

Application of Exposure Biomarkers in Clinical Studies of Potential Reduced-Exposure Products

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Clinical study requirements

Smoke chemistry characterisation

- PREP and reference cigarette(s)
- Percentage reductions in compounds A, B, C, ..

Biomarker(s) for reduced smoke constituent(s)

- ADME of compounds A, B, C, ..
- Validated analytical method(s)

Clinical study design concept

- Biomarker(s) → study design, size and power

Smoke chemistry characterisation

Tar, nicotine and carbon monoxide

Harmful smoke constituents ('Hoffmann list')¹

- Nitrosamines (11)
- Polycyclic aromatic hydrocarbons (10)
- Hetrocyclic compounds (8)
- Aromatic / heterocyclic amines (12)
- Aldehydes / volatile hydrocarbons (8)
- Organic compounds (17)
- Inorganic compounds / metals (16)

¹Hoffmann and Hoffmann, Beitr. Tabakforsch. Int. 18, 49-52, 1998

Representative biomarkers

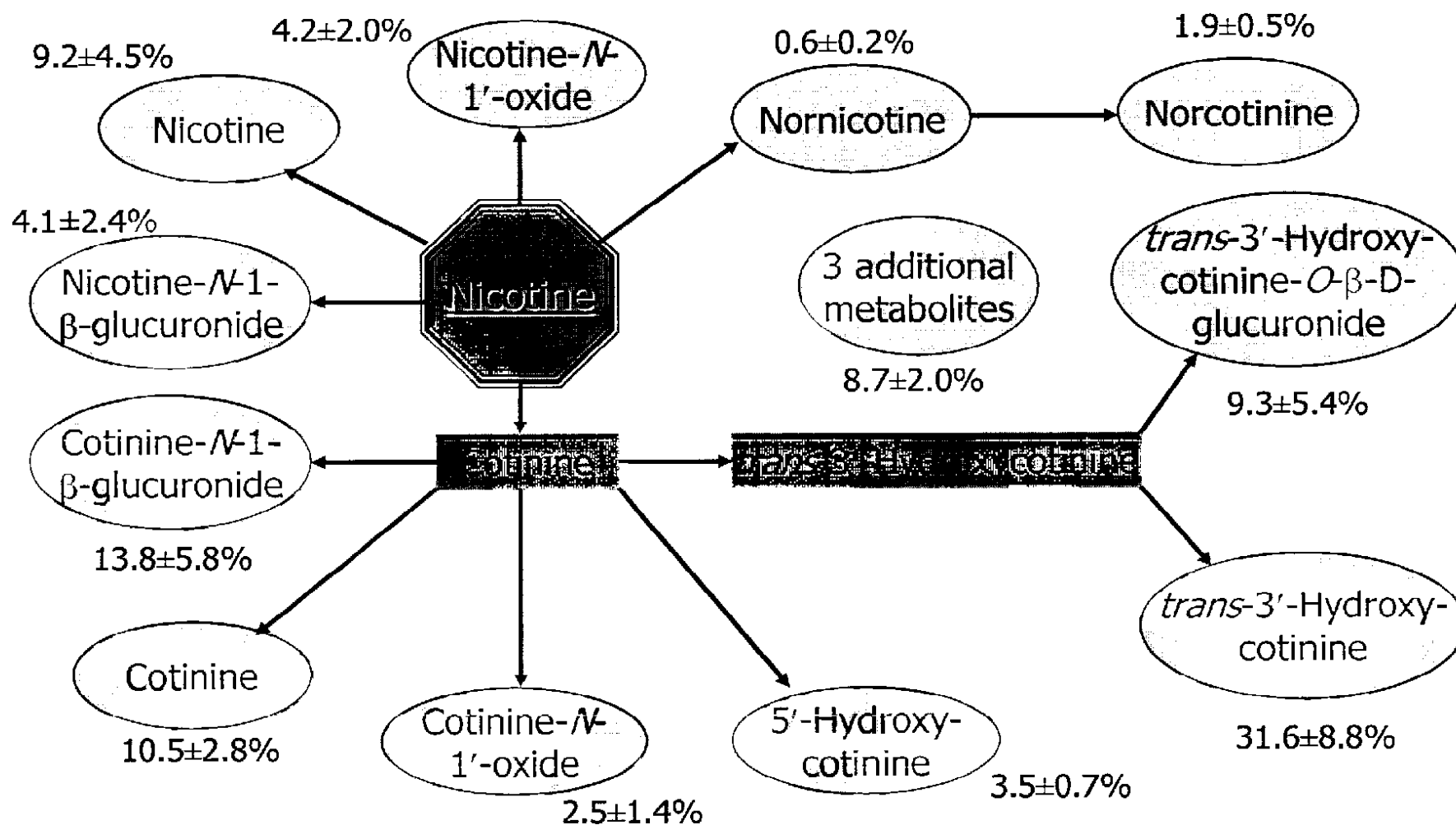
Smoke constituents	Biomarker / matrix		
	Breath	Blood	Urine
Tar	-	-	(Ames ?)
Nicotine	-	Metabolites	Metabolites
Carbon monoxide	CO	COHb	-
Nitrosamines (e.g., NNK)	-	Adducts	Metabolites
Polycyclic aromatic hydrocarbons (e.g., pyrene)	-	Adducts	Metabolites
Heterocyclic compounds (e.g., pyridine, furan)	?	?	?
Aromatic / heterocyclic amines (e.g., 4-ABP)	-	Adducts	Metabolites
Aldehydes (e.g., acrolein)	-	Adducts	Metabolites
Volatile hydrocarbons (e.g., benzene, toluene, styrene, 1,3-butadiene)	✓	Adducts and metabolites	Metabolites
Organic compounds (e.g., ethylene oxide)	?	Adducts	-
Inorganic compounds / metals	-	-	-

'Tar'

Determination of Ames mutagenicity of urine may give an indication of exposure to mutagenic and promutagenic material present in the particulate phase ('tar') of cigarette smoke

- Controlled experimental and dietary conditions
- *Salmonella typhimurium* TA98 and TA100
- *Salmonella typhimurium* YG1024

Nicotine



Nicotine – blood measurements

Nicotine

- Short half-life ($t_{1/2} \sim 2$ h), low sensitivity
- Rate of metabolism (*CYP2A6* polymorphism)
- Background nicotine contamination
- 'Nicotine boost'

Cotinine (proximal metabolite of nicotine)

- Long half-life ($t_{1/2} \sim 18$ h), high sensitivity

trans-3'-Hydroxycotinine and cotinine-*N*-glucuronide

'Nicotine boost' (Nicotine_{boost})

Experimental estimation of blood nicotine uptake

$$\text{'Nicotine}_{\text{boost}}' = \text{Post-cigarette}_{\text{blood nicotine}} - \text{Pre-cigarette}_{\text{blood nicotine}}$$

- Results dependent on experimental design
- Time of blood sampling
- Subject size and weight

Nicotine – urine measurement

Nicotine	9.2±4.5	} 80.7±3.4%
Nicotine- <i>N</i> -1-β-glucuronide	4.1±2.4	
Cotinine	10.5±2.8	
Cotinine- <i>N</i> -1-β-glucuronide	13.8±5.8	
<i>trans</i> -3'-Hydroxycotinine	31.6±8.8	
<i>trans</i> -3'-Hydroxycotinine- <i>O</i> -β-D-glucuronide	9.3±5.4	
5'-Hydroxycotinine	3.5±0.7	
Nicotine- <i>N</i> -1'-oxide	4.2±2.0	
Cotinine- <i>N</i> -1-oxide	2.5±1.4	
Nornicotine	0.6±0.2	
Norcotinine	1.9±0.5	
4-(3-Pyridyl)-4-oxobutyric acid	0.8±0.6	
4-(3-Pyridyl)-4-hydroxybutyric acid	6.6±1.2	
3-Pyridylacetic acid	1.3±0.4	
Total	100%	

Nicotine – urine measurements

24-hour urine and spontaneous urine samples

- Correction of metabolite excretion by urine specific gravity or creatinine excretion

Method of analysis for glucuronide conjugates

- Direct analysis by LC-MS/MS
- Indirect analysis (+/- β -glucuronidase)

'Nicotine equivalents' estimate of nicotine uptake

- NEq = molar sum of nicotine + 5 metabolites
- Comparison of nicotine uptake/cigarette

Carbon monoxide

Carbon monoxide in exhaled breath (CO_{ex}) and bound carboxyhemoglobin (COHb) nonspecific

- Short half-lives ($t_{1/2}$ CO_{ex} and COHb $\sim 2-4$ h)
- $\text{COHb} (\%) = 0.6 + 0.3 \times \text{CO}_{\text{ex}} (\text{ppm})$

	Standard reference values	
	CO_{ex} (ppm)	COHb (%)
Nonsmokers	3.0 – 7.0	0.8 – 1.5
Smokers	20 - 40	4.0 – 8.0

'CO_{boost}'

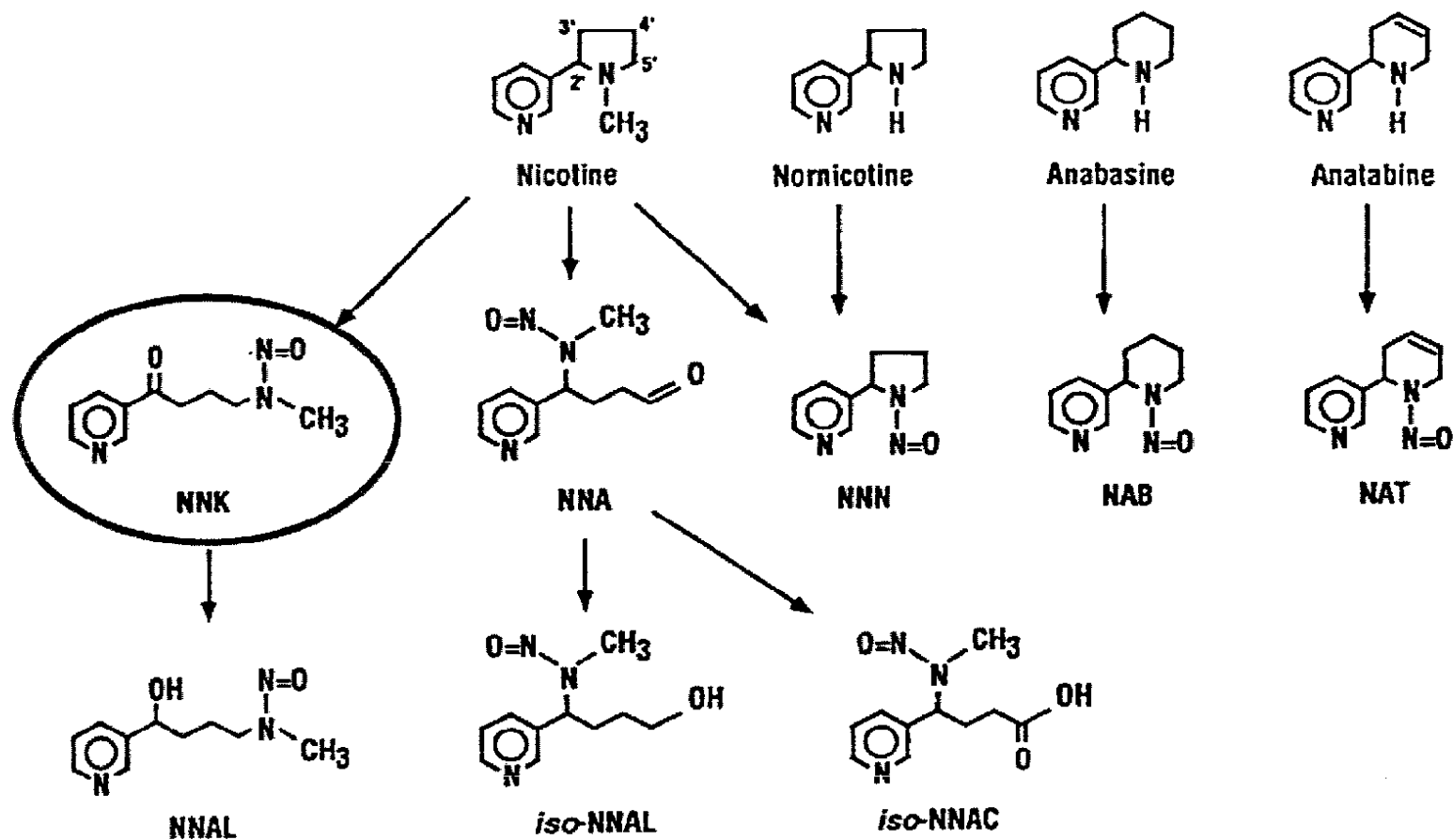
Experimental estimation of CO uptake

$$\text{'CO}_{\text{boost}}' = \text{Post-cigarette}_{\text{COex}} - \text{Pre-cigarette}_{\text{COex}}$$

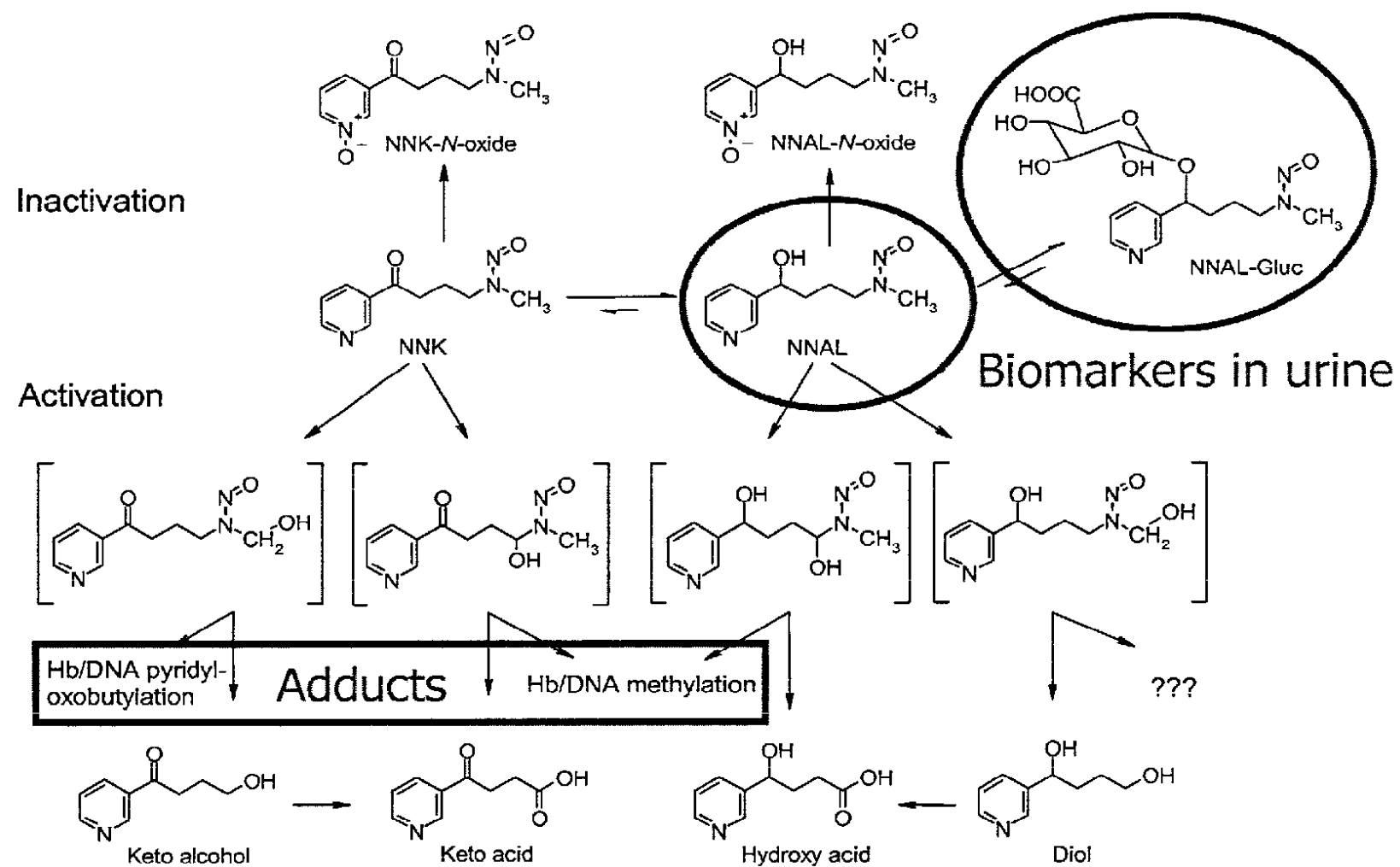
'Inhalation index' (I) or 'inhalation efficiency'

$$I = \text{'CO}_{\text{boost}}' / \text{CO}_{\text{cigarette yield}}$$

(Tobacco-specific) nitrosamines



Metabolism of NNK



Adducts as biomarkers

Smoke constituents	Biomarker / matrix		
	Breath	Blood	Urine
Nitrosamines (e.g., NNK)	-	Adducts	Metabolites
Polycyclic aromatic hydrocarbons (e.g., pyrene)	-	Adducts	Metabolites
Aromatic / heterocyclic amines (e.g., 4-ABP)	-	Adducts	Metabolites
Aldehydes (e.g., acrolein)	-	Adducts	Metabolites
Volatile hydrocarbons (e.g., benzene, toluene, styrene, 1,3-butadiene)	✓	Adducts and metabolites	Metabolites
Organic compounds (e.g., ethylene oxide)	?	Adducts	-

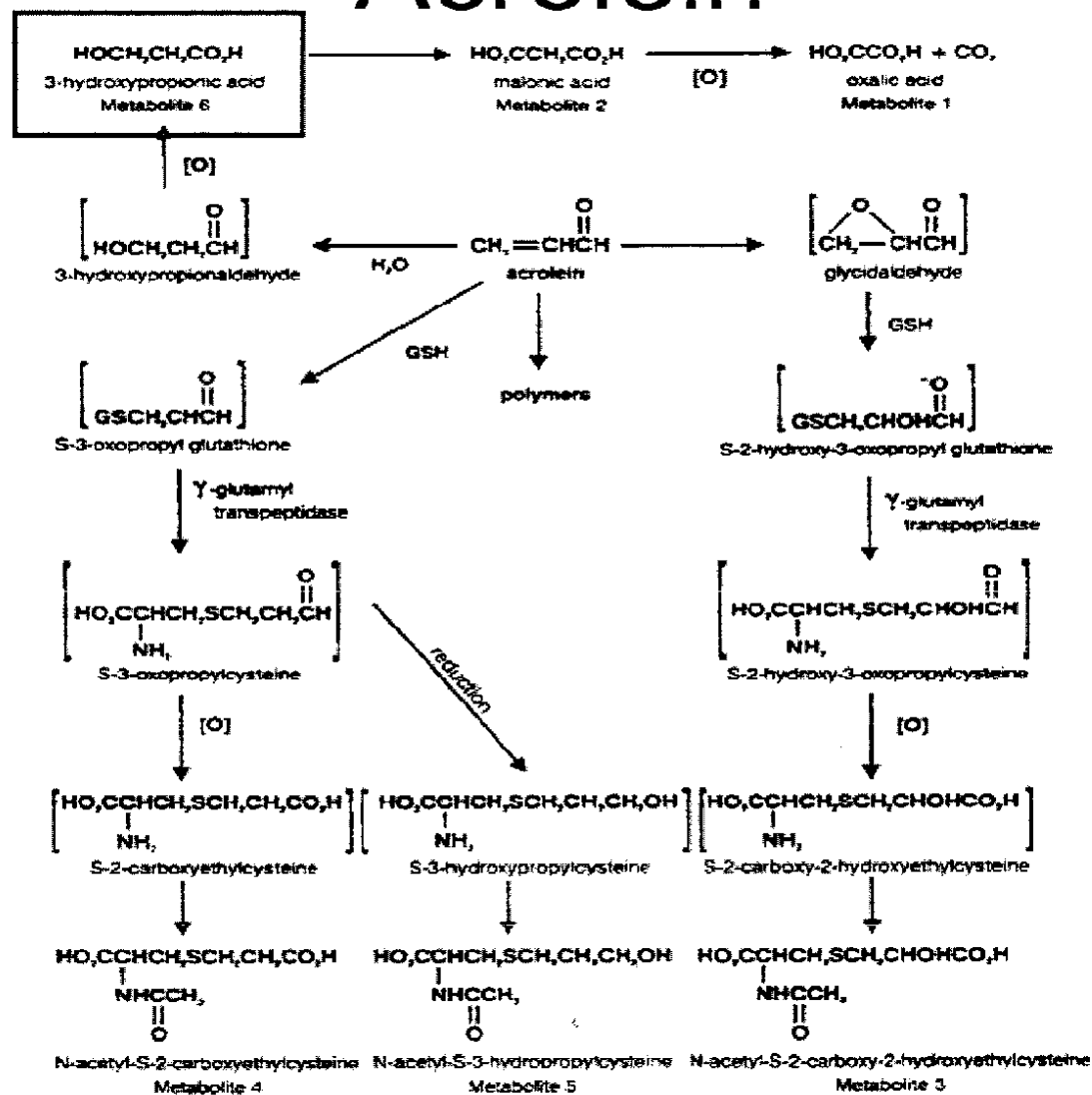
- The same adduct with DNA, albumin and hemoglobin may be formed by several different compounds
- Long-term (surveillance) studies

Polycyclic aromatic hydrocarbons

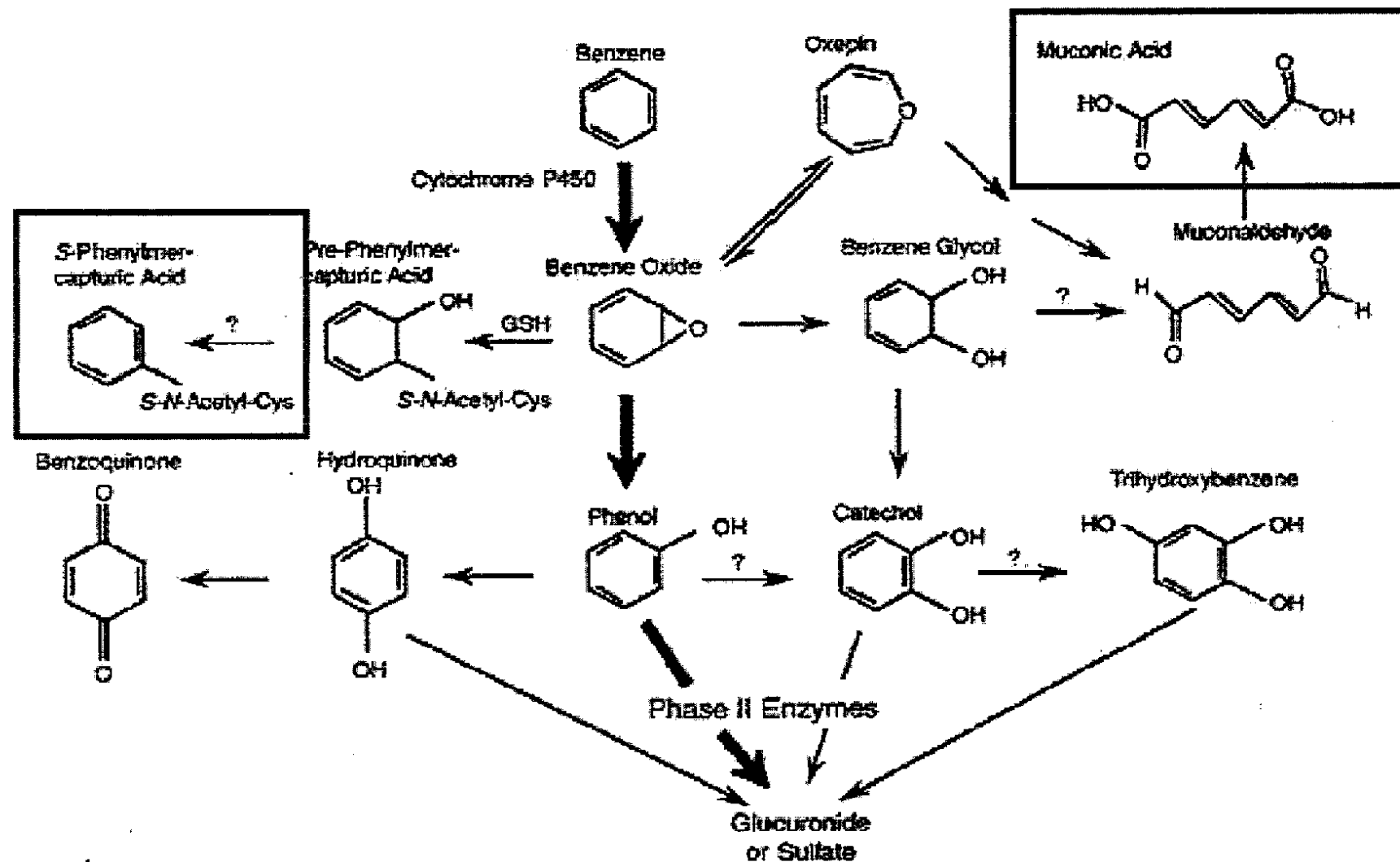
PAH	Major urinary metabolites
Benzo[a]anthracene	1- and 3-Hydroxybenzo[a]anthracene
Benzo[c]phenanthrene	1-, 2- and 3-Hydroxybenzo[c]phenanthrene
Chrysene	3- and 6-Hydroxychrysene
Fluoranthene	3-Hydroxyfluoranthene
Fluorene	2, and 3-Hydroxyfluorene
Phenanthrene	1-, 2- and 3-Hydroxyphenanthrene
Pyrene	1-Hydroxypyrene

- Hydroxypyrene (1-OHP) marker compound for total PAH
- Exposure to pyrene from diet and environment

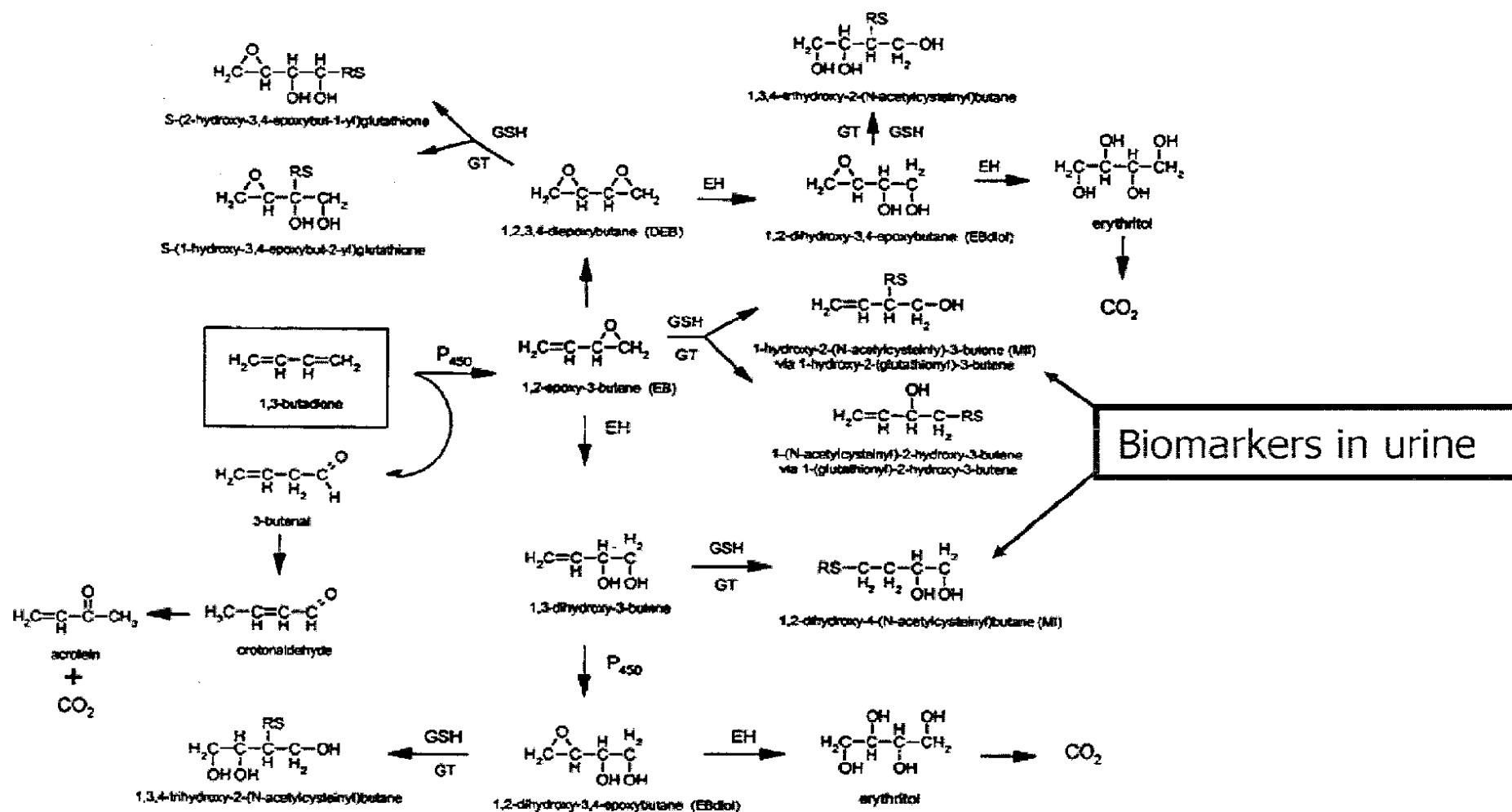
Acrolein



Benzene



1,3-butadiene



Clinical study example

- Smoke chemistry characterisation
- Biomarker selection
- Study design
- Results

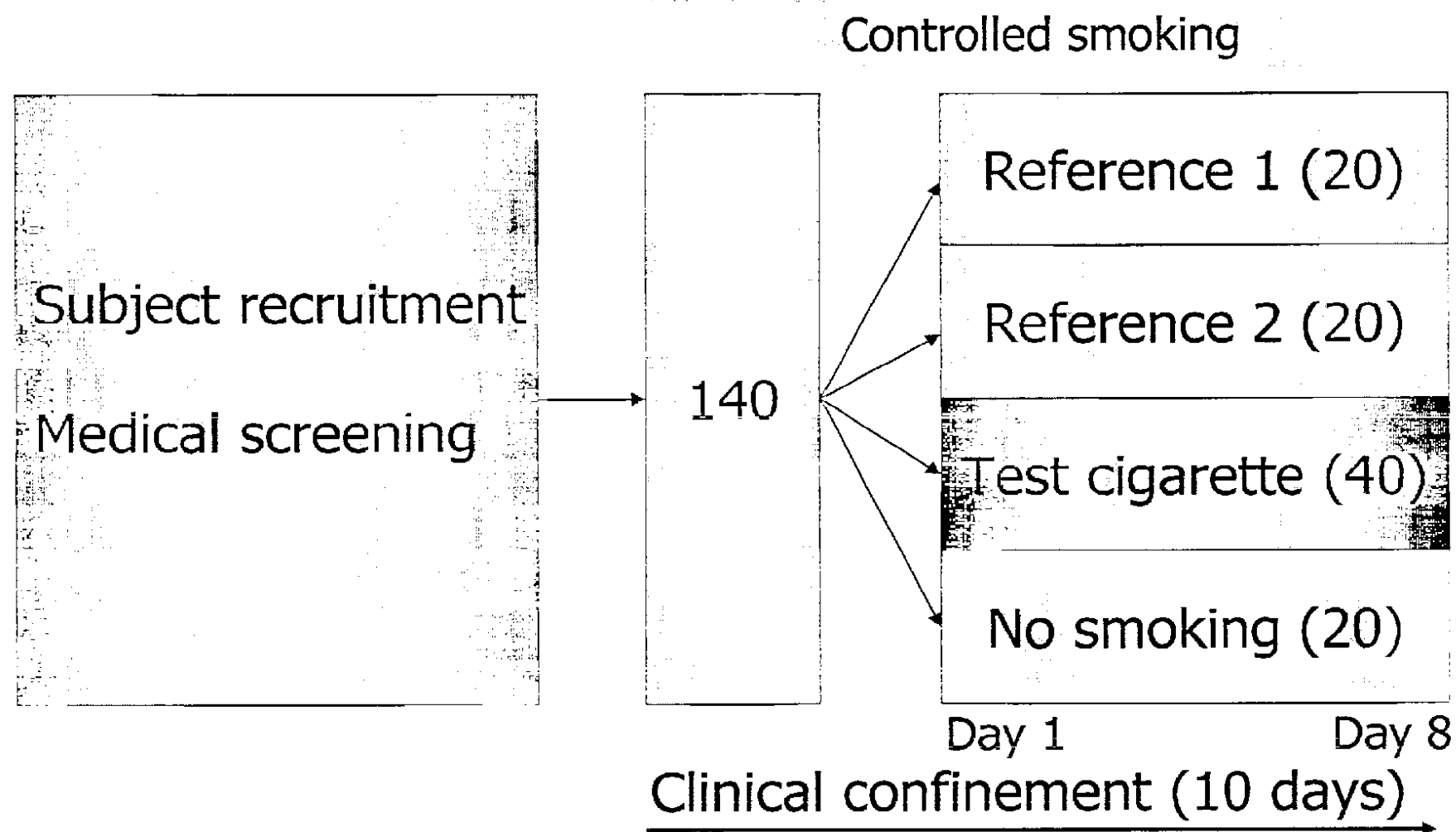
Smoke chemistry characterisation

Smoke constituent	Cigarette yield		
	Reference 1	Reference 2	Test (PREP prototype)
Tar (mg)	6.0	1.0	6.0
Nicotine (mg)	0.5	0.1	0.5
Carbon monoxide (mg)	7.0	2.0	7.0
NNK (ng)	43	18	35
Pyrene (ng)	28	6	26
4-aminobiphenyl (ng)	0.8	0.3	0.9
Acrolein (μg)	50	10	1
Benzene (μg)	31	11	1
1,3-butadiene (μg)	28	9	1

Biomarker selection

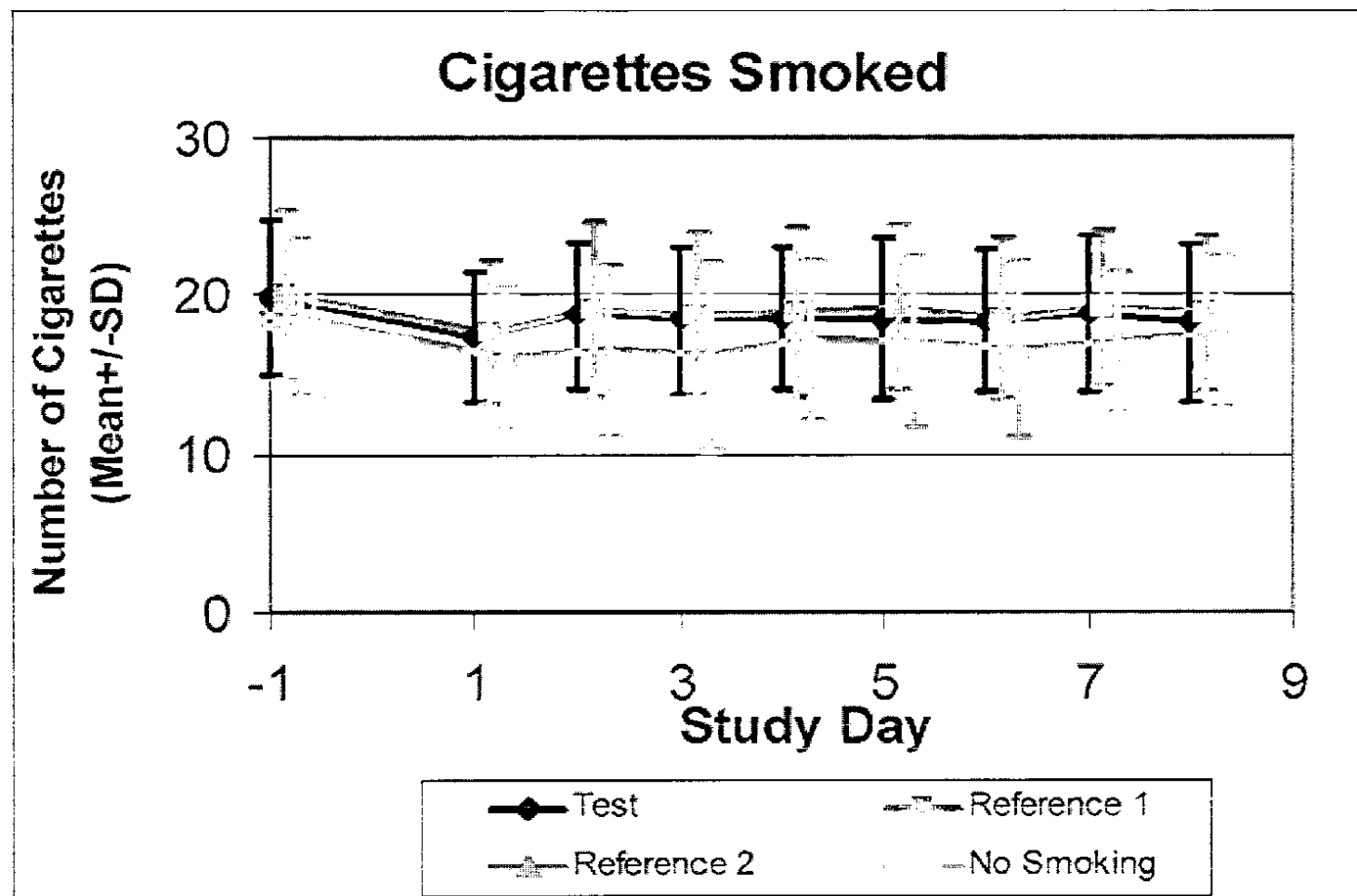
Smoke constituent	Cigarette yield			Biomarker
	Reference 1	Reference 2	Test (PREP prototype)	
Tar (mg)	6.0	1.0	6.0	No suitable biomarker
Nicotine (mg)	0.5	0.1	0.5	NEq (nicotine + 5 metabolites) $t_{1/2}$ all metabolites <24 h
Carbon monoxide (mg)	7.0	2.0	7.0	Sensitivity: COHb >> CO _{ex}
NNK (ng)	43	18	35	Total NNAL + NNAL-Gluc Slow elimination kinetics
Pyrene (ng)	28	6	26	Represents total PAH
4-aminobiphenyl (ng)	0.8	0.3	0.9	No suitable biomarker for short-term studies
Acrolein (μg)	50	10	1	3-HPMA (not specific to acrolein)
Benzene (μg)	31	11	1	Specificity: <i>S</i> -PMA >> <i>t,t</i> -MA
1,3-butadiene (μg)	28	9	1	Sensitivity: MHBMA >> DHBMA

Study design



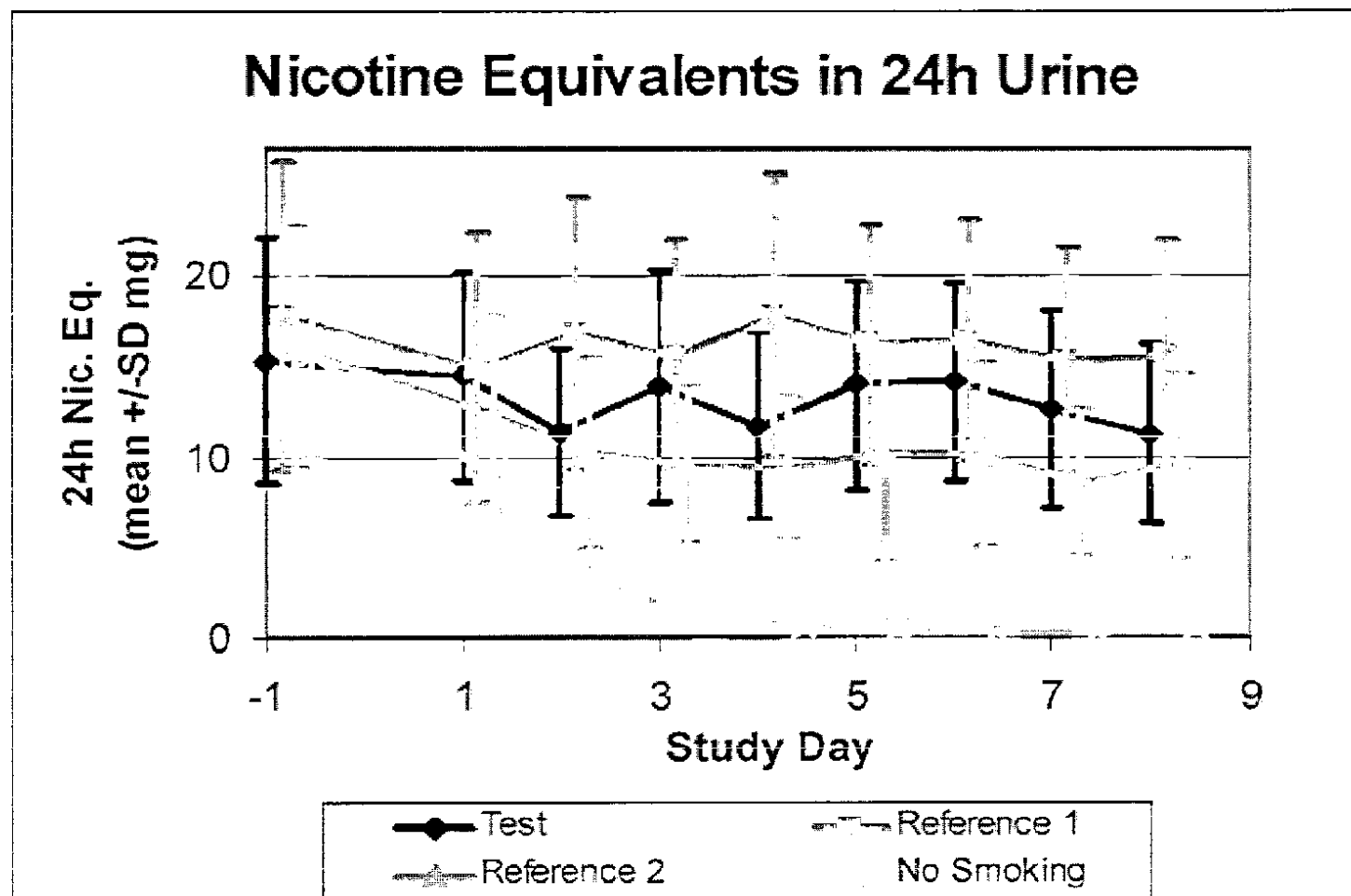
Cigarettes per day (CPD)

Smoking group
Ref. 1
Ref. 2
Test
No smoking



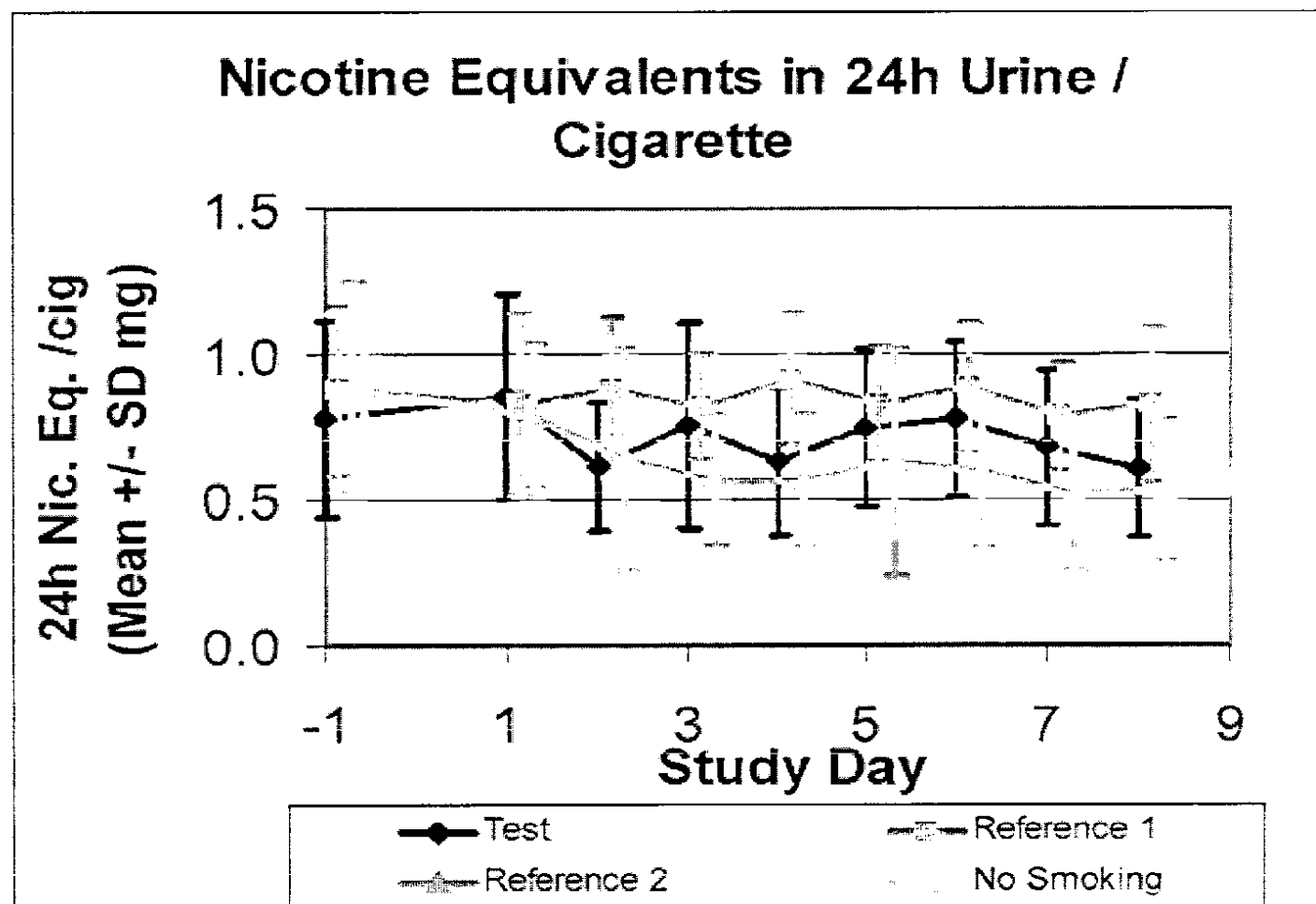
Nicotine

Cigarette yield (mg)
0.5
0.1
0.5
No smoking



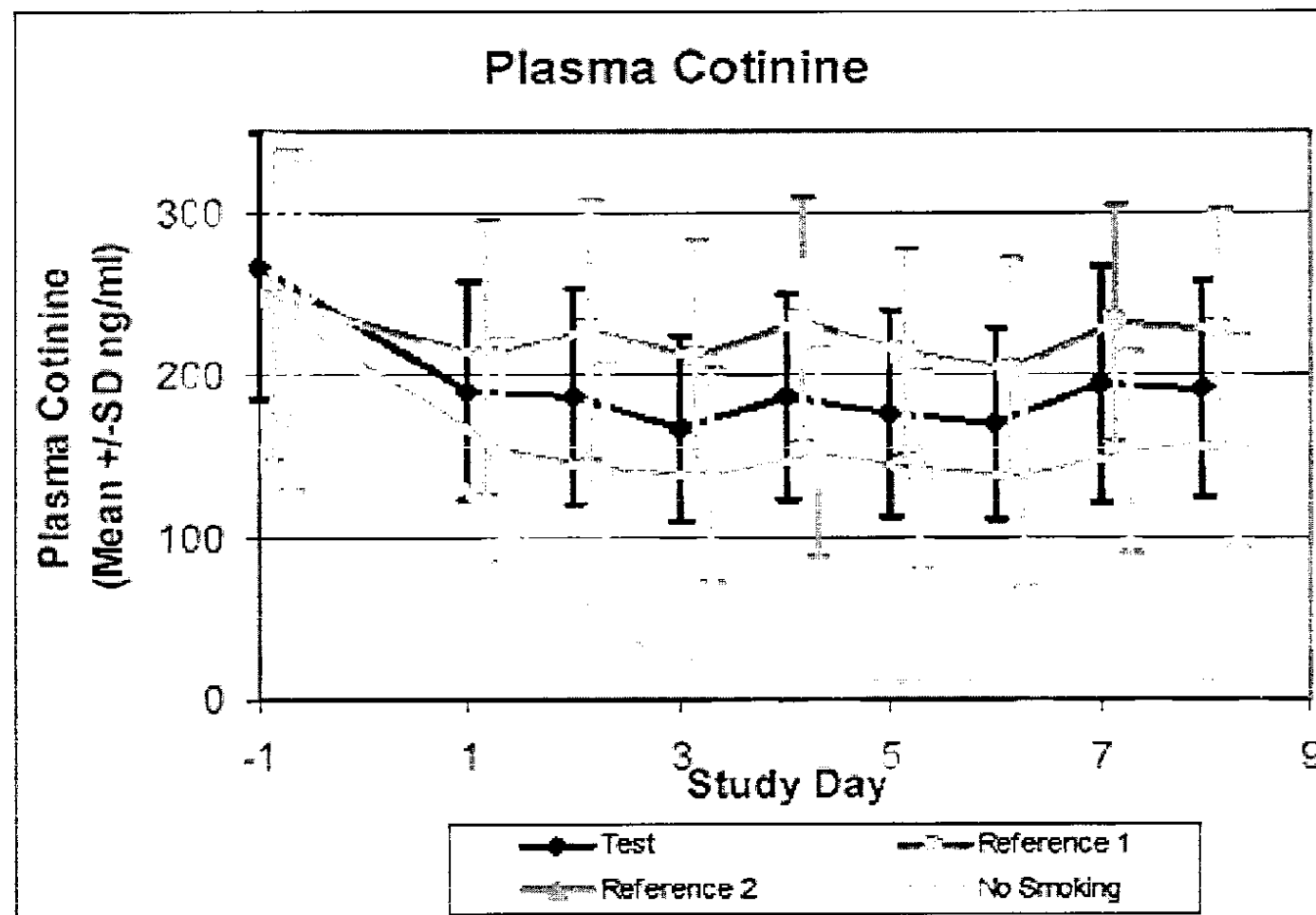
Nicotine

Cigarette yield (mg)
0.5
0.1
0.5
No smoking



Nicotine

Cigarette yield (mg)
0.5
0.1
0.5
No smoking



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Hangzhou 11th to 13th October, 2004

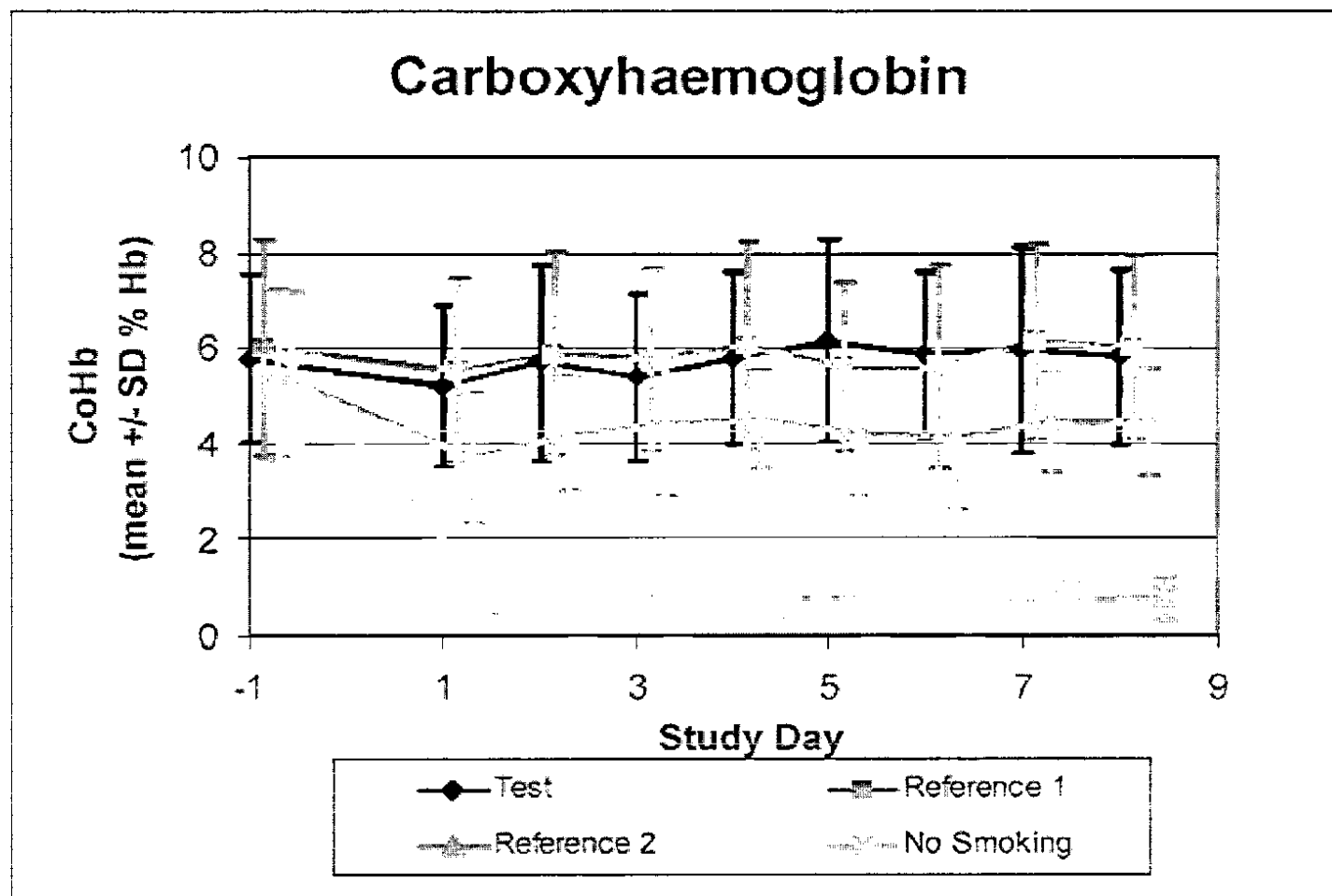
ATR

27

53287 9795

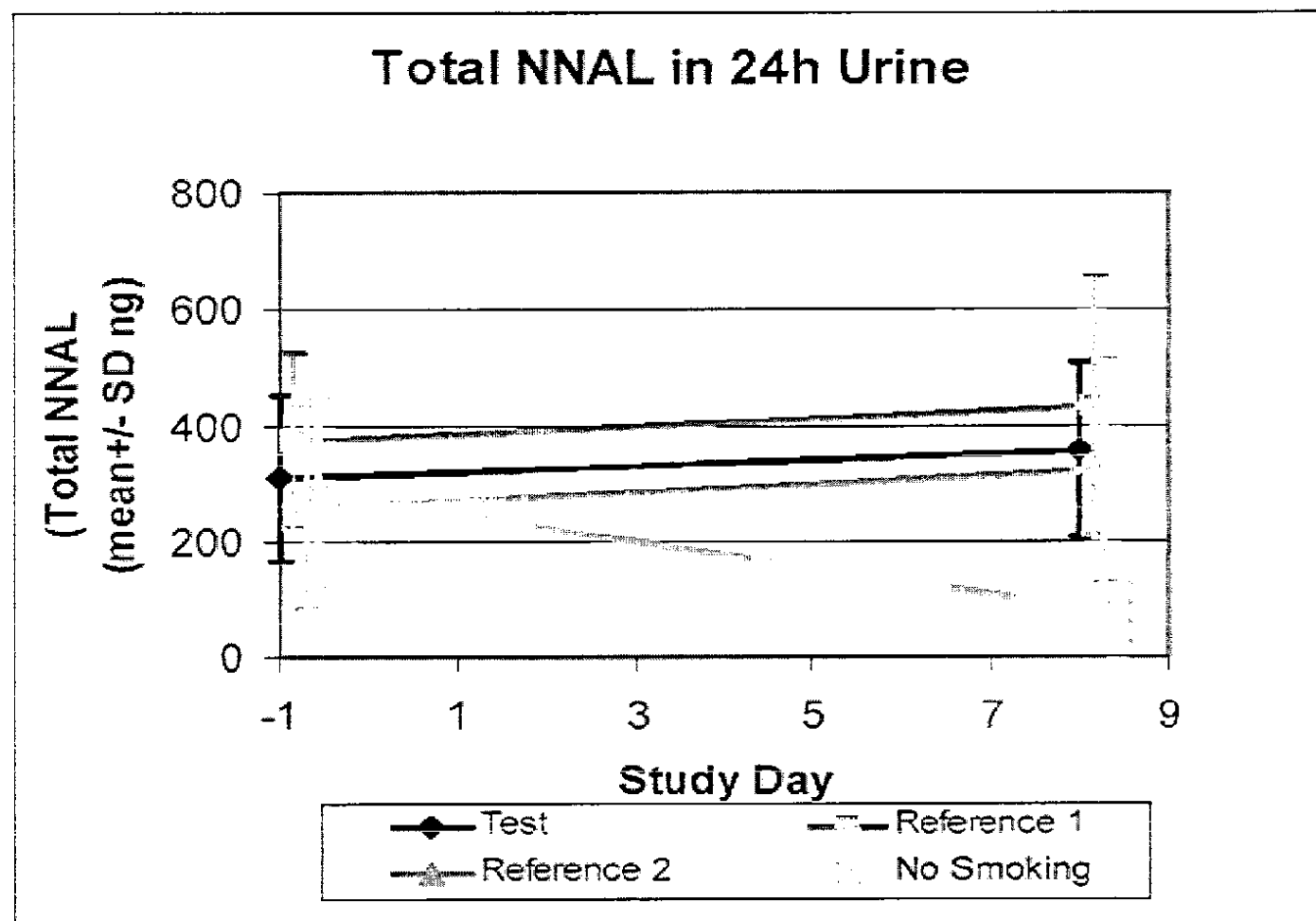
Carbon monoxide

Cigarette yield (mg)
7.0
2.0
7.0
No smoking



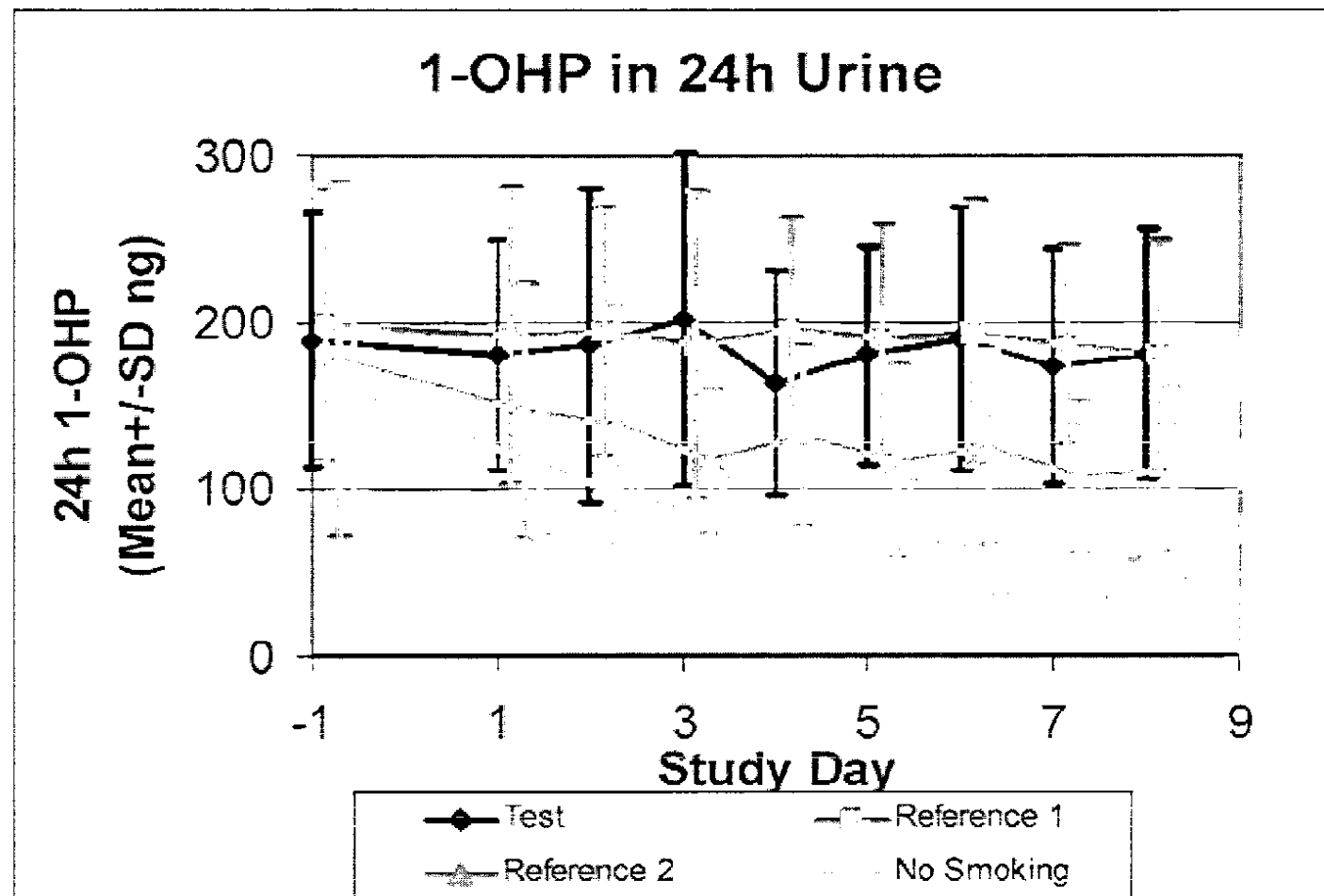
Nitrosamines (e.g., NNK)

Cigarette yield (ng)
43
18
35
No smoking



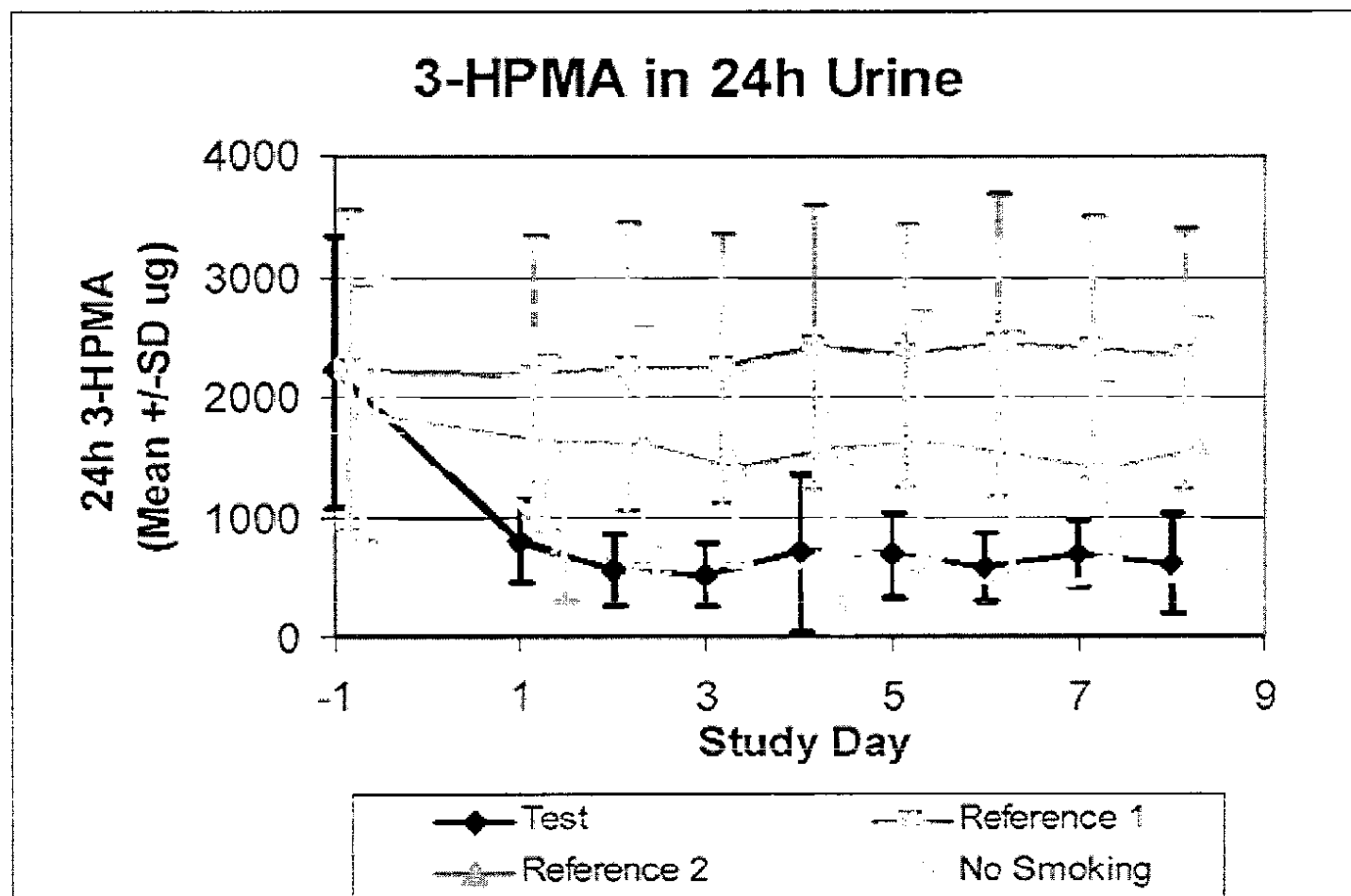
PAH (e.g., pyrene)

Cigarette yield (ng)
28
6
26
No smoking



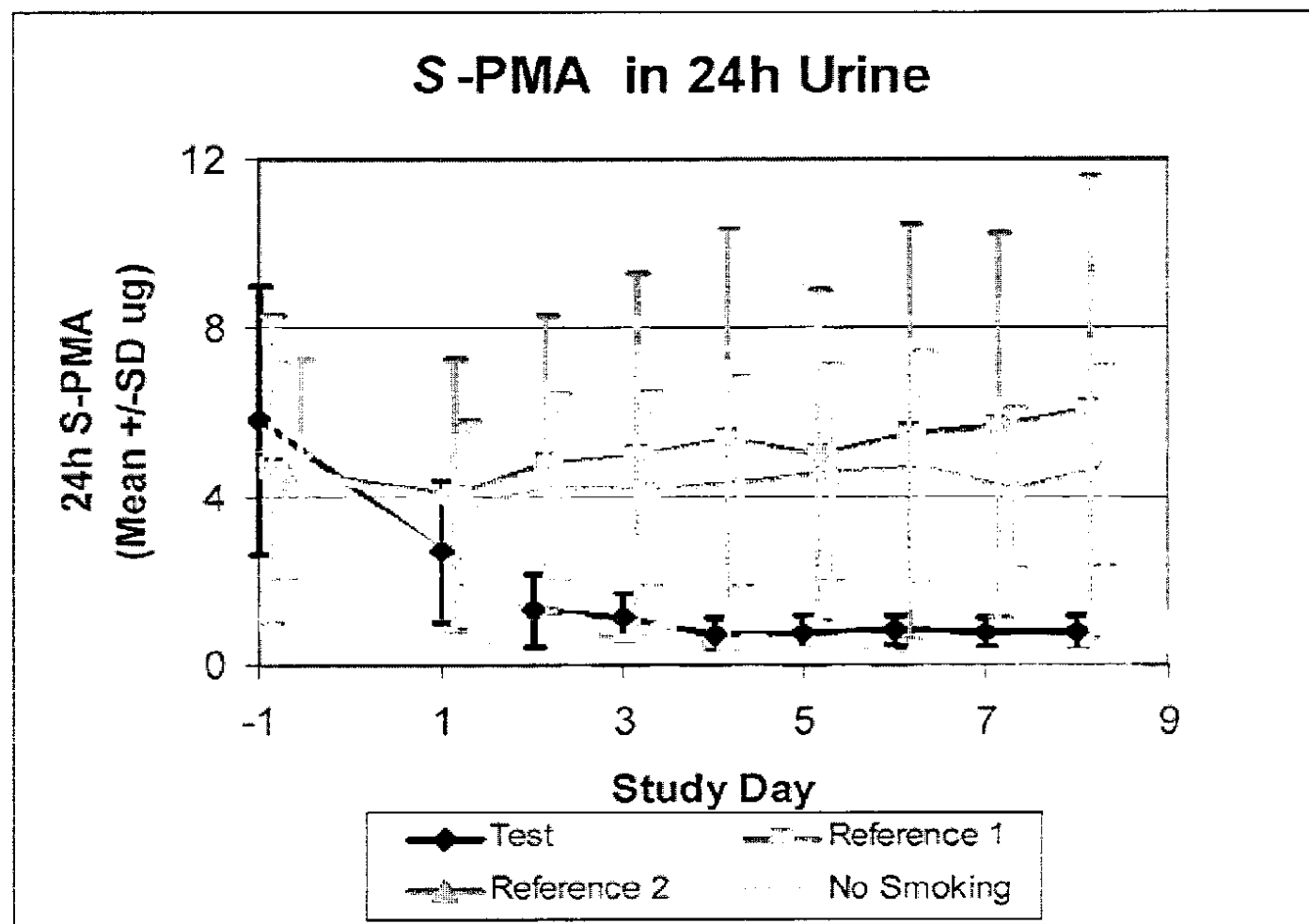
Aldehydes (e.g., acrolein)

Cigarette yield (μg)
50
10
1
No smoking



Benzene

Cigarette yield (μg)
31
11
1
No smoking



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Hangzhou 11th to 13th October, 2004

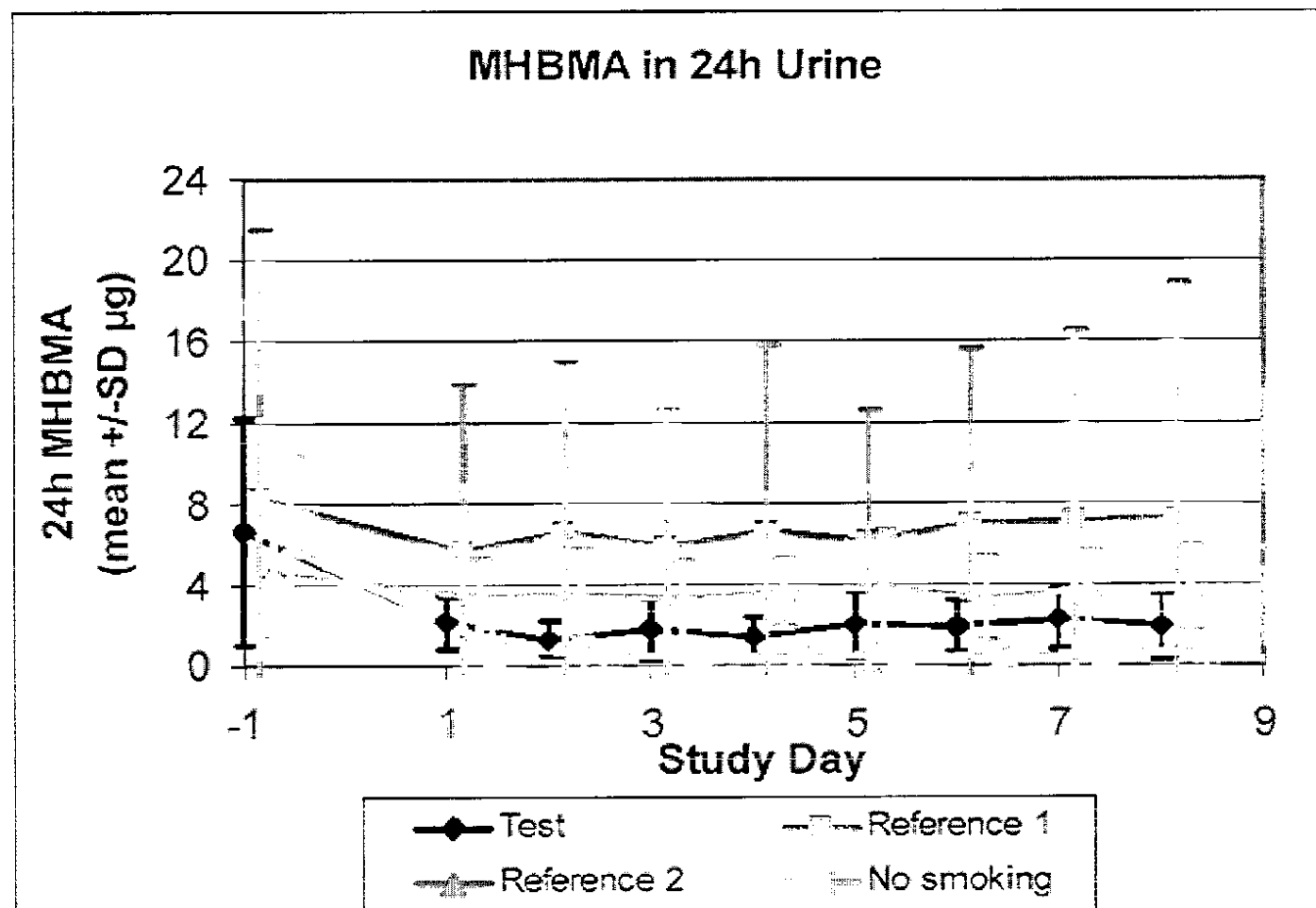
ATR

32

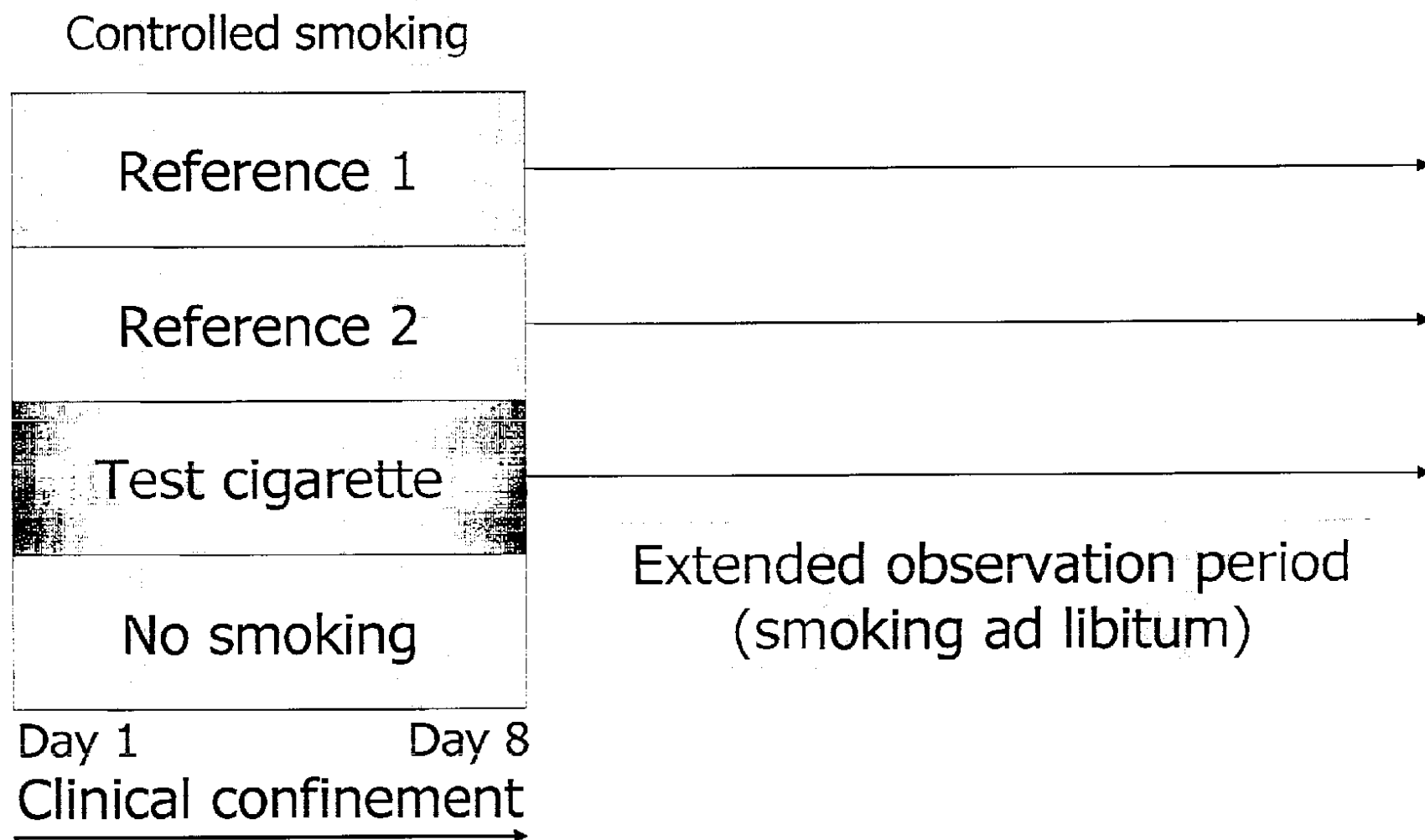
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1,3-butadiene

Cigarette yield (μg)
28
9
1
No smoking



Controlled clinical study follow-up



Clinical study summary

Smoke chemistry characterisation

- Percentage reductions in smoke constituents
- ADME of smoke constituents must be known
- Validated analytical methods for biomarkers

Biomarker selection

- Study objective and practical considerations

Clinical study design

- Biomarker(s) → study design, size and power