Biomarkers of exposure to environmental tobacco smoke

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IEA Committee

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Overview

- Concept of human biomonitoring
- Exposure to environmental tobacco smoke (ETS) as a risk factor
  - ETS: Biomarkers of exposure
  - ETS: Biomarkers of effect
- Conclusions
- References
Assessing the exposure of humans

Methods of assessing the exposure in human studies

1. Questionnaires
2. Ambient air measurements & Time activity diaries
3. Personal monitoring
4. Biomarkers
Paradigm of biomonitoring and biomarkers

- Exposure (ext. dose) → Internal dose → Biolog. effect. dose → Biolog. effects (revers.) → Irrev. Biolog. effects → Chronic diseases
- Susceptibility factors → Interventions

- Time: ~1 Day to ~30 Years
Environmental tobacco smoke (ETS) (1)

- Since 1980 almost 100 epidemiological studies on the effects (primarily lung cancer) of exposure to ETS (“passive smoking”) have been performed.
Environmental tobacco smoke (ETS) (2)

- As a consequence, ETS has been classified as a ‘human carcinogen’ by many agencies, e.g.:
  - US EPA, 1992
  - German MAK, 1998
  - National Toxicology Program, 2000
  - IARC, 2004
Environmental tobacco smoke (ETS) (3)

- ETS is a dynamic mixture consisting of 80 – 90 % of diluted sidestream smoke and 10 – 20 % exhaled mainstream smoke.

- The chemical composition of ETS is almost identical to mainstream smoke in qualitative terms, but different in quantitative terms.

  In principle, the same biomarkers are suitable for both active and passive smoking (however, a much higher sensitivity is required for biomonitoring the exposure to ETS!)

- Unlike other complex mixtures (e.g., polluted ambient air, diesel exhaust), ETS contains some source-specific compounds (e.g., nicotine, tobacco-specific nitrosamines) which give rise to specific biomarkers for ETS exposure (e.g., cotinine, NNAL).

- Except for assessing the extent of ETS exposure, cotinine in body fluids can be also used to identify misclassified smokers.
Biomarkers of exposure
“The evidence presented in this review indicates that cotinine levels provide a valid and quantitative measure of average human ETS exposure over time. Cotinine is clearly the best available biomarker of ETS exposure at present.”

ETS: Cotinine in the (US) population

Pirkle et al. (1996), JAMA 275: 1233-1240

- Representative US population, age $\geq$ 4 years, 10642 had cotinine measurements

<table>
<thead>
<tr>
<th>Group ($\geq$17 years)</th>
<th>Cotinine (ng/ml) Geom. Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ETS exposure</td>
<td>0.132</td>
</tr>
<tr>
<td>ETS at work only</td>
<td>0.318</td>
</tr>
<tr>
<td>ETS at home only</td>
<td>0.651</td>
</tr>
<tr>
<td>ETS at home and at work</td>
<td>0.926</td>
</tr>
</tbody>
</table>
ETS: Cotinine and self-reported exposure

Heller et al. (1993), Indoor air '93, Proceeding Vol 3: 361-365

- MONICA Study in Southern Germany, 1490 never smokers (1989/90)

Mean serum cotinine levels (ng/ml) with 95 % confidence intervals

- Are there smokers in the household?
  - (984) no
  - (317) yes
  - (175) living alone

- How much do other people smoke in your household?
  - (984) none
  - (132) a little
  - (175) some
  - (317) a lot

- How much do other people smoke at your work place?
  - (463) none
  - (208) a little
  - (132) some
  - (66) a lot

- Do you feel annoyed by other people's smoke?
  - (354) never
  - (552) sometimes
  - (568) often
# Cotinine in body fluids: Ratio: Smokers/Nonsmokers

<table>
<thead>
<tr>
<th>Study</th>
<th>Body fluid</th>
<th>Not exposed to ETS</th>
<th>Exposed to ETS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvis et al., 1984</td>
<td>Plasma</td>
<td>344</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>927</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>443</td>
<td>124</td>
</tr>
<tr>
<td>Wald et al., 1984</td>
<td>Urine</td>
<td>914</td>
<td>56</td>
</tr>
<tr>
<td>Thompson et al., 1990</td>
<td>Urine</td>
<td>384</td>
<td>148</td>
</tr>
<tr>
<td>Tunstall-Pedoe et al, 1991</td>
<td>Plasma</td>
<td>Males: 353</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females: 2430</td>
<td></td>
</tr>
</tbody>
</table>
Carbon monoxide (CO): *Levels in ETS*

CO yields in mainstream smoke of cigarettes: 11.0 – 40.7 mg/cig*

CO yields in sidestream smoke of cigarettes: 31.5 – 54.1 mg/cig*

**CO in ETS:**

<table>
<thead>
<tr>
<th></th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.25 ppm</td>
<td>2.81 ppm</td>
<td>Scherer &amp; Adlkofer, 1999 /</td>
</tr>
<tr>
<td></td>
<td>0.02 ppm</td>
<td>3.08 ppm</td>
<td>Average in realistic rooms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ABF 2004 /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experim. room, low IAQ</td>
</tr>
</tbody>
</table>

10 cig/5 h

**Other sources:**

- Traffic exhausts

* Massachusetts smoking parameters (IARC, 2004)
Carbon monoxide (CO): \textit{Biomarkers}

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Blood (invasive)</th>
<th>Exhaled air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half live</td>
<td>2 – 4 h (depending on physical activity)</td>
<td>2 – 3 ppm</td>
</tr>
<tr>
<td>Background levels</td>
<td>~ 1 %</td>
<td>4 – 8 %</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td></td>
<td>2 – 3 ppm</td>
</tr>
<tr>
<td>Interference</td>
<td>Endogenous CO formation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>COHb (%)</td>
<td>0.72 (N = 41)</td>
<td>0.63 (N = 130)</td>
<td>Szadkowski et al., 1976</td>
</tr>
<tr>
<td>COex (ppm)</td>
<td>7.1 (N = 828)</td>
<td>7.7*** (N = 244)</td>
<td>Svendsen et al., 1987</td>
</tr>
<tr>
<td>COex (ppm)</td>
<td>2.5 (N = 100)</td>
<td>5.0*** (N = 100)</td>
<td>Laranjeira et al., 2000</td>
</tr>
</tbody>
</table>

***: p < 0.001
Benzene: Levels in ETS

Benzene yields in mainstream smoke of cigarettes: 28.0 - 105.9 µg/cig*

Benzene yields in sidestream smoke of cigarettes: 70.7 – 134.3 µg/cig*

Benzene in ETS:

<table>
<thead>
<tr>
<th></th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µg/m³)</td>
<td>5.9</td>
<td>9.4</td>
<td>Scherer &amp; Adlkofeir, 1999 /</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>16</td>
<td>Average in realistic rooms</td>
</tr>
</tbody>
</table>

Other sources:
- Traffic exhausts
- Fuels

* Massachusetts smoking parameters (IARC, 2004)
## Benzene: Biomarkers

<table>
<thead>
<tr>
<th></th>
<th>trans,trans-Muconic Acid (t,t-MA)</th>
<th>S-Phenylmercapturic acid (SPMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological matrix</td>
<td>Urine</td>
<td>Urine</td>
</tr>
<tr>
<td>Half live</td>
<td>5 - 8 h</td>
<td>9 h</td>
</tr>
<tr>
<td>Background levels</td>
<td>50 - 60 µg/g crea.</td>
<td>0.1 µg/24 h</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td>100 – 300 µg/g crea.</td>
<td>2 – 10 µg/24h</td>
</tr>
<tr>
<td>Interference</td>
<td>Sorbic acid</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS (µg/g, N)</th>
<th>Nonsmokers Exposed to ETS (µg/g, N)</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>t,t-MA</td>
<td></td>
<td></td>
<td>Scherer et al., 1995</td>
</tr>
<tr>
<td>µg/g</td>
<td>92 (N = 39)</td>
<td>126 (N = 43)</td>
<td></td>
</tr>
<tr>
<td>t,t-MA</td>
<td></td>
<td></td>
<td>Weaver et al., 1996</td>
</tr>
<tr>
<td>µg/g</td>
<td>64 (N = 39)</td>
<td>91 (N = 39)</td>
<td></td>
</tr>
<tr>
<td>t,t-MA</td>
<td></td>
<td></td>
<td>Buratti et al., 1996</td>
</tr>
<tr>
<td>µg/g</td>
<td>76 (N = 60)</td>
<td>77 (N = 22)</td>
<td></td>
</tr>
</tbody>
</table>
Benzene: Relationship between tt-MA excretion and ETS exposure

Scherer et al, 1995
### Benzene: Biomarkers

![Benzene: Biomarkers](image)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t,t$-MA (µg/l)</td>
<td>44 (Median) (N = 42)</td>
<td>63 (Median)* (N = 27)</td>
<td>Carrer et al., 2000</td>
</tr>
<tr>
<td>SPMA (µg/24h)</td>
<td>0.27 (N = 54)</td>
<td>0.33* (N = 44)</td>
<td>Scherer et al., unpubl.</td>
</tr>
</tbody>
</table>

*: p < 0.05

![Graph: SPMA versus cotinine in saliva](image)

**Graph:**

- **Title:** SPMA versus cotinine in saliva
- **Y-axis:** SPMA in urine (ng/24h)
- **X-axis:** Cotinine in saliva (ng/ml)
- **Data points:** N = 98, $r = 0.226^*$
# Acrolein: Levels in ETS

Acrolein yields in mainstream smoke of cigarettes: 51.2 - 223.4 µg/cig*

Acrolein yields in sidestream smoke of cigarettes: 342.1 – 522.7 µg/cig*

<table>
<thead>
<tr>
<th>Acrolein in ETS:</th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.4 µg/m³</td>
<td>10.5 µg/m³</td>
<td>Scherer &amp; Adlkofer, 1999 / Average in ca. 70 realistic rooms</td>
</tr>
<tr>
<td></td>
<td>0.4 µg/m³</td>
<td>8.8 µg/m³</td>
<td>ABF 2004 / Experim. room, low IAQ</td>
</tr>
</tbody>
</table>

**Other sources:**
- Traffic exhausts
- Heating of fat

* Massachusetts smoking parameters (IARC, 2004)
**Acrolein: Biomarkers**

### 3-Hydroxypropylmercapturic Acid (HPMA)

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half live</strong></td>
<td><strong>6 - 9 h</strong></td>
</tr>
<tr>
<td><strong>Background levels</strong></td>
<td><strong>150 - 450 µg/24 h</strong></td>
</tr>
<tr>
<td><strong>Levels in smokers</strong></td>
<td><strong>500 – 1500 µg/24 h</strong></td>
</tr>
<tr>
<td><strong>Interference</strong></td>
<td><strong>Endogenous formation</strong> (Lipid peroxidation)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Non-smokers not exposed to ETS</th>
<th>Non-smokers exposed to ETS</th>
<th>Reference / Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMA (µg/24 h)</td>
<td><strong>200</strong> (N = 5)</td>
<td><strong>750</strong>* (N = 5)</td>
<td>Scherer et al., 1992  / *</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental study with <strong>high</strong> ETS exposure</td>
</tr>
<tr>
<td>HPMA (µg/24 h)</td>
<td><strong>324</strong> (N = 55)</td>
<td><strong>353</strong> (N = 45)</td>
<td>Scherer et al., unpubl. *</td>
</tr>
</tbody>
</table>

*: p < 0.05
Acrolein: Biomarkers

Scherer et al, 1992
Acrolein:  

**Biomarkers**

3-Hydroxypropyl mercapturic acid, HPMA  

versus cotinine in saliva

\[ N = 100 \]
\[ r = 0.007 \]

Scherer et al, unpublished
**Pyrene (surrogate for polycyclic aromatic hydrocarbons): Levels in ETS**

Pyrene yields in mainstream smoke of cigarettes: 45 ng/cig*

Pyrene yields in sidestream smoke of cigarettes: 476 ng/cig*

<table>
<thead>
<tr>
<th>Pyrene in ETS:</th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.6 – 9.3 ng/m³</td>
<td>4.3 - 11 ng/m³</td>
<td>Chuang et al., 1991 / 8 homes</td>
</tr>
<tr>
<td></td>
<td>2.7 – 11.8 ng/m³</td>
<td></td>
<td>Husgafvel-Pursiainen et al., 1986 / Restaurants</td>
</tr>
<tr>
<td></td>
<td>18.9 ng/m³</td>
<td>21.8 ng/m³</td>
<td>ABF 2004 / Experim. room, low IAQ</td>
</tr>
</tbody>
</table>

**Other sources:**
- Traffic exhausts
- Heating exhausts

* ISO/FTC smoking parameters (Grimmer et al., 1987)
**Pyrene (surrogate for PAH): Biomarkers**

1-Hydroxypyrene (1-OHP)

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half live</td>
<td>20 h</td>
</tr>
<tr>
<td>Background levels</td>
<td>0.05 µg/24 h</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td>1.00 µg/24 h</td>
</tr>
<tr>
<td>Interference</td>
<td>Diet</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Non-smokers not exposed to ETS</th>
<th>Non-smokers exposed to ETS</th>
<th>Reference / Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-OHP (µg/24 h)</td>
<td>0.171 (N = 23)</td>
<td>0.140 (N = 19)</td>
<td>Scherer et al., 2000 ⇒</td>
</tr>
<tr>
<td>1-OHP (µmol/mol crea.)</td>
<td>0.32 (N = 126)</td>
<td>0.36 (N = 286)</td>
<td>Siwinska et al., 1999</td>
</tr>
</tbody>
</table>
Pyrene: Relationship between 1-OHP excretion and ETS exposure

Scherer et al, 2000
Benzo[a]pyrene (BaP):  Levels in ETS

BaP yields in mainstream smoke of cigarettes:  5.6 - 41.5 ng/cig*

BaP yields in sidestream smoke of cigarettes:  51.8 – 94.5 ng/cig*

BaP in ETS:

<table>
<thead>
<tr>
<th></th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.27 – 0.58 ng/m³</td>
<td>0.37 – 1.7 ng/m³</td>
<td>Chuang et al., 1991 /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 homes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Husgafvel-Pursiainen et al., 1986 / Restaurants</td>
</tr>
<tr>
<td></td>
<td>2.2 – 13.3 ng/m³</td>
<td></td>
<td>ABF 2004 /</td>
</tr>
<tr>
<td></td>
<td>1.74 ng/m³</td>
<td>5.45 ng/m³</td>
<td>Experim. room, low IAQ</td>
</tr>
</tbody>
</table>

Other sources:
- Traffic exhausts
- Heating exhausts

* Massachusetts smoking parameters (IARC, 2004)
### Benzo[a]pyrene (BaP): Biomarkers

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Blood</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half live</td>
<td>4 months (life-time)</td>
<td>20 d</td>
</tr>
<tr>
<td>Background levels</td>
<td>variable (dependent on the method)</td>
<td>variable (dependent on the method)</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td>variable (dependent on the method)</td>
<td>variable (dependent on the method)</td>
</tr>
<tr>
<td>Interference</td>
<td>Diet</td>
<td>Diet</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP-Alb (fmol/μg)</td>
<td>0.15 (N = 23)</td>
<td>0.35* (N = 31)</td>
<td>Crawford et al., 1994 Children</td>
</tr>
<tr>
<td>BaP-Alb (fmol/μg)</td>
<td>0.185 (N = 24)</td>
<td>0.437* (N = 82)</td>
<td>Tang et al., 1999 Children</td>
</tr>
</tbody>
</table>

*: p < 0.05
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP-Alb (fmol/mg)</td>
<td>0.019 (N = 23)</td>
<td>0.021 (N = 19)</td>
<td>Scherer et al., 2000</td>
</tr>
<tr>
<td>BaP-Hb (fmol/mg)</td>
<td>0.083 (N = 23)</td>
<td>0.049 (N = 19)</td>
<td>Scherer et al., 2000</td>
</tr>
</tbody>
</table>

Biomarkers of Benzo[a]pyrene (BaP): Nonsmokers: 0.105

Exposed to ETS: Smokers: 0.042

Nonsmokers: 0.019

Not exposed to ETS: Smokers: 0.021

N = 42, r = -0.15

N = 42, r = 0.20
Benzo[a]pyrene (BaP) and PAH: Biomarkers

- *Mooney et al. (1995):* PAH-DNA adducts (determined by ELISA) were significantly higher when there was another smoker at home.

- *Petruzelli et al. (1998):* Anti-BPDE*-DNA antibodies in serum were not associated with passive smoking.

- *Shinozaki et al. (1999):* BPDE-DNA adducts in peripheral lymphocytes were not associated with passive smoking.

- *Zenžes et al. (1998):* PAH-DNA adduct levels in granulosa-lutein cell of IVF-patients were twice as high in passive smokers compared to nonsmokers. Passive smokers had cotinine concentrations in follicular fluid 1/10 of active smoker!

*BPDE: Benzo[a]pyrene-diol-epoxide*
PAH / $^{32}$P-Postlabelling: Biomarkers

- **Holz et al. (1990):** No increase of DNA adducts in peripheral monocytes after high experimental exposure to ETS.

- **Georgiadis et al. (2001):** DNA adduct levels in lymphocytes paralleled the ETS exposure as determined by reported times of ETS exposure 24 h prior to blood sampling, serum cotinine or chrysene/benzo[g,h,i]perylene ratio.

- **Everson et al. (1986):** DNA adducts in placenta of nonsmokers are possibly related to ETS exposure (N = 3!).

- **Daube et al. (1997):** No evidence for elevated DNA adduct levels after exposure to tobacco smoke (active and passive smoking).
4-Aminobiphenyl (4-ABP): Levels in ETS

4-ABP yields in mainstream smoke of cigarettes: 1.8 - 7.8 ng/cig*

BaP yields in sidestream smoke of cigarettes: 20.8 – 31.8 ng/cig*

<table>
<thead>
<tr>
<th>4-ABP in ETS:</th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.051 ng/m³</td>
<td>0.11 – 0.20 ng/m³</td>
<td>Luceri et al., 1993</td>
<td></td>
</tr>
<tr>
<td>(Train)</td>
<td>(2 Offices, 1 hair dresser saloon)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 – 11 ng/m³</td>
<td>15 – 33 ng/m³</td>
<td>Palmiotto et al., 2001 / 9 Homes</td>
<td></td>
</tr>
<tr>
<td>(sum of 9 amines)</td>
<td>(sum of 9 amines)</td>
<td>ABF 2004 / Experim. room, low IAQ</td>
<td></td>
</tr>
<tr>
<td>0.026 ng/m³</td>
<td>0.582 ng/m³</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other sources: ?

* Massachusetts smoking parameters (IARC, 2004)
### 4-Aminobiphenyl (4-ABP): Biomarkers

**4-ABP-Hemoglobin adducts**

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half live</strong></td>
<td><strong>4 months</strong> (life-time)</td>
</tr>
<tr>
<td><strong>Background levels</strong></td>
<td><strong>10 – 50 pg/g</strong></td>
</tr>
<tr>
<td><strong>Levels in smokers</strong></td>
<td><strong>50 – 500 pg/g</strong></td>
</tr>
<tr>
<td><strong>Interference</strong></td>
<td><strong>4-NBP&lt;sup&gt;1&lt;/sup&gt; (exhausts), diet, hair dyes</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-ABP-Hb (pg/g)</td>
<td><strong>42 - 50</strong> (N = 44)</td>
<td><strong>45 – 54(*)</strong> (N = 31)</td>
<td>MaClure et al., 1989 ⇒</td>
</tr>
<tr>
<td>4-ABP-Hb (pg/g)</td>
<td><strong>17.6</strong> (N = 7)</td>
<td><strong>27.8</strong>* (N = 9)</td>
<td>Hammond et al., 1993 (Pregnant women)</td>
</tr>
<tr>
<td>4-ABP-Hb (pg/g)</td>
<td><strong>10.6</strong> (N = 27)</td>
<td><strong>9.3 – 10.6</strong> (N = 9)</td>
<td>Branner et al., 1998 ⇒ (Pregnant women)</td>
</tr>
</tbody>
</table>

<sup>1</sup> 4-NBP: 4-Nitrobiphenyl

(*): p = 0.06;  *: p < 0.05
4-Aminobiphenyl (4-ABP): Biomarkers

MaClure et al., 1989

Branner et al., 1998

MaClure et al., 1989
### 4-Aminobiphenyl (4-ABP): Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-ABP-Hb (pg/g)</td>
<td>23.8 *(N = 10)</td>
<td>34.3* *(N = 41)</td>
<td>Tang et al., 1999 / Infants</td>
</tr>
</tbody>
</table>

*: p < 0.05
### NNK: Levels in ETS

**NNK yields in mainstream smoke of cigarettes:** 53.5 - 220.7 ng/cig*

**NNK yields in sidestream smoke of cigarettes:**
- 50.7 – 95.7 ng/cig*
- 386 – 1444 ng/cig**

<table>
<thead>
<tr>
<th>NNK in ETS:</th>
<th>Control (No smoking)</th>
<th>Smoking (29 Rooms)</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 ng/m³ (11 Rooms)</td>
<td>15.5 ng/m³</td>
<td>Scherer &amp; Adlkofer, 1999 / Average in realistic rooms</td>
</tr>
<tr>
<td></td>
<td>0.18 ng/m³</td>
<td>7.12 ng/m³</td>
<td>ABF 2004 / Experim. room, low IAQ</td>
</tr>
</tbody>
</table>

**Other sources:**
- None

* Massachusetts smoking parameters (IARC, 2004); ** ISO/FTC smoking parameters
### NNK: Biomarkers

#### NNAL/NNAL-Glucuronide

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half live</td>
<td>1 d (Phase 2: 6 weeks)</td>
</tr>
<tr>
<td>Background levels</td>
<td>&lt; LOD (&lt; 3 pmol/24 h)</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td>3200 pmol/24 h</td>
</tr>
<tr>
<td>Interference</td>
<td>None</td>
</tr>
</tbody>
</table>

#### NNAL-Gluc (pmol/ml)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNAL-Gluc</td>
<td>0.012 (N = 5)</td>
<td>0.059** (N = 9)</td>
<td>Parsons et al., 1998 ⇒</td>
</tr>
<tr>
<td>Total NNAL (pmol/24 h)</td>
<td>&lt; 3 (N = 12)</td>
<td>43.3* (N = 17)</td>
<td>Meger et al., 2000 ⇒</td>
</tr>
<tr>
<td>Total NNAL (pmol/ml)</td>
<td>0.007 (N = 22)</td>
<td>0.050* (N = 23)</td>
<td>Anderson et al., 2001</td>
</tr>
<tr>
<td>Total NNAL (pmol/ml)</td>
<td>0.035 (N = 35)</td>
<td>0.095* (N = 38)</td>
<td>Hecht et al., 2001 / Children</td>
</tr>
</tbody>
</table>

*: p < 0.05; **: p < 0.01
NNK: Biomarkers

Meger et al., 2000
**NNK:** Biomarkers

*Fig. 3.* Relationship between urinary cotinine and NNAL-Gluc in nonsmokers exposed to ETS ($r = 0.51; P = 0.029$).

Parsons et al., 1998

*Fig. 5.* Relationship between levels of total cotinine and NNAL plus NNAL-Gluc in the urine of 74 children. $r = 0.71; P < 0.0001$.

Hecht et al., 2001
**Ethylene (E) / Ethylene oxide (EO):**

**Levels in ETS**

E (EO) yields in mainstream smoke of cigarettes: 300 (7) µg/cig*

E yields in sidestream smoke of cigarettes: 2000 µg/cig*

<table>
<thead>
<tr>
<th>E in ETS:</th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 µg/m³</td>
<td>100 - 250 µg/m³</td>
<td>Persson et al., 1988 / Experimental room</td>
</tr>
</tbody>
</table>

**Other sources:**

- Traffic exhausts
- Terrestrial and marine organisms

* ISO/FTC smoking parameters
### Ethylene / Ethylene oxide: Biomarkers

<table>
<thead>
<tr>
<th>N-(2-Hydroxyethyl)valine Hemoglobin adducts (OHEtVal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological matrix</td>
</tr>
<tr>
<td>Half live</td>
</tr>
<tr>
<td>Background levels</td>
</tr>
<tr>
<td>Levels in smokers</td>
</tr>
<tr>
<td>Interference</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference / Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHEtVal (pmol/g)</td>
<td>17.0 (N = 74)</td>
<td>16.6 (N = 28)</td>
<td>Bono et al. et al., 1999 / No difference in urinary cotinine was found!</td>
</tr>
<tr>
<td>OHEtVal (pmol/g)</td>
<td>21.3 (N = 55)</td>
<td>20.8 (N = 45)</td>
<td>Scherer et al., unpubl. ⇔</td>
</tr>
</tbody>
</table>
Ethylene / Ethylene oxide: Biomarkers

Hydroxyethylvaline Hb adducts versus cotinine in saliva

Scherer et al., unpublished
Acrylonitrile (AN): Levels in ETS

AN yields in mainstream smoke of cigarettes: 7.8 - 39.1 µg/cig*
AN yields in sidestream smoke of cigarettes: 24.1 – 43.9 µg/cig*

<table>
<thead>
<tr>
<th>AN in ETS:</th>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8 µg/m³</td>
<td></td>
<td>Guerin et al, 1992</td>
</tr>
<tr>
<td></td>
<td>(Family room )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.6 µg/m³</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(upstairs bedroom )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other sources:
- Certain workplaces
- ?

* Massachusetts smoking parameters (IARC, 2004)
**Acrylonitrile: Biomarkers**

Cyanoethylvaline Hemoglobin adducts (CyEtVal)

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half live</td>
<td>4 months (life-time)</td>
</tr>
<tr>
<td>Background levels</td>
<td>2 - 3 pmol/g</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td>30 - 250 pmol/g</td>
</tr>
<tr>
<td>Interference</td>
<td>None</td>
</tr>
</tbody>
</table>

**Biomarker**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference / Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CyEtVal (pmol/g)</td>
<td>5.4 (N = 55)</td>
<td>7.8 (*) (N = 45)</td>
<td>Scherer et al., unpubl. ⇒</td>
</tr>
</tbody>
</table>

(*): p = 0.061
Acrylonitrile: Biomarkers

Scherer et al., unpublished

Cyanoethylvaline Hb adducts versus cotinine in saliva

N = 100
r = 0.240*

Scherer et al., unpublished
Methylating and ethylating agents (MA and EA): Levels in ETS

**MA**: e.g., *N*-nitrosodimethylamine (NDMA), NNK, methyl halides
- **NDMA yields in mainstream smoke of cigarettes**: ~ 100 ng/cig*
- **NDMA yields in sidestream smoke of cigarettes**: 200 – 1040 ng/cig*

**EA**: unknown! *N*-nitrosodimethylamine (NDEA)?, ethyl chloride?, NMEA?
- **NDEA yields in mainstream smoke of cigarettes**: ~ 5 ng/cig*
- **NDEA yields in sidestream smoke of cigarettes**: ~ 50 ng/cig*

**NDMA/NDEA in ETS:**

<table>
<thead>
<tr>
<th>Control (No smoking)</th>
<th>Smoking</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA 10.4 ng/m³</td>
<td>31.2 ng/m³</td>
<td>Scherer &amp; Adlkofer, 1999 / Average in realistic rooms</td>
</tr>
<tr>
<td>(14 Rooms)</td>
<td>(55 Rooms)</td>
<td></td>
</tr>
<tr>
<td>NDEA nd – 8.6 ng/m³</td>
<td></td>
<td>Klus et al., 1987 /</td>
</tr>
<tr>
<td>(Office, 9 conditions)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Other sources:**
- Cooking
- Rubber

* ISO/FTC smoking parameters
## Methylating/ethylating agents: Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>3-Methyladenine (3-MeA)</th>
<th>3-Ethyladenine (3-EtA)</th>
<th>Methyl-valine Hb MeVal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological matrix</td>
<td>Urine</td>
<td>Urine</td>
<td>Blood</td>
</tr>
<tr>
<td>Half live</td>
<td>~ 12 h</td>
<td>~ 12 h</td>
<td>4 months</td>
</tr>
<tr>
<td>Background levels</td>
<td>1 – 5 µg/24h</td>
<td>10 – 30 ng/24h</td>
<td>300 pmol/g</td>
</tr>
<tr>
<td>Levels in smokers</td>
<td>10 - 20 µg/24 h</td>
<td>100 - 200 ng/24h</td>
<td>400 pmol/g</td>
</tr>
<tr>
<td>Interference</td>
<td>Diet</td>
<td>Diet</td>
<td>Endogenous</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers not exposed to ETS</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference /Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MeA (µg/24h)</td>
<td>4.7 – 5.9 (N = 5)</td>
<td>4.8 – 4.9 (N = 5)</td>
<td>Kopplin et al., 1995 ⇔</td>
</tr>
<tr>
<td>3-EtA (ng/24 h)</td>
<td>14 - 31 (N = 5)</td>
<td>18 - 25 (N = 5)</td>
<td>Diet controlled study with high experimental ETS exposure</td>
</tr>
<tr>
<td>MeVal (pmol/g)</td>
<td>309 (N = 55)</td>
<td>298 (N = 45)</td>
<td>Scherer et al., unpubl. ⇔</td>
</tr>
</tbody>
</table>
Methylating/ethylating agents: Biomarkers

Kopplin et al., 1995
**Methylating agents:**  
*Biomarkers*

**Methylvaline Hb adducts versus cotinine in saliva**

![Graph showing the relationship between methylvaline Hb adducts (MeVal) and cotinine in saliva.](image)

- **MeVal** (pmol/g) vs. **Cotinine in saliva (ng/ml)**
- **N = 100**
- **r = -0.015**

*Scherer et al., unpublished*
Mutagens: Properties in ETS

• No unique class of compounds in tobacco smoke.

• PAH, N-heterocyclic amines, aromatic amines etc. contribute to the mutagenic activity of tobacco smoke.

• Mutagens are mainly located in the particulate phase of ETS (90 %) (Salomaa et al, 1988).

• Mutagens in tobacco smoke are indirect mutagens, i.e. they require metabolic activation before being mutagenic.

• Other sources for airborne mutagens: Organic combustion products (heating, combustion engines, cooking, etc.)
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nonsmokers Exposed to ETS</th>
<th>Reference / Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagenic activity with TA98 +S9 (cigarette equivalents)</td>
<td>0.8* (N = 8)</td>
<td>Bos et al., 1983 Experimental exposure to ETS</td>
</tr>
<tr>
<td></td>
<td>4 – 5* (N = 6)</td>
<td>Mohtashamipur et al., 1987 High exp. exposure to ETS</td>
</tr>
<tr>
<td></td>
<td>0.2 (N = 5)</td>
<td>Scherer et al., 1990 High exp. exposure to ETS</td>
</tr>
</tbody>
</table>

*: p < 0.05
## Mutagens: Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Non-smokers not exposed to ETS</th>
<th>Non-smokers exposed to ETS</th>
<th>Reference / Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rev/25 µl urine</td>
<td>4.2 (N = 20)</td>
<td>4.7 (N = 27)</td>
<td>Husgafvel-Pursiainen et al., 1987 / ETS exposed restaurant personnel</td>
</tr>
<tr>
<td>with TA98+S9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rev/µmol crea.</td>
<td>No correlation with urinary cotinine</td>
<td></td>
<td>Kado et al., 1987 ⇒ Pilot study with clerks</td>
</tr>
<tr>
<td>With TA98+S9</td>
<td>(N = 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rev/mmol crea.</td>
<td>0 (N = 35)</td>
<td>182 (N = 4)</td>
<td>Bartsch et al., 1990 / ETS exposed restaurant personnel</td>
</tr>
<tr>
<td>With TA98+S9</td>
<td>(N = 27)</td>
<td>509 (N = 11)</td>
<td></td>
</tr>
<tr>
<td>Rev/g crea.</td>
<td>9944 (N = 10)</td>
<td>15130 (N = 11)</td>
<td>Scherer et al., 1996 ⇒ ETS classification: &lt; 5 versus ≥ 5 µg/g crea. cotinine</td>
</tr>
<tr>
<td>With YG1024+S9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Reporting ETS exposure and nicotine or cotinine detectable in urine
2 Reporting ETS exposure, but no nicotine or cotinine detectable in urine
Mutagens: Biomarkers

Kado et al., 1987
Mutagens: Biomarkers

Scherer et al., 1996
**ETS: Biomarkers of exposure (except nicotine metabolites) (1)**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Precursor in ETS</th>
<th>Other sources</th>
<th>Significant increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>COHb, COex</td>
<td>CO</td>
<td>Traffic, endogenous</td>
<td>↑→</td>
</tr>
<tr>
<td>SCN in body fluids</td>
<td>HCN</td>
<td>Diet</td>
<td>→</td>
</tr>
<tr>
<td>Benzene in blood or exhalate</td>
<td>Benzene</td>
<td>Traffic, fuels</td>
<td>↑→</td>
</tr>
<tr>
<td>t,t-MA in urine</td>
<td>Benzene</td>
<td>Traffic, fuels, sorbic acid</td>
<td>↑→</td>
</tr>
<tr>
<td>SPMA in urine</td>
<td>Benzene</td>
<td>Traffic, fuels</td>
<td>↑→</td>
</tr>
<tr>
<td>HPMA in urine</td>
<td>Acrolein</td>
<td>Traffic, heated fat, endogenous</td>
<td>↑→</td>
</tr>
<tr>
<td>1-Hydroxypyrene in urine</td>
<td>Pyrene (PAH)</td>
<td>Traffic, diet</td>
<td>↑→</td>
</tr>
<tr>
<td>Hydroxy-phenanthrene in urine</td>
<td>Phenanthrene (PAH)</td>
<td>Traffic, diet</td>
<td>→</td>
</tr>
<tr>
<td>BaP adducts (Hb, albumin)</td>
<td>BaP</td>
<td>Traffic, diet</td>
<td>↑→</td>
</tr>
</tbody>
</table>

*No data shown in this presentation*

*Scherer & Richter, 1997 (modified)*
### ETS: Biomarkers of exposure (except nicotine metabolites) (2)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Precursor in ETS</th>
<th>Other sources</th>
<th>Significant increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulky DNA adducts (WBC, placenta)</td>
<td>PAH (probably)</td>
<td>Traffic, diet</td>
<td>↑→</td>
</tr>
<tr>
<td>4-ABP adducts (Hb)</td>
<td>4-ABP</td>
<td>Gas or kerosene heaters, diesel exhaust?, diet?</td>
<td>↑→</td>
</tr>
<tr>
<td>NNAL/NNAL-gluc in urine</td>
<td>NNK</td>
<td>None</td>
<td>↑</td>
</tr>
<tr>
<td>HPB adducts (Hb)</td>
<td>NNK, NNN</td>
<td>Myosmine in diet?</td>
<td>→</td>
</tr>
<tr>
<td>2-Hydroxyethylvaline (Hb)</td>
<td>Ethylene oxide, ethylene</td>
<td>Ambient air, endogenous</td>
<td>→</td>
</tr>
<tr>
<td>Cyanoethylvaline (Hb)</td>
<td>Acrylonitrile</td>
<td>(Workplace)</td>
<td>(↑)</td>
</tr>
</tbody>
</table>

*Scherer & Richter, 1997 (modified)*
**ETS: Biomarkers of exposure (except nicotine metabolites) (3)**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Precursor in ETS</th>
<th>Other sources</th>
<th>Significant increase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3-Methyl-/3-Ethyl-adenine in urine</strong></td>
<td>Methylating and ethylating compounds</td>
<td>Diet</td>
<td>→</td>
</tr>
<tr>
<td><strong>Mutagenicity in urine</strong></td>
<td>PAH, HHA, AA</td>
<td>Diet</td>
<td>↑→</td>
</tr>
<tr>
<td><strong>Thioethers in urine</strong></td>
<td>Electrophiles</td>
<td>Diet</td>
<td>→</td>
</tr>
</tbody>
</table>
### ETS: Biomarkers of effect (1)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Causing agent in ETS</th>
<th>Other factors</th>
<th>Significant effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG in urine, WBC, placenta</td>
<td>(Oxidative stress)</td>
<td>Many endogenous and exogenous factors</td>
<td>↑→</td>
</tr>
<tr>
<td>5-HMUrA in urine</td>
<td>(Oxidative stress)</td>
<td>Many endogenous and exogenous factors</td>
<td>(↑)</td>
</tr>
<tr>
<td>Nitrated proteins in plasma</td>
<td>(Inflammation)</td>
<td>Many endogenous and exogenous factors</td>
<td>(↑)</td>
</tr>
<tr>
<td>Induction of AHH in placenta</td>
<td>PAHs, others</td>
<td>Traffic, diet, medications</td>
<td>(↑)</td>
</tr>
<tr>
<td>Hydroxyproline in urine</td>
<td>NO₂ (?)</td>
<td>Traffic, heating</td>
<td>↑→</td>
</tr>
<tr>
<td>Total cholesterol in blood</td>
<td>?</td>
<td>Diet, predisposition</td>
<td>↓→</td>
</tr>
<tr>
<td>HDL in blood</td>
<td>?</td>
<td>Diet, predisposition</td>
<td>↓→</td>
</tr>
<tr>
<td>LDL in blood</td>
<td>?</td>
<td>Diet, predisposition</td>
<td>→</td>
</tr>
<tr>
<td>Triglycerides in blood</td>
<td>?</td>
<td>Diet, predisposition</td>
<td>→</td>
</tr>
</tbody>
</table>

Scherer & Richter, 1997 (modified)
### Biomarkers of effect (2)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Causing agent in ETS</th>
<th>Other factors</th>
<th>Significant effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet aggregation</td>
<td>?</td>
<td>Diet, medication</td>
<td>(↑)</td>
</tr>
<tr>
<td>Fibrinogen in plasma</td>
<td>?</td>
<td>Age, BMI, alcohol etc.</td>
<td>(↑)</td>
</tr>
<tr>
<td>Carotid wall thickness</td>
<td>?</td>
<td>Diet, predisposition</td>
<td>↑</td>
</tr>
</tbody>
</table>

Scherer & Richter, 1997 (modified)
Conclusions (1)

- Biomonitoring can significantly improve the assessment of the exposure to environmental tobacco smoke (ETS). This is particularly true because source-specific biomarkers are available.

- Source-specific biomarkers for ETS are nicotine metabolites (particularly cotinine) and NNAL/NNAL-glucuronide (metabolites of NNK).

- The exposure dose ratio smoking/passive smoking for the ETS-specific biomarkers is in the range 100 – 200.

- For almost all other biomarkers of exposure to ETS, there is significant interference from background exposure (ambient air, diet, endogenous formation).

- Results of ETS biomarker of exposure studies are partly controversial mainly due to difficulties in controlling the background exposure.
Conclusions (2)

• In principle, biomarkers of effect are unspecific for the underlying exposure(s).

• When studying biomarkers of ETS-related effects, it is essential (and also extremely difficult) to select ETS exposed and suitable unexposed control groups.

• Not unexpectedly, results of studies on biomarkers of ETS-related effects are controversial. In particular, the extent of the observed effects was often similar or only slightly lower than in active smokers.

• This discrepancy has to dissolved in future studies.
References (1)

General literature and Reviews


References (2)

Cited papers

References (3)

Cited papers


References (4)

Cited papers


Cited papers


Cited papers


Cited papers


References (8)

Cited papers


References (9)

Cited papers


