)
- Toxicity, mutation, etc
- Individual susceptibility

Detoxification → DNA repair → Apoptosis

Cell cycle arrest
Example of synergy
aflatoxin B1 urinary DNA adduct

P450

N-7-deoxyguanosine adduct

excreted in urine
Example of synergy

Combined effects of HBsAg positivity and aflatoxin biomarkers on hepatocellular carcinoma

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Aflatoxin negative Relative risk (95% CI)</th>
<th>Aflatoxin positive Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1.0</td>
<td>3.4 (1.1, 10.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>7.3 (2.2, 24.4)</td>
<td>59.4 (16.6, 212.0)</td>
</tr>
</tbody>
</table>

Summary

• Analytical methods for detecting carcinogen metabolites or DNA damage often exceed the sensitivity of biological assays, i.e. need to know more about low level dose-response relationships to improve cancer risk estimates for environmentally exposed populations.

• ‘Despite the substantial progress which has been achieved in the development of analytical methodologies, few biomarkers ......can be considered as adequately validated and mature for use in risk assessment’

S Kyrtopoulos  ECNIS, 2007
Major aspects in interpretation of carcinogen biomarker data:

The analytical integrity of data
• *Is the answer right?*

The data's ability to describe exposure
• *Is the answer specific and selective?*

The relationship between the biomarker and effects
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An overall evaluation and weight of evidence
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### Guidance for the Interpretation of Biomonitoring Data

ECETOC, Document 44, 2005

Proposed framework for the evaluation of biomonitoring data

<table>
<thead>
<tr>
<th>Purpose of study</th>
<th>Required knowledge</th>
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<tbody>
<tr>
<td></td>
<td>Analytical integrity</td>
</tr>
<tr>
<td>Trends in exposures</td>
<td>✗</td>
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<tr>
<td>Characterisation of exposures</td>
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<tr>
<td>Investigation of health impacts</td>
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<tr>
<td>Risk assessment and standard setting</td>
<td>✗</td>
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</tbody>
</table>
### Human Biomonitoring for Environmental Chemicals

**The National Academies, USA, 2006**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Group</th>
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<tbody>
<tr>
<td>Reproducible sampling/analytical methodology</td>
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<td>VI</td>
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<td></td>
<td>VII</td>
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<tr>
<td>External dose-[BM] relationship in animals</td>
<td>R</td>
</tr>
<tr>
<td>External dose-[BM] relationship in humans</td>
<td>R</td>
</tr>
<tr>
<td>[BM] – biological effect relationship in animals</td>
<td>O</td>
</tr>
<tr>
<td>[BM] – biological effect relationship in humans</td>
<td>R</td>
</tr>
<tr>
<td>External dose-response relationship in animals</td>
<td>O</td>
</tr>
<tr>
<td>External dose-response relationship in humans</td>
<td>O</td>
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<tr>
<td>Biomarker informs on</td>
<td></td>
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<tr>
<td>Internal Dose</td>
<td>†</td>
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<tr>
<td>External Dose</td>
<td>†</td>
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<tr>
<td>Biological effects$^3$</td>
<td>†</td>
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<tr>
<td>Potential for risk assessment</td>
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</table>
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