Biomarkers of exposure to environmental tobacco smoke

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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

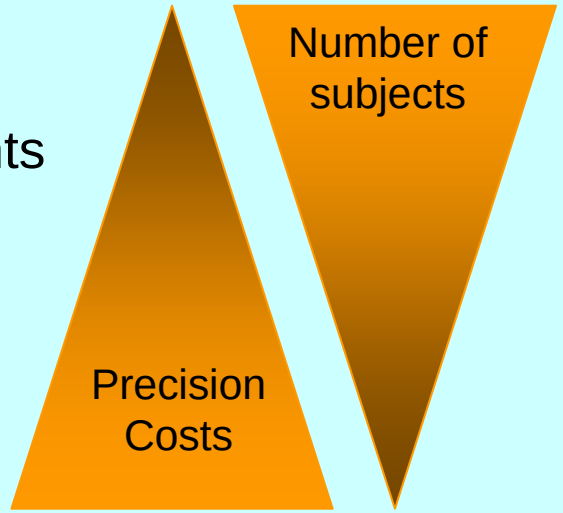
Methods of assessing the exposure in human studies

1 Questionnaires

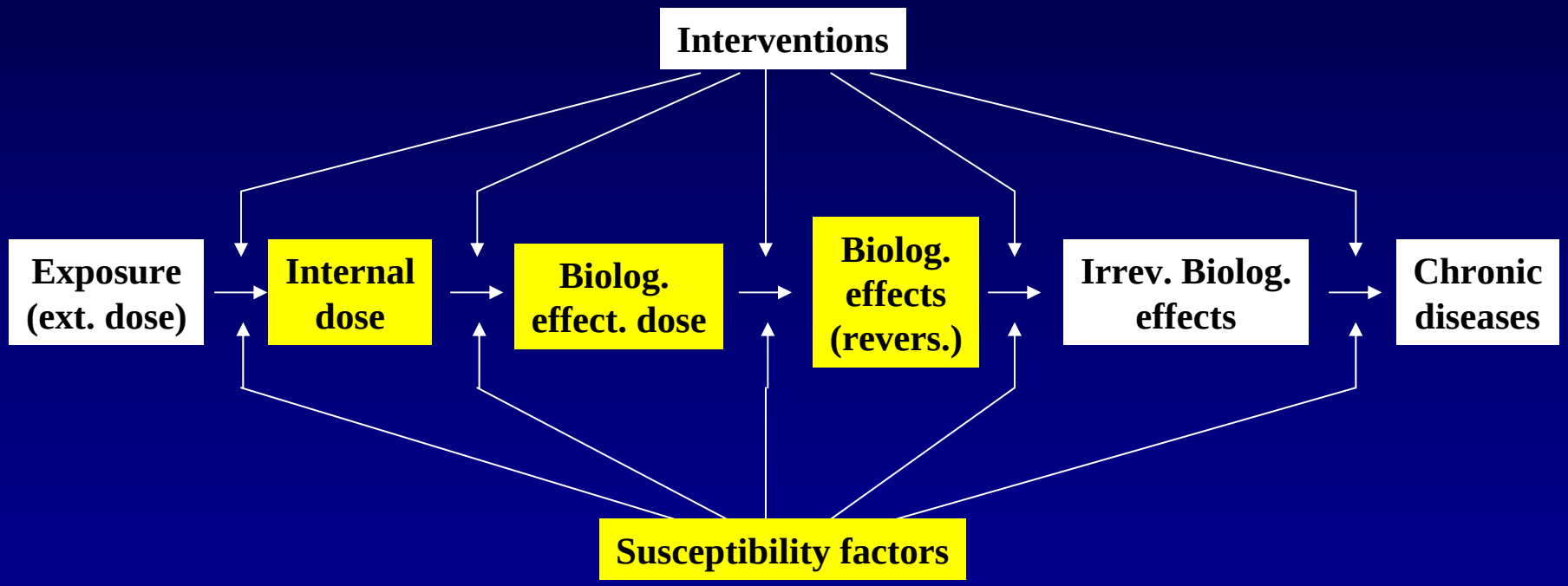
2 Ambient air measurements & Time activity diaries

3 Personal monitoring

4 Biomarkers



Paradigm of biomonitoring and biomarkers

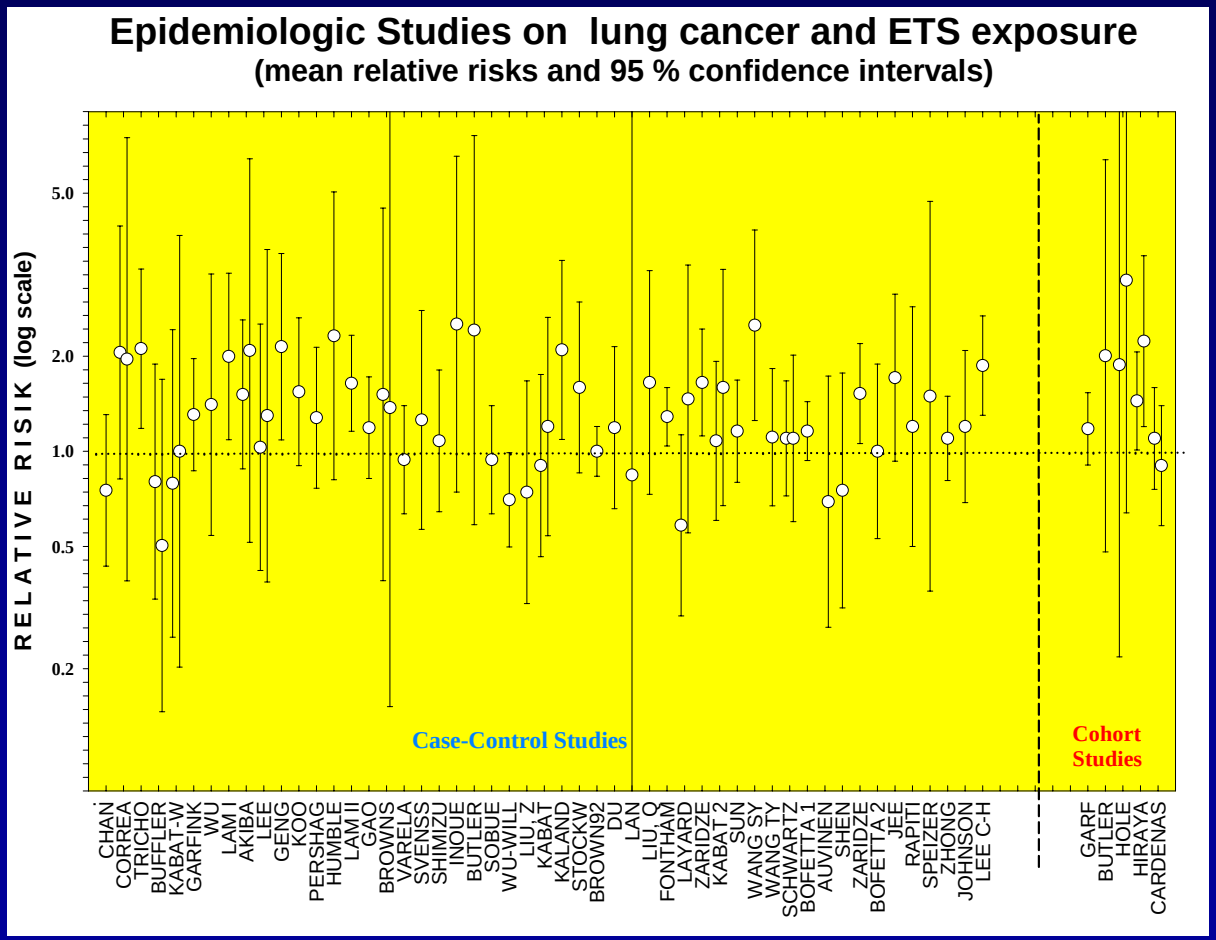


Time

~ 1 Day ..... ~ 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

ETS: *Cotinine as a biomarker of exposure*

"The evidence presented in this review indicates that cotinine levels provide a valid and quantititve measure of average human ETS exposure over time. Cotinine is clearly the best available biomarker of ETS exposure at present."

Benowitz, N.L. (1996) Cotinine as a biomarker for Environmental Tobacco Smoke Exposure. *Epidemiologic Reviews*, 18: 188-204

ETS: *Cotinine in the (US) population*

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

- **Representative US population, age ≥ 4 years, 10642 had cotinine measurements**

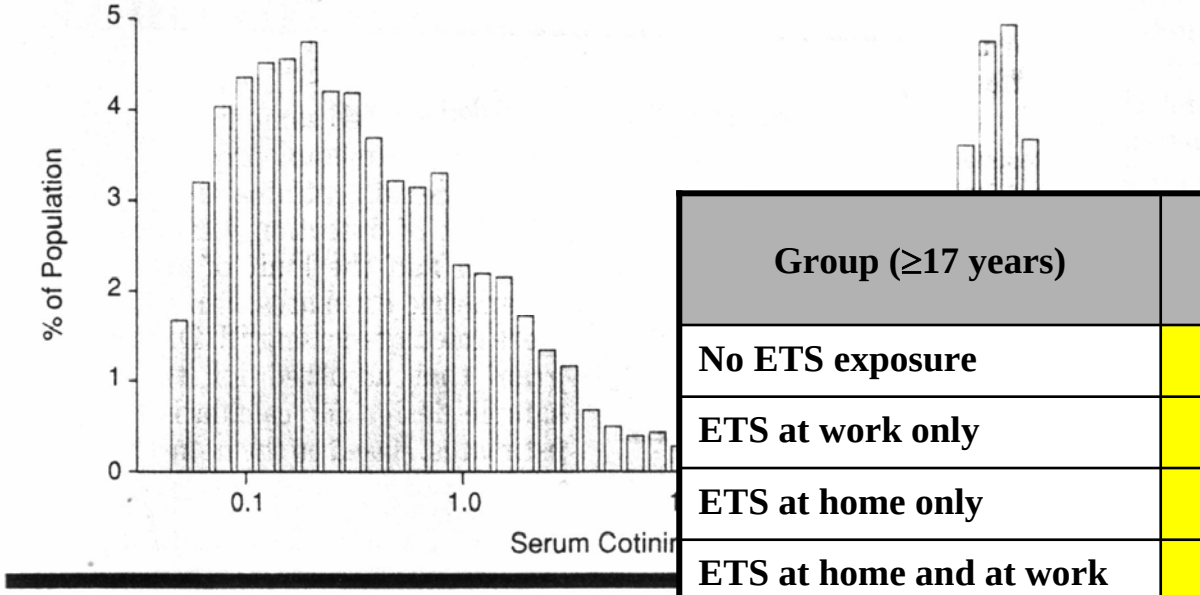


Figure 1.—Distribution of serum cotinine levels in the US population aged 4 years and older: Third National Health and Nutrition Examination Survey, October 25, 1988, to October 21, 1991.

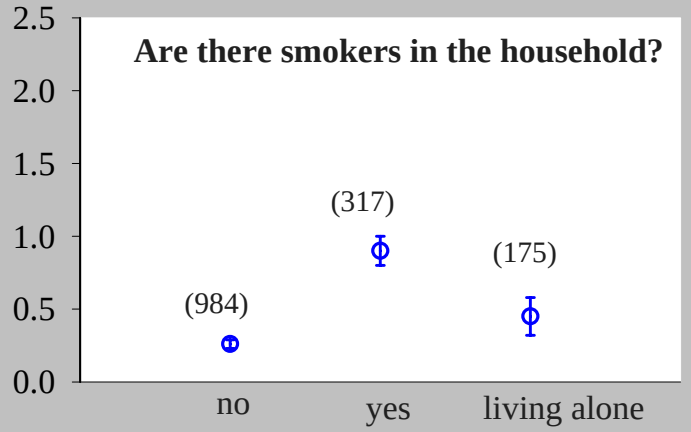
Group (≥17 years)	Cotinine (ng/ml) Geom. Mean
No ETS exposure	0.132
ETS at work only	0.318
ETS at home only	0.651
ETS at home and at work	0.926

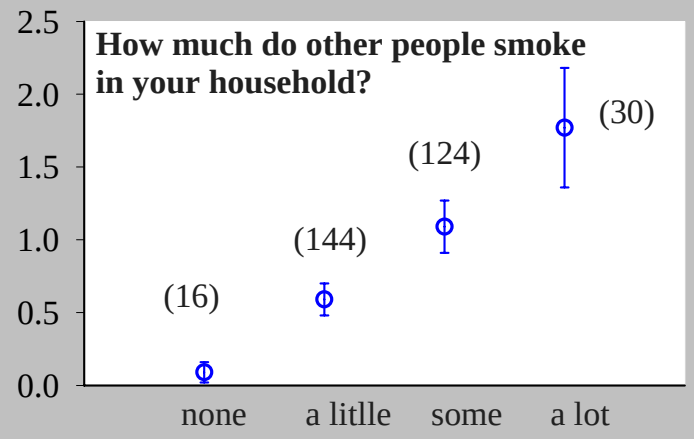
ETS: *Cotinine and self-reported exposure*

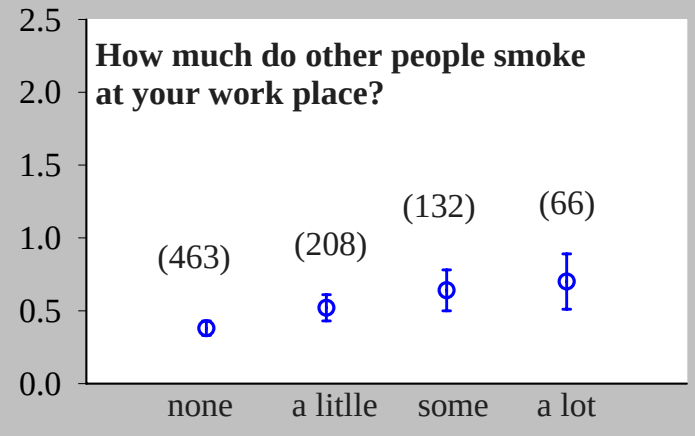
Heller et al. (1993), Indoor air '93, Proceeding Vol 3: 361-365

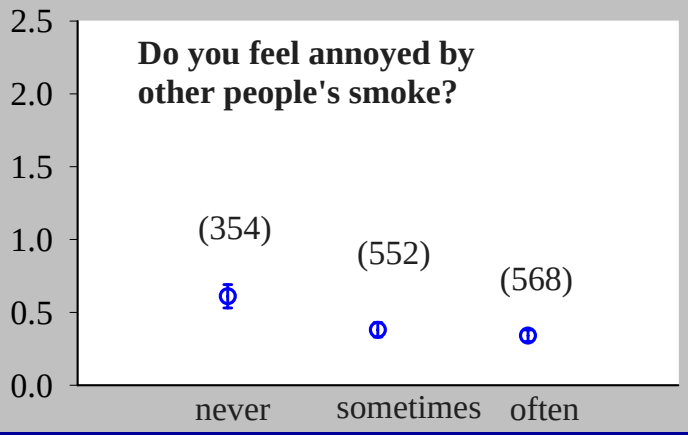
- **MONICA Study in Southern Germany, 1490 never smokers (1989/90)**

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









Cotinine in body fluids: *Ratio: Smokers/Nonsmokers*

Study	Body fluid	Not exposed to ETS	Exposed to ETS
Jarvis et al., 1984	Plasma	344	138
	Urine	927	181
	Saliva	443	124
Wald et al., 1984	Urine	914	56
Thompson et al., 1990	Urine	384	148
Tunstall-Pedoe et al, 1991	Plasma	Males: 353 Females: 2430	

Carbon monoxide (CO): *Levels in ETS*

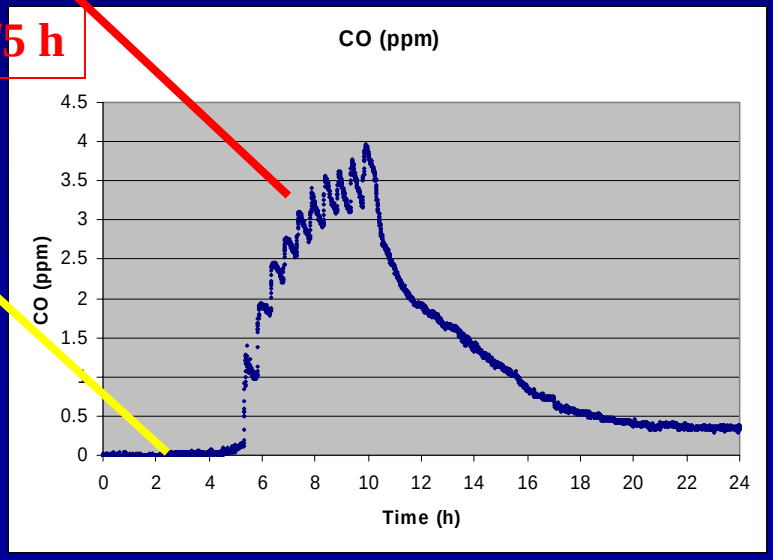
CO yields in mainstream smoke of cigarettes: 11.0 - 40.7 mg/cig*

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

CO in ETS:

Control (No smoking)	Smoking	Reference /Remarks
2.25 ppm	**2.81 ppm**	Scherer & Adlkofer, 1999 / Average in realistic rooms
0.02 ppm	**3.08 ppm**	ABF 2004 / Experim. room, low IAQ

10 cig/5 h



Other sources:

- Traffic exhausts

* Massachusetts smoking parameters (IARC, 2004)

Carbon monoxide (CO): *Biomarkers*

	Carboxyhemoglobin (COHb)	CO in exhaled air (COex)
Biological matrix	**Blood** (invasive)	**Exhaled air**
Half live	**2 – 4 h** (depending on physical activity)	
Background levels	**~ 1 %**	**2 – 3 ppm**
Levels in smokers	**4 – 8 %**	**10 – 50 ppm**
Interference	**Endogenous CO formation**	

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

***: $p < 0.001$

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:

Control (No smoking)	Smoking	Reference /Remarks
5.9 µg/m^3	**9.4 µg/m^3**	Scherer & Adlkofer, 1999 / Average in realistic rooms
1.6 µg/m^3	**16 µg/m^3**	ABF 2004 / Experim. room, low IAQ

Other sources:

- Traffic exhausts
- Fuels

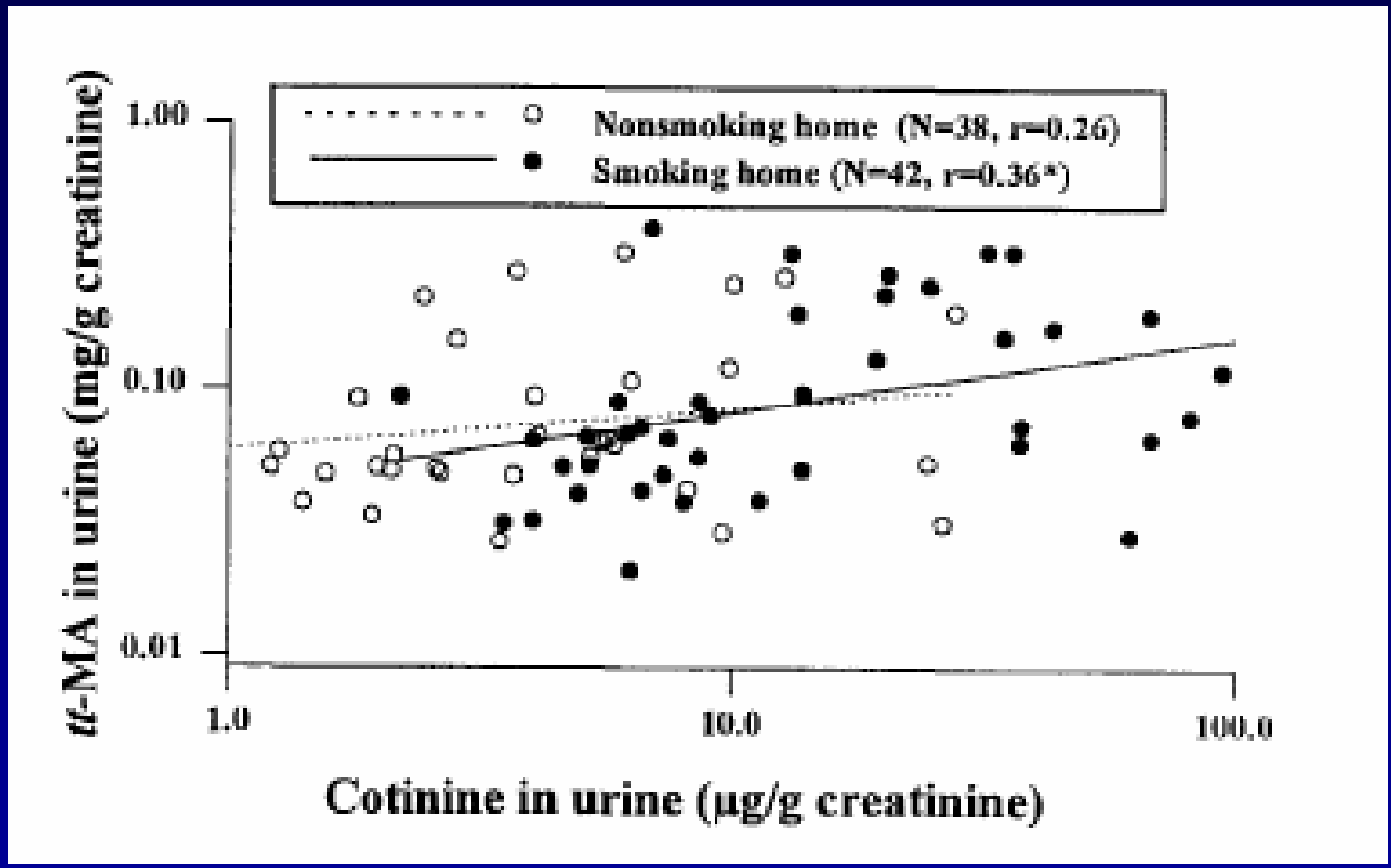
* Massachusetts smoking parameters (IARC, 2004)

Benzene: *Biomarkers*

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

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
t,t-MA (µg/g)	92 (N = 39)	126 (N = 43)	Scherer et al., 1995 ⇨
t,t-MA (µg/g)	64 (N = 39)	91 (N = 39)	Weaver et al., 1996
t,t-MA (µg/g)	76 (N = 60)	77 (N = 22)	Buratti et al., 1996

Benzene: *Relationship between tt-MA excretion and ETS exposure*

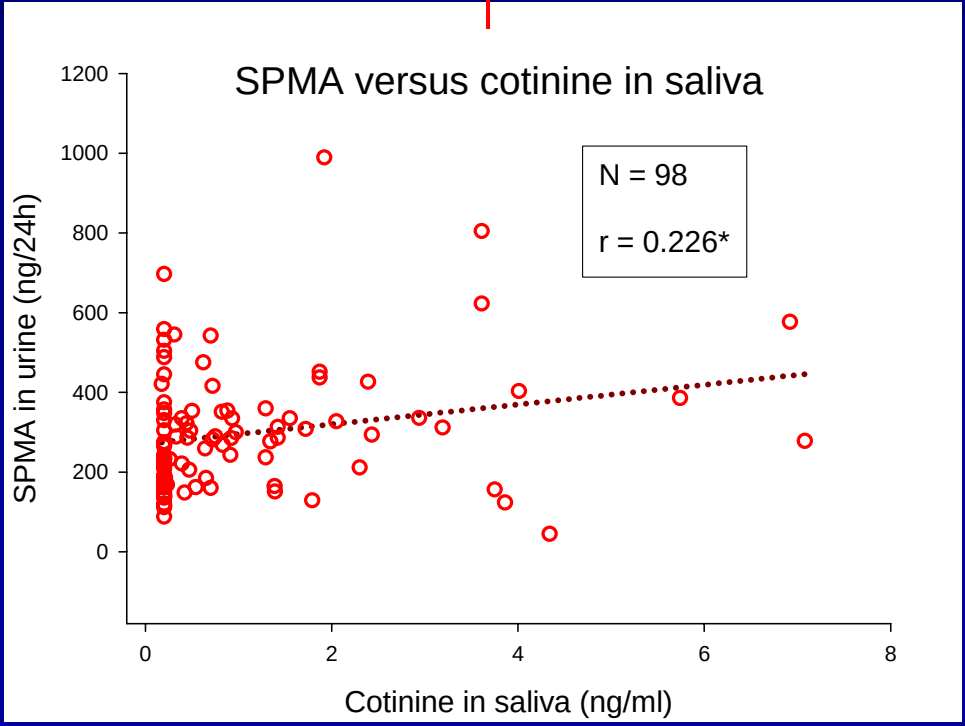


Scherer et al, 1995

Benzene: *Biomarkers*

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

*: $p < 0.05$



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*

Acrolein in ETS:	Control (No smoking)	Smoking	Reference /Remarks
	8.4 µg/m^3	**10.5 µg/m^3**	Scherer & Adlkofer, 1999 / Average in ca. 70 realistic rooms
	0.4 µg/m^3	**8.8 µg/m^3**	ABF 2004 / Experim. room, low IAQ

Other sources:

- Traffic exhausts
- Heating of fat

* Massachusetts smoking parameters (IARC, 2004)

Acrolein: *Biomarkers*

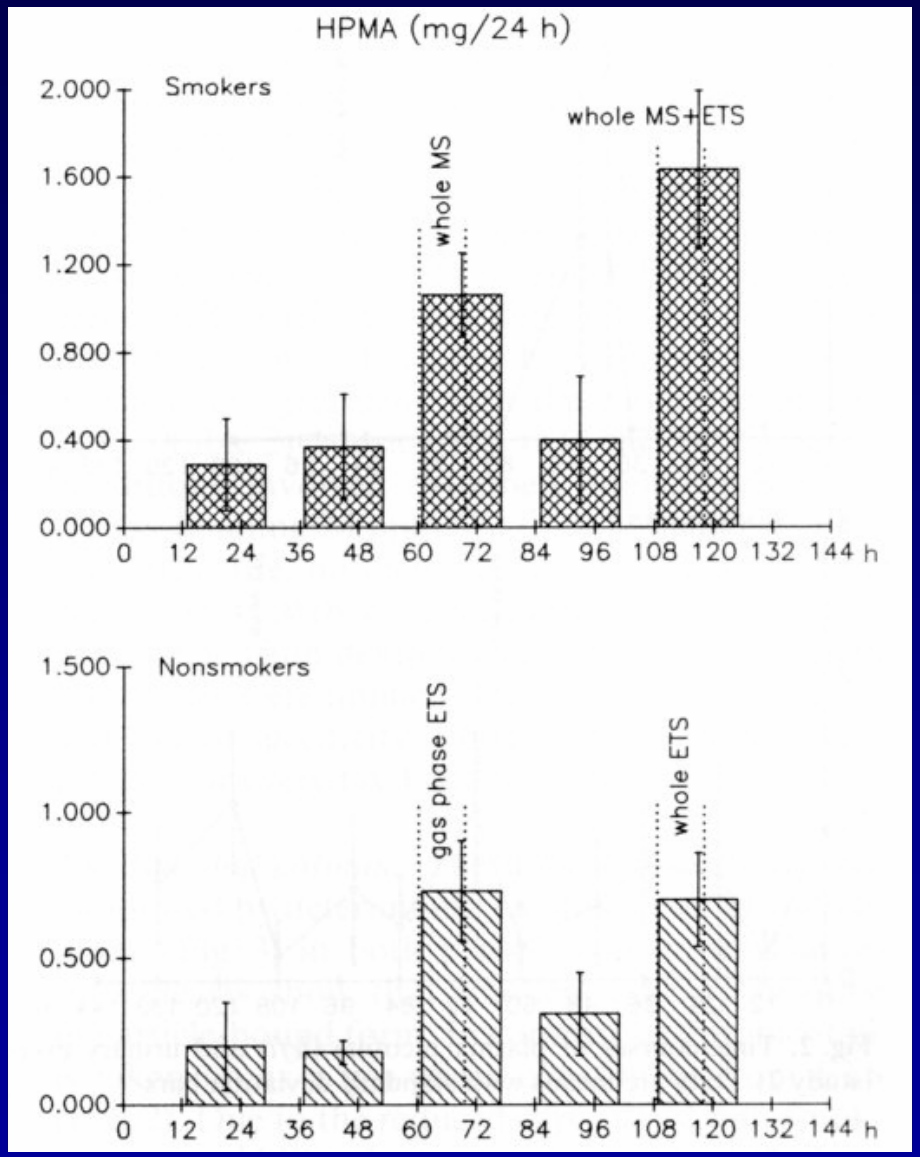
3-Hydroxypropylmercapturic Acid (HPMA)

Biological matrix	**Urine**
Half live	**6 - 9 h**
Background levels	**150 - 450 µg/24 h**
Levels in smokers	**500 – 1500 µg/24 h**
Interference	**Endogenous formation (Lipid peroxidation)**

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
HPMA (µg/24 h)	**200** (N = 5)	**750*** (N = 5)	Scherer et al., 1992 / ⇨ Experimental study with **high** ETS exposure
HPMA (µg/24 h)	**324** (N = 55)	**353** (N = 45)	Scherer et al., unpubl. ⇨

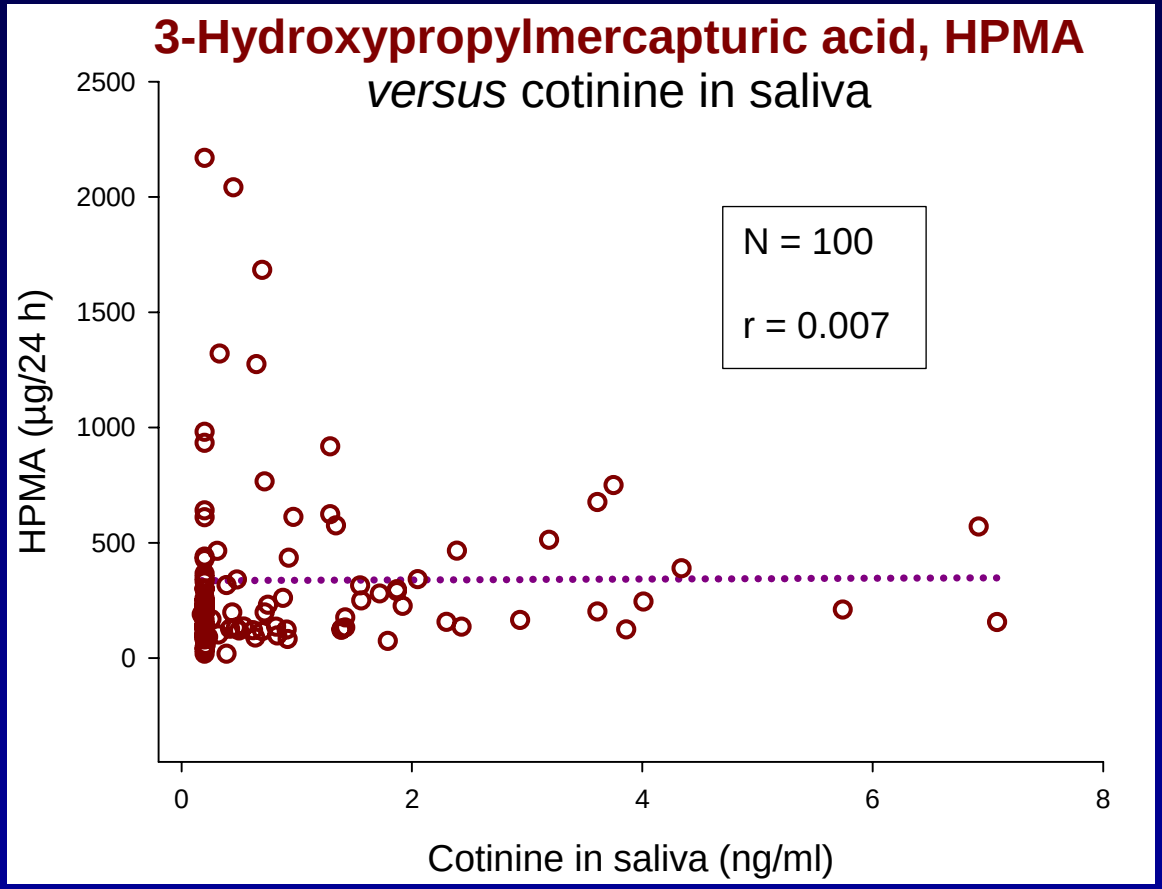
*: $p < 0.05$

Acrolein: *Biomarkers*



Scherer et al, 1992

Acrolein: *Biomarkers*



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***

Pyrene in ETS:

Control (No smoking)	Smoking	Reference /Remarks
4.6 – 9.3 ng/m^3	**4.3 - 11 ng/m^3**	Chuang et al., 1991 / 8 homes
	2.7 – 11.8 ng/m^3	Husgafvel-Pursiainen et al., 1986 / Restaurants
18.9 ng/m^3	**21.8 ng/m^3**	ABF 2004 / Experim. room, low IAQ

Other sources:

- Traffic exhausts
- Heating exhausts

* ISO/FTC smoking parameters (Grimmer et al., 1987)

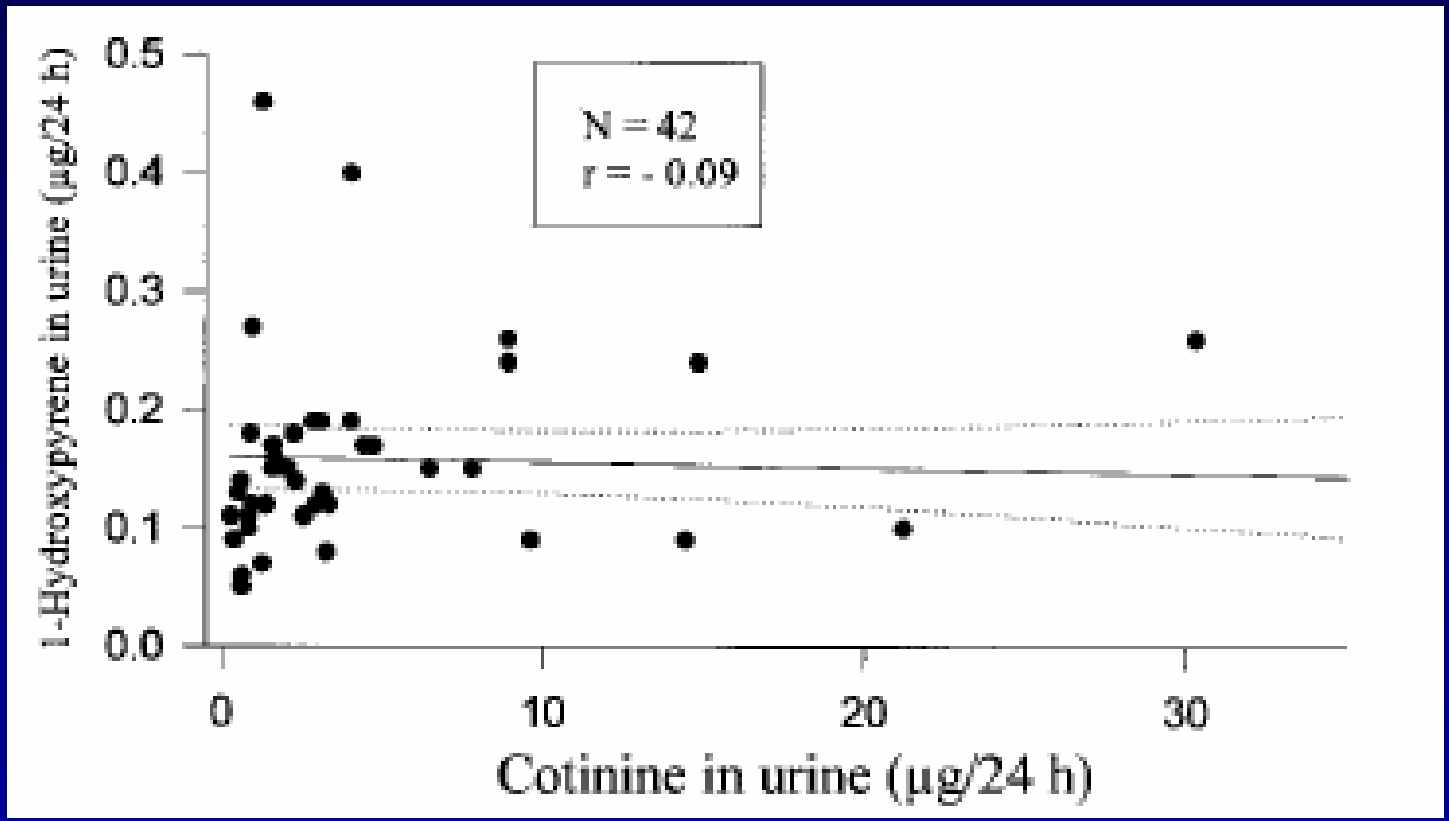
Pyrene (surrogate for PAH) : *Biomarkers*

1-Hydroxypyrene (1-OHP)

Biological matrix	**Urine**
Half live	**20 h**
Background levels	**0.05 µg/24 h**
Levels in smokers	**1.00 µg/24 h**
Interference	**Diet**

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
1-OHP (µg/24 h)	**0.171** (N = 23)	**0.140** (N = 19)	Scherer et al., 2000 ⇨
1-OHP (µmol/mol crea.)	**0.32** (N = 126)	**0.36** (N = 286)	Siwinska et al., 1999

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:

Control (No smoking)	Smoking	Reference /Remarks
0.27 – 0.58 ng/m^3	**0.37 – 1.7 ng/m^3**	Chuang et al., 1991 / 8 homes
	2.2 – 13.3 ng/m^3	Husgafvel-Pursiainen et al., 1986 / Restaurants
1.74 ng/m^3	**5.45 ng/m^3**	ABF 2004 / Experim. room, low IAQ

Other sources:

- Traffic exhausts
- Heating exhausts

* Massachusetts smoking parameters (IARC, 2004)

Benzo[a]pyrene (BaP): *Biomarkers*

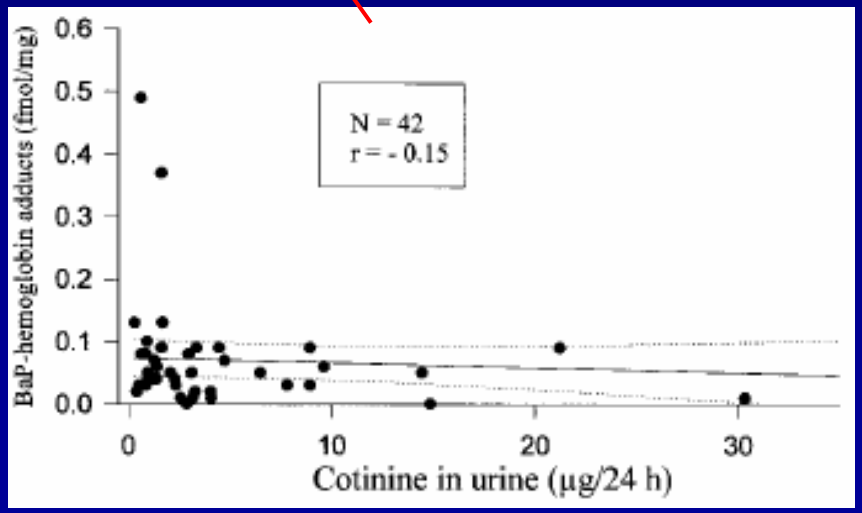
	BaP-Hemoglobin adducts (BaP-Hb)	BaP-Albumin adducts (BaP-Alb)
Biological matrix	**Blood**	**Plasma**
Half live	**4 months** (life-time)	**20 d**
Background levels	**variable** (dependent on the method)	
Levels in smokers	**variable** (dependent on the method)	
Interference	**Diet**	**Diet**

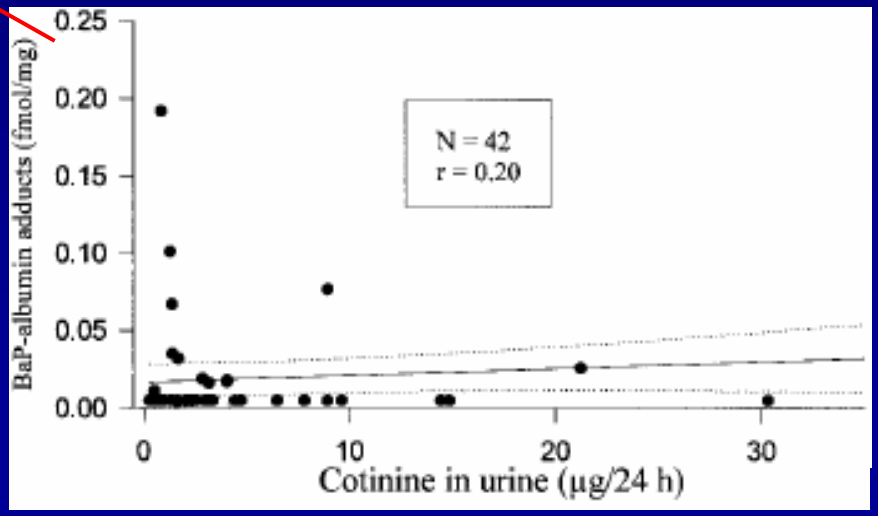
Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
BaP-Alb (fmol/µg)	**0.15** (N = 23)	**0.35*** (N = 31)	Crawford et al., 1994 Children
BaP-Alb (fmol/µg)	**0.185** (N = 24)	**0.437*** (N = 82)	Tang et al., 1999 Children

*: $p < 0.05$

Benzo[a]pyrene (BaP): *Biomarkers*

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
BaP-Alb (fmol/mg)	**0.019** (N = 23)	**0.021** (N = 19) Smokers: 0.042	Scherer et al., 2000
BaP-Hb (fmol/mg)	**0.083** (N = 23)	**0.049** (N = 19) Smokers: 0.105	Scherer et al., 2000





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**

- ***Petruzzelli 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.**

- ***Zenzes 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*

4-ABP in ETS:

Control (No smoking)	Smoking	Reference /Remarks
0.051 ng/m^3 (Train)	**0.11 – 0.20 ng/m^3 (2 Offices, 1 hair dresser saloon)**	Luceri et al., 1993
5 – 11 ng/m^3 (sum of 9 amines)	**15 – 33 ng/m^3 (sum of 9 amines)**	Palmiotto et al., 2001 / 9 Homes
0.026 ng/m^3	**0.582 ng/m^3**	ABF 2004 / Experim. room, low IAQ

<u>Other sources:</u> **?**

* Massachusetts smoking parameters (IARC, 2004)

4-Aminobiphenyl (4-ABP): *Biomarkers*

4-ABP-Hemoglobin adducts (4-ABP-Hb)

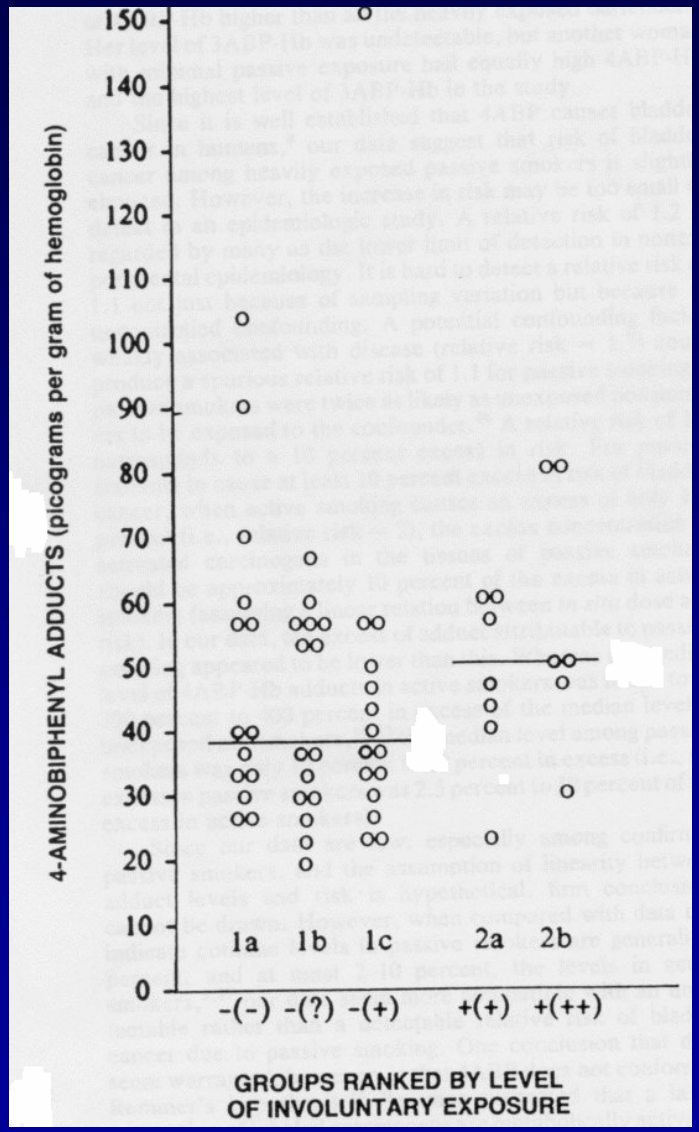
Biological matrix	**Blood**
Half live	**4 months** (life-time)
Background levels	**10 – 50 pg/g**
Levels in smokers	**50 – 500 pg/g**
Interference	**4-NBP[1] (exhausts), diet, hair dyes**

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

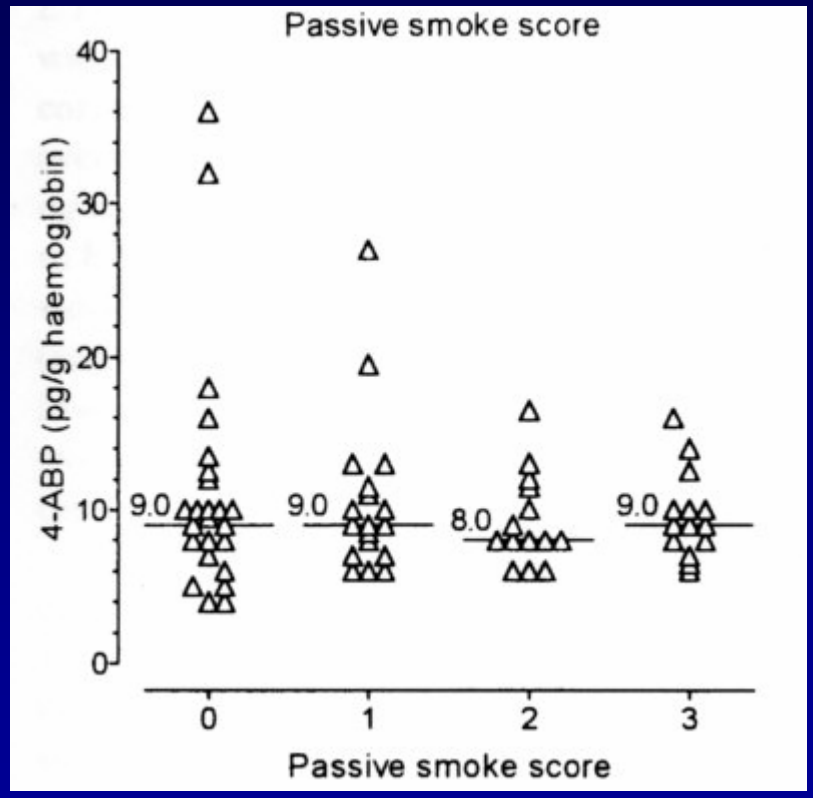
[1] 4-NBP: 4-Nitrobiphenyl

(*): p = 0.06; *: p < 0.05

4-Aminobiphenyl (4-ABP): *Biomarkers*



MaClure et al., 1989

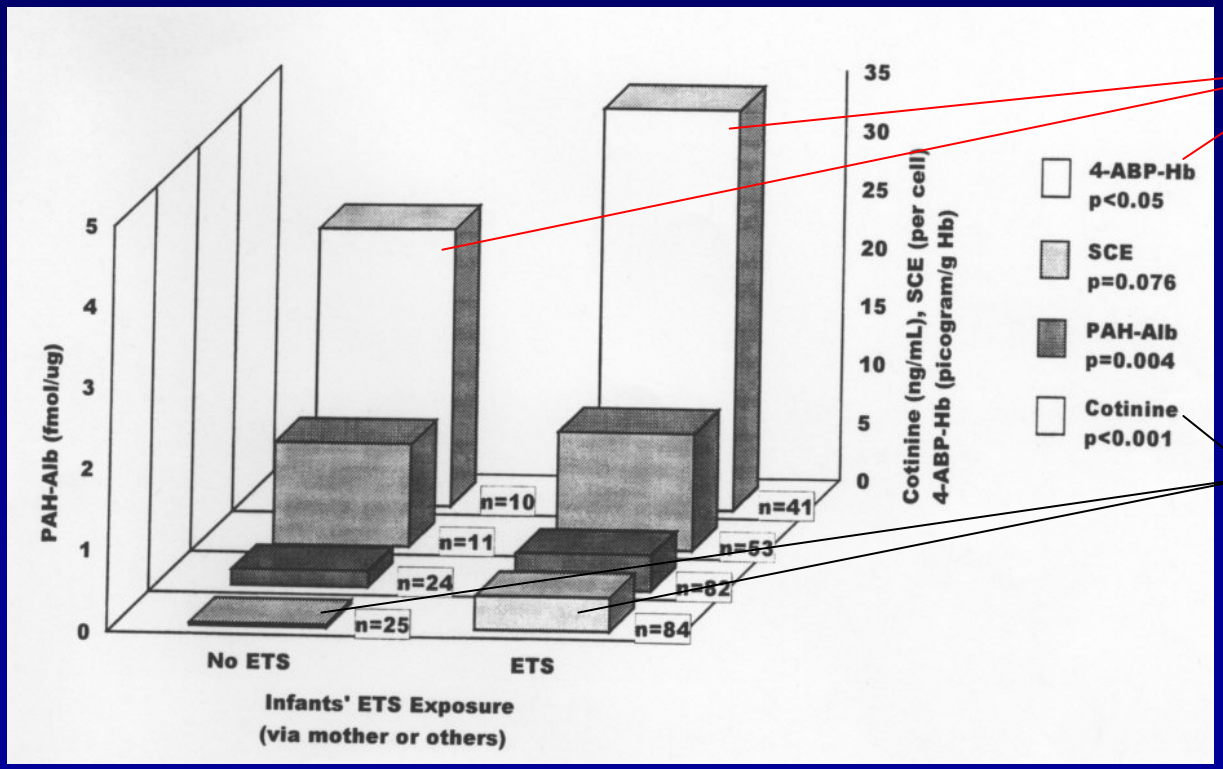


Branner et al., 1998

4-Aminobiphenyl (4-ABP): *Biomarkers*

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
4-ABP-Hb (pg/g)	**23.8** (N = 10)	**34.3*** (N = 41)	Tang et al., 1999 / ⇨ Infants

*: $p < 0.05$



4-ABP (pg/g)

Cotinine in plasma (ng/g)

NNK: *Levels in ETS*

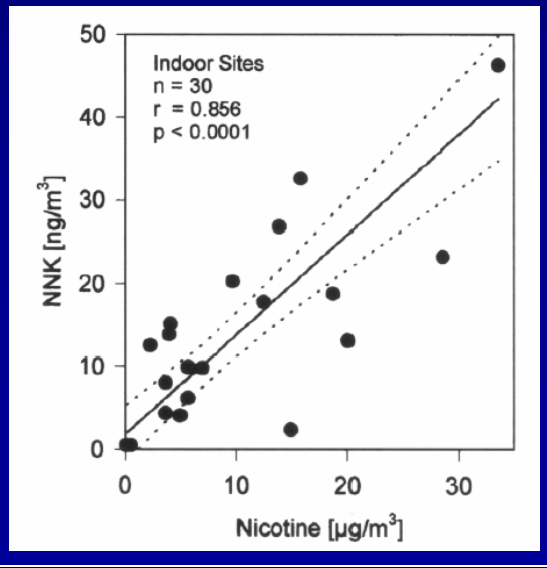
NNK yields in mainstream smoke of cigarettes: **53.5 - 220.7 ng/cig***

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

NNK in ETS:	Control (No smoking)	Smoking	Reference /Remarks
	0.25 ng/m^3 **(11 Rooms)**	**15.5 ng/m^3** **(29 Rooms)**	Scherer & Adlkofer, 1999 / Average in realistic rooms
	0.18 ng/m^3	**7.12 ng/m^3**	ABF 2004 / Experim. room, low IAQ

Other sources:

- None



Meger et al., 2000

* Massachusetts smoking parameters (IARC, 2004); ** ISO/FTC smoking parameters

NNK: *Biomarkers*

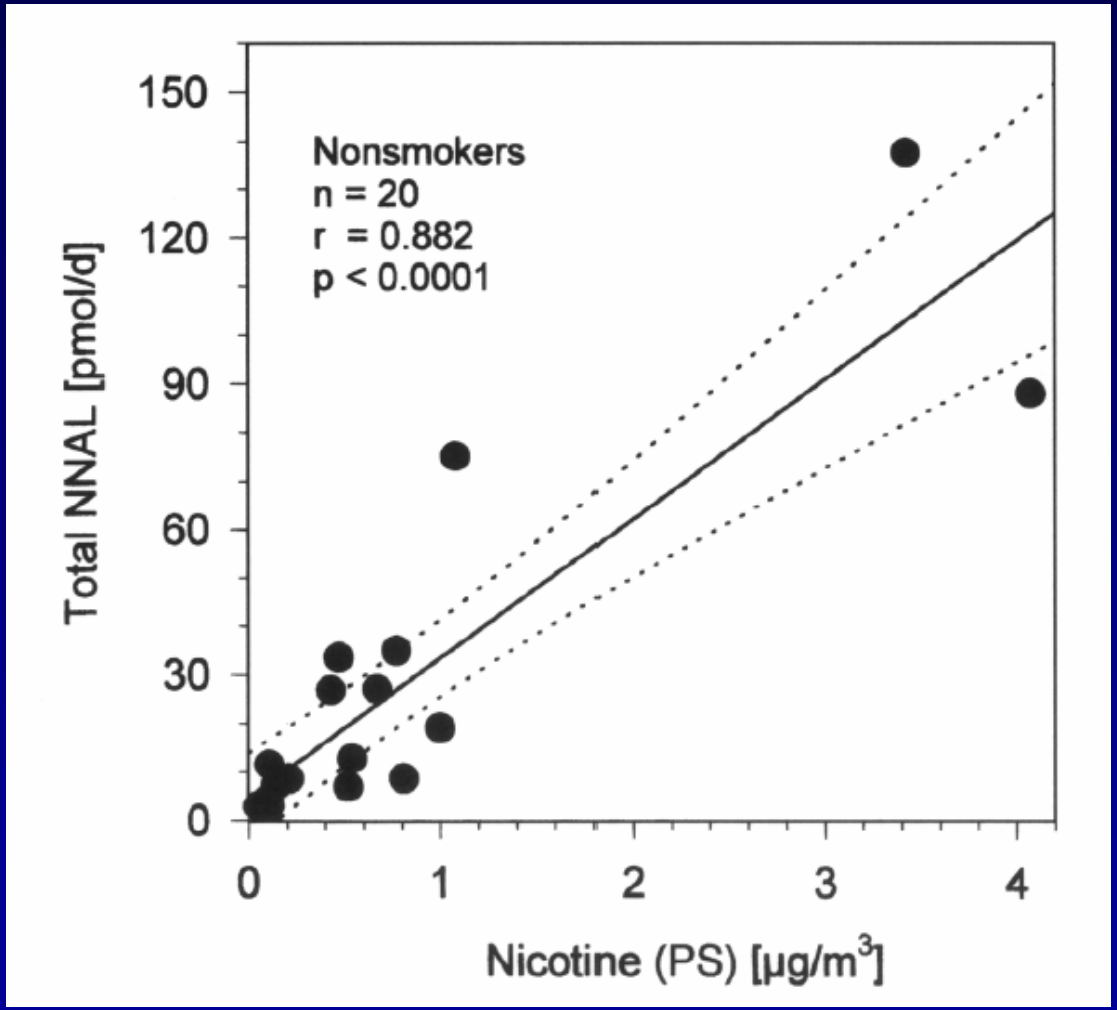
NNAL/NNAL-Glucuronide (Total NNAL)

Biological matrix	**Urine**
Half live	**1 d** (Phase 2: 6 weeks)
Background levels	**< LOD (< 3 pmol/24 h)**
Levels in smokers	**3200 pmol/24 h**
Interference	**None**

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

*: $p < 0.05$; **: $p < 0.01$

NNK: *Biomarkers*



Meger et al., 2000

NNK: *Biomarkers*

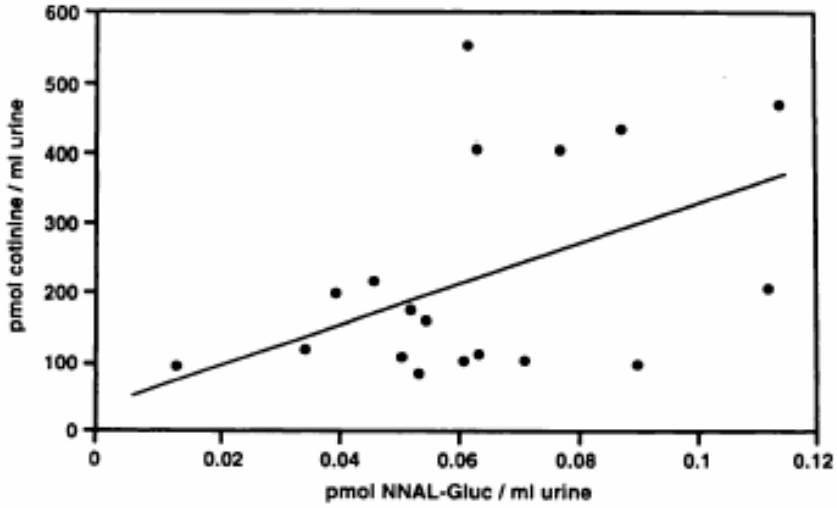


Fig. 3. Relationship between urinary cotinine and NNAL-Gluc in nonsmokers exposed to ETS ($r = 0.51$; $P = 0.029$).

Parsons et al., 1998

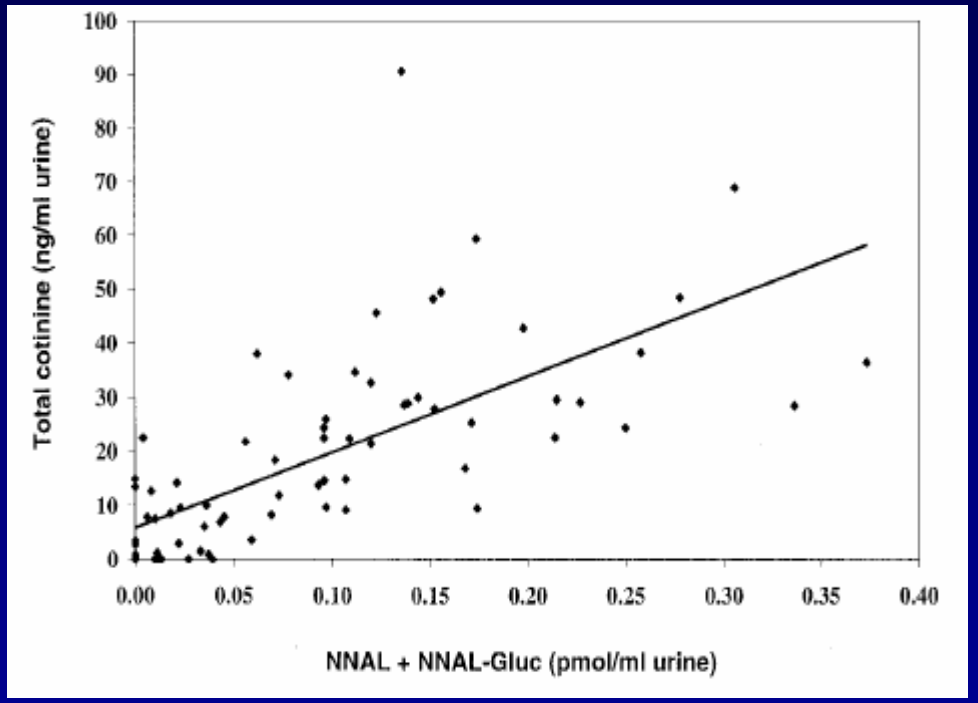


Fig. 5. Relationship between levels of total cotinine and NNAL plus NNAL-Gluc in the urine of 74 children. $r = 0.71$; $P < 0.0001$.

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*

E in ETS:	Control (No smoking)	Smoking	Reference /Remarks
	5 µg/m^3	**100 - 250 µg/m^3**	Persson et al., 1988 / Experimental room

<u>Other sources:</u>

- Traffic exhausts
- Terrestrial and marine organisms

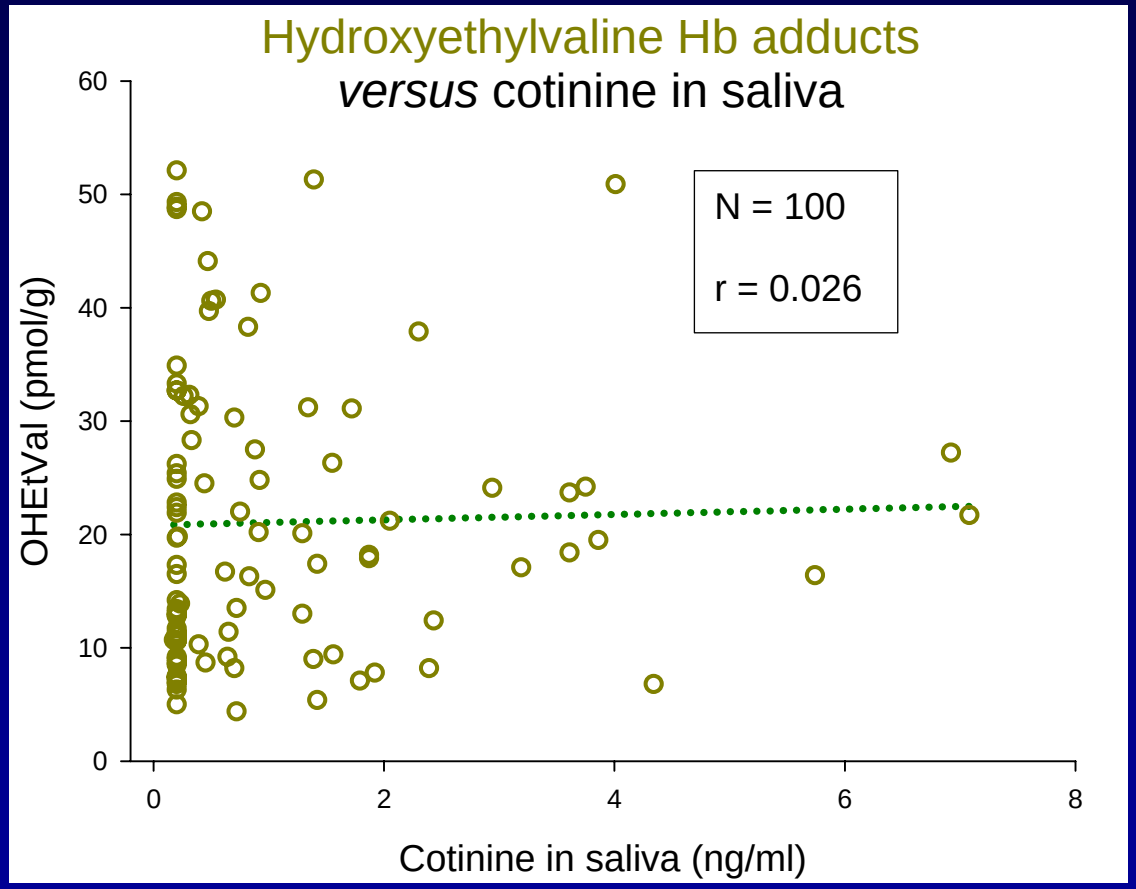
* ISO/FTC smoking parameters

Ethylene / Ethylene oxide: *Biomarkers*

	N-(2-Hydroxyethyl)valine Hemoglobin adducts (OHEtVal)
Biological matrix	**Blood**
Half live	**4 months** (life-time)
Background levels	**10 -20 pmol/g**
Levels in smokers	**50 - 200 pmol/g**
Interference	**Endogenous formation**

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference / Remarks
OHEtVal (pmol/g)	**17.0** (N = 74)	**16.6** (N = 28)	Bono et al et al., 1999 / No difference in urinary cotinine was found!
OHEtVal (pmol/g)	**21.3** (N = 55)	**20.8** (N = 45)	Scherer et al., unpubl. ⇨

Ethylene / Ethylene oxide: *Biomarkers*



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***

AN in ETS:	Control (No smoking)	Smoking	Reference /Remarks
	-	**0.8 µg/m^3 (Family room)** **0.6 µg/m^3 (upstairs bedroom)**	Guerin et al, 1992

Other sources:

- Certain workplaces
- ?

* Massachusetts smoking parameters (IARC, 2004

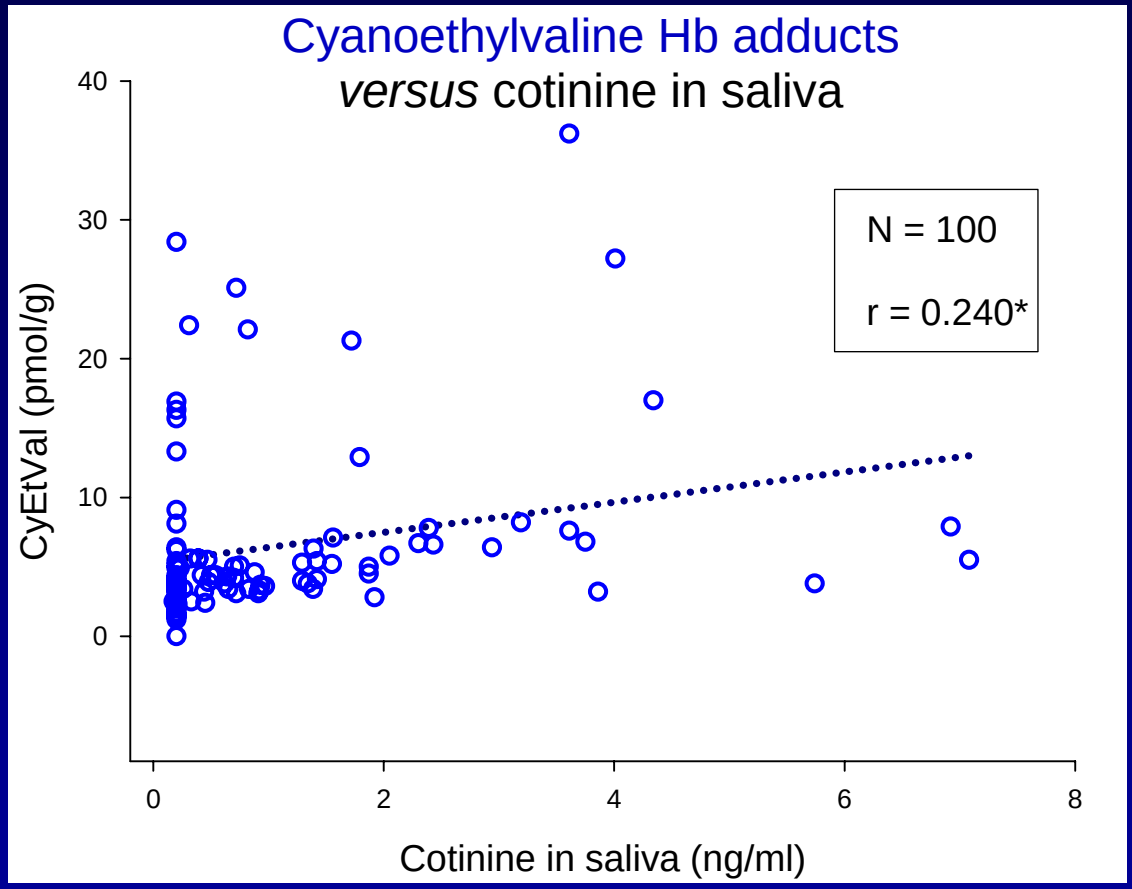
Acrylonitrile: *Biomarkers*

	Cyanoethylvaline Hemoglobin adducts (CyEtVal)
Biological matrix	**Blood**
Half live	**4 months** (life-time)
Background levels	**2 - 3 pmol/g**
Levels in smokers	**30 - 250 pmol/g**
Interference	**None**

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference / Remarks
CyEtVal (pmol/g)	**5.4** (N = 55)	**7.8(*)** (N = 45)	Scherer et al., unpubl. ⇨

(*): p = 0.061

Acrylonitrile: *Biomarkers*



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*-nitrosodiethylamine (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:

	Control (No smoking)	Smoking	Reference /Remarks
NDMA	**10.4 ng/m^3 (14 Rooms)**	**31.2 ng/m^3 (55 Rooms)**	Scherer & Adlkofer, 1999 / Average in realistic rooms
NDEA	**-**	**nd – 8.6 ng/m^3 (Office, 9 conditions)**	Klus et al., 1987 /

Other sources:

- Cooking
- Rubber

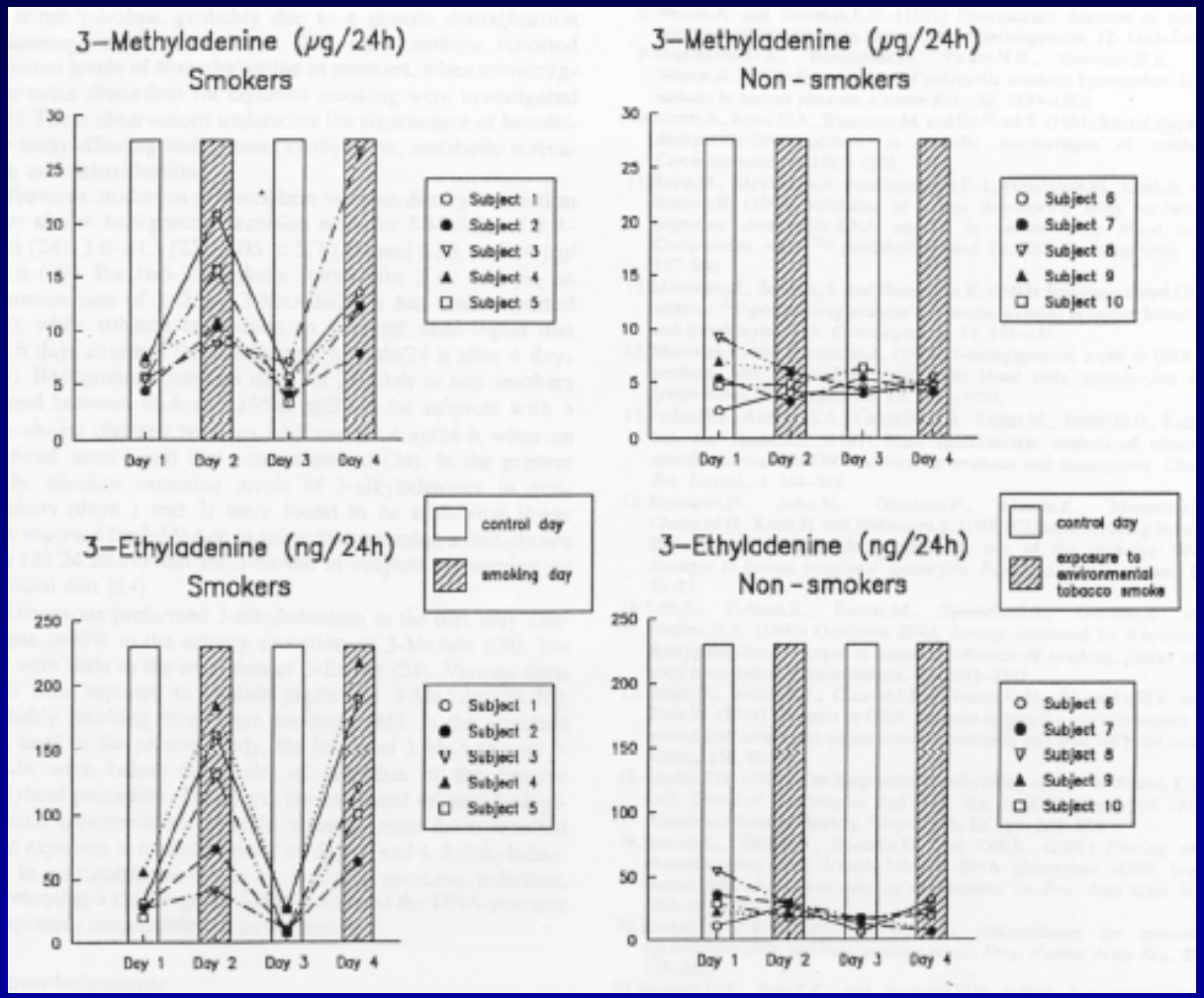
* ISO/FTC smoking parameters

Methylating/ethylating agents: *Biomarkers*

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

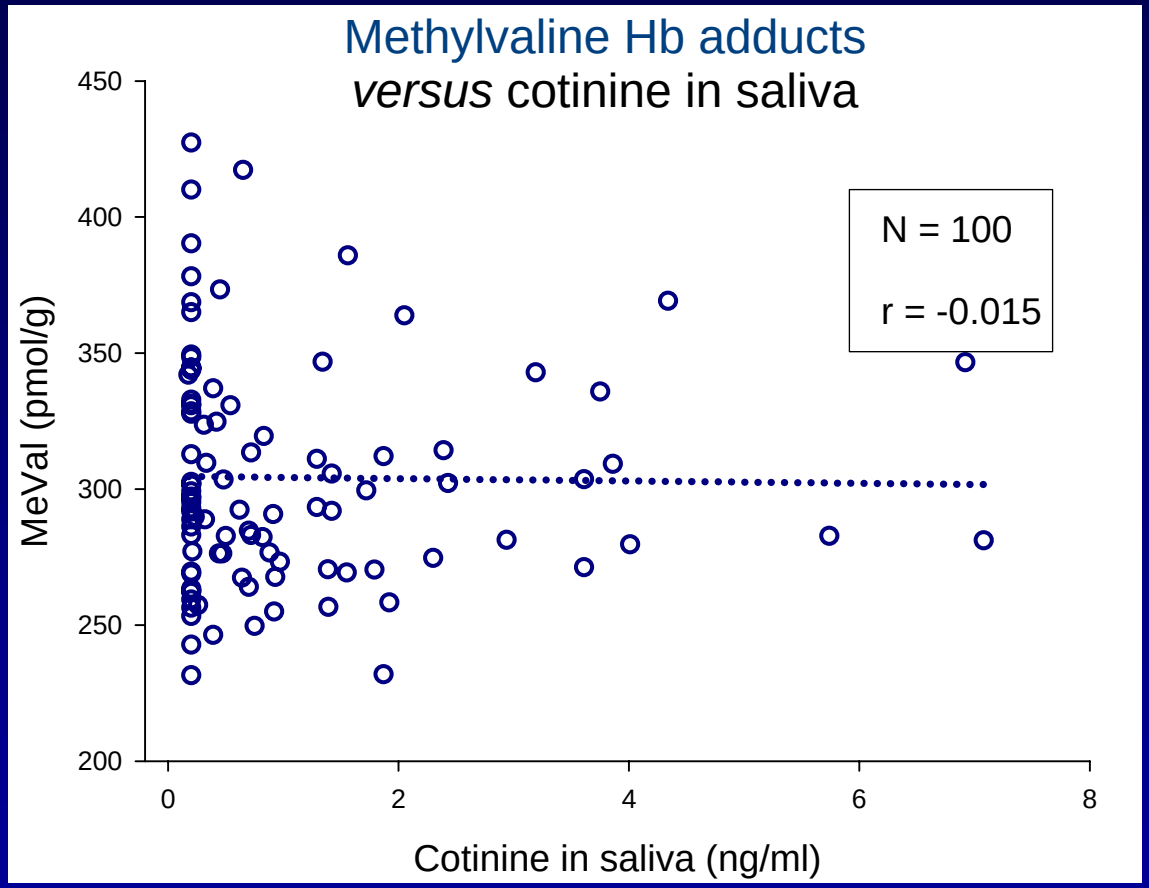
Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference /Remarks
3-MeA (µg/24h)	**4.7 – 5.9** (N = 5)	**4.8 – 4.9** (N = 5)	Kopplin et al., 1995 ⇨ Diet controlled study with high experimental ETS exposure
3-EtA (ng/24 h)	**14 - 31** (N = 5)	**18 - 25** (N = 5)	
MeVal (pmol/g)	**309** (N = 55)	**298** (N = 45)	Scherer et al., unpubl. ⇨

Methylating/ethylating agents: *Biomarkers*



Kopplin et al., 1995

Methylating agents: *Biomarkers*



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.)**

Mutagens: *Biomarkers*

Mutagenic activity of urine extracts	
Biological matrix	**Urine**
Half live	**7 h**
Background levels	**depending in bacterial strain (TA98, YG1024)**
Levels in smokers	**10 – 20-fold of background**
Interference	**Diet**

Biomarker	Nonsmokers Exposed to ETS	Reference / Remarks
Mutagenic activity with TA98 +S9 (cigarette equivalents)	**0.8*** (N = 8)	Bos et al., 1983 Experimental exposure to ETS
"	**4 – 5*** (N = 6)	Mohtashamipur et al., 1987 High exp. exposure to ETS
"	**0.2** (N = 5)	Scherer et al., 1990 High exp. exposure to ETS

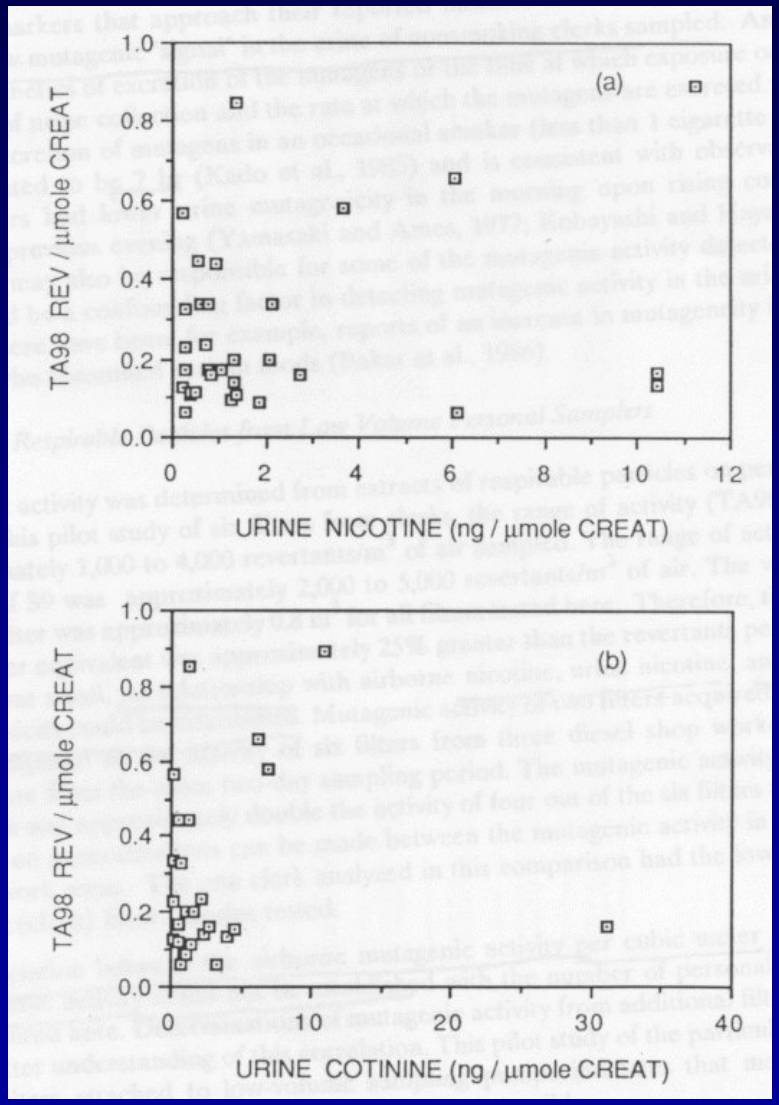
*: $p < 0.05$

Mutagens: *Biomarkers*

Biomarker	Nonsmokers not exposed to ETS	Nonsmokers Exposed to ETS	Reference / Remarks
Rev/25 µl urine with TA98+S9	**4.2** (N = 20)	**4.7** (N = 27)	Husgafvel-Pursiainen et al., 1987 / ETS exposed restaurant personnel
Rev/µmol crea. With TA98+S9	**No correlation with urinary cotinine** (N = 13)		Kado et al., 1987 ⇨ Pilot study with clerks
Rev/mmol crea. With TA98+S9	**0** (N = 35)	**182** (N = 4)[1] **509** (N = 11)[2]	Bartsch et al., 1990 / ETS exposed restaurant personnel
Rev/g crea. With YG1024+S9	**9944** (N = 10)	**15130** (N = 11)	Scherer et al., 1996 ⇨ ETS classification: < 5 *versus* ≥ 5 µg/g crea. cotinine

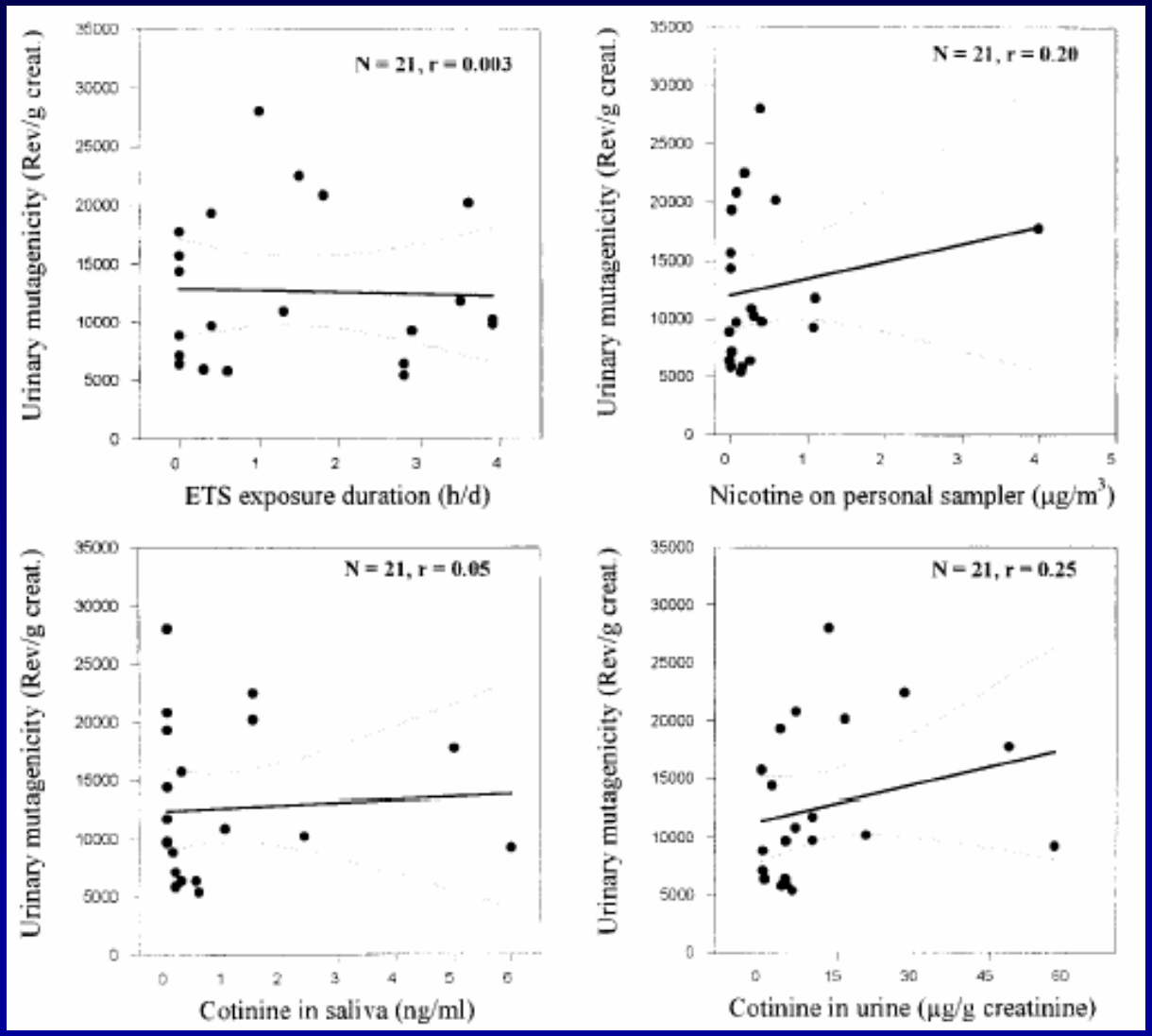
[1] Reporting ETS exposure and nicotine or cotinine detectable in urine
[2] Reporting ETS exposure, but no nicotine or cotinine detectable in urine

Mutagens: *Biomarkers*



Kado et al., 1987

Mutagens: *Biomarkers*



Scherer et al., 1996

ETS: *Biomarkers of exposure* *(except nicotine metabolites)* *(1)*

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

○ *No data shown in this presentation*

Scherer & Richter, 1997 (modified)

ETS: *Biomarkers of exposure (except nicotine metabolites) (2)*

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

Scherer & Richter, 1997 (modified)

ETS: *Biomarkers of exposure (except nicotine metabolites) (3)*

Biomarker	Precursor in ETS	Other sources	Significant increase
3-Methyl-/3-Ethyl-adenine in urine	Methylating and ethylating compounds	Diet	→
Mutagenicity in urine	PAH, HHA, AA	Diet	↑→
Thioethers in urine	Electrophiles	Diet	→

Scherer & Richter, 1997 (modified)

ETS: *Biomarkers of effect (1)*

Biomarker	Causing agent in ETS	Other factors	Significant effect
8-OHdG in urine, WBC, placenta	(Oxidative stress)	Many endogenous and exogenous factors	↑→
5-HMUra in urine	(Oxidative stress)	Many endogenous and exogenous factors	(↑)
Nitrated proteins in plasma	(Inflammation)	Many endogenous and exogenous factors	(↑)
Induction of AHH in placenta	PAHs, others	Traffic, diet, medications	(↑)
Hydroxyproline in urine	NO_2 (?)	Traffic, heating	↑→
Total cholesterol in blood	?	Diet, predisposition	↓→
HDL in blood	?	Diet, predisposition	↓→
LDL in blood	?	Diet, predisposition	→
Triglycerides in blood	?	Diet, predispostion	→

Scherer & Richter, 1997 (modified)

ETS: *Biomarkers of effect (2)*

Biomarker	Causing agent in ETS	Other factors	Significant effect
Platelet aggregation	?	Diet, medication	(↑)
Fibrinogen in plasma	?	Age, BMI, alcohol etc.	(↑)
Carotid wall thickness	?	Diet, predisposition	↑

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.**

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