Clinical Evaluation Methodology

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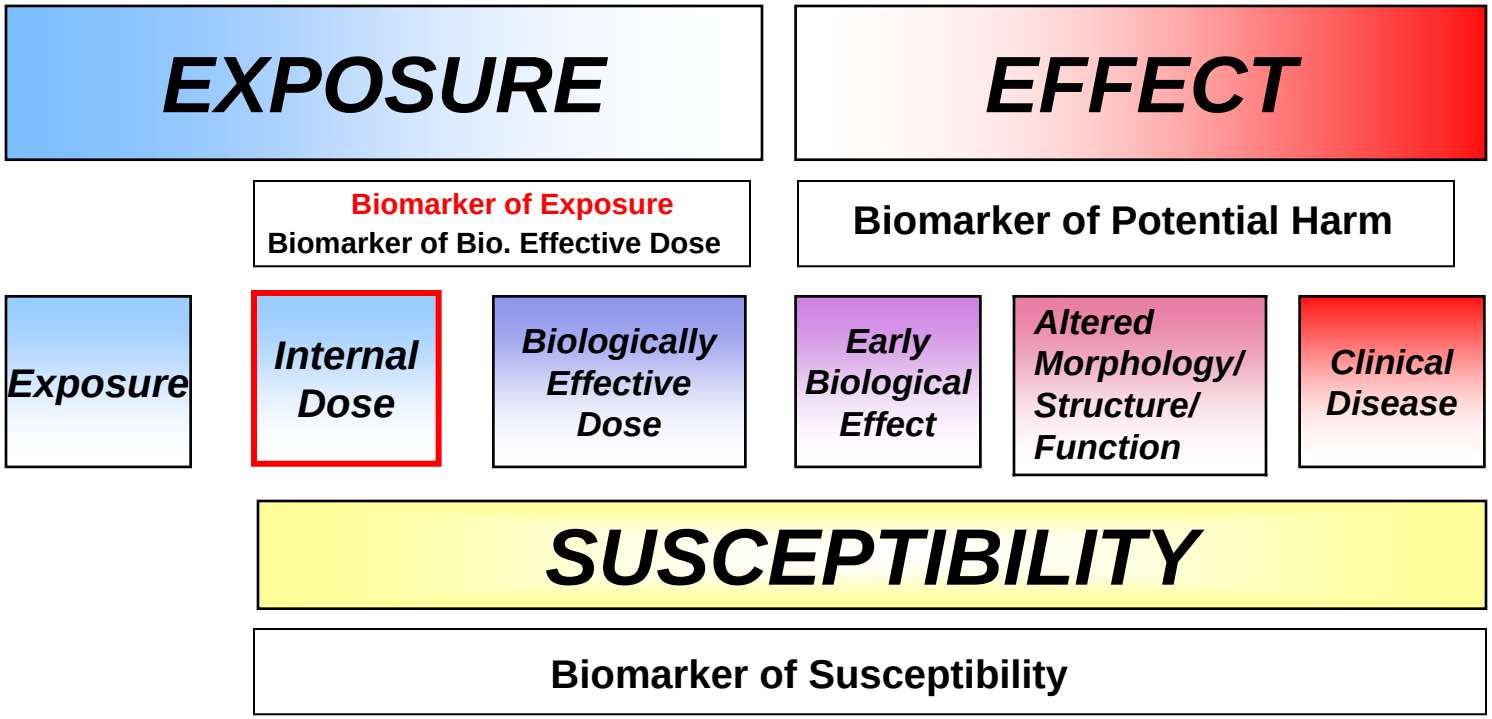
Clinical Exposure Evaluation: Objective

To measure the exposure of adult smokers who switch to a potential reduced-exposure product (PREP) to determine if exposure of the adult smoker to measured smoke constituents is reduced when compared to smoking a conventional cigarette.

Clinical Evaluation: Exposure



National Research Council Committee on Biomarkers, 1987

Biomarker of Exposure

"A tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule;

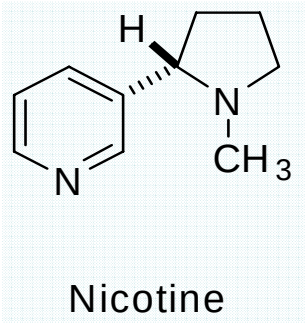
"Sometimes considered a measure of internal dose"

Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction (The Institute of Medicine, 2001, p. 150)

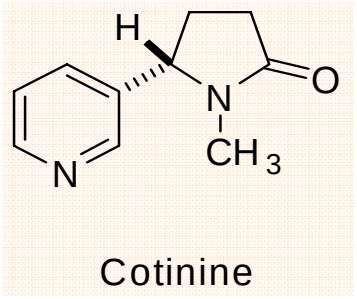
Biomarkers of Exposure

Biomarker	Matrix	Smoke Constituent
Particulate Phase		
Nicotine and 5 Major Metabolites *(Molar sum expressed as Nicotine Equivalents)* • Nicotine and Nicotine-*N*-glucuronide • Cotinine and Cotinine-*N*-glucuronide • *trans*-3'-Hydroxycotinine and *trans*-3'-Hydroxycotinine-*O*-glucuronide	Urine	Nicotine
Cotinine	Serum	Nicotine
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and NNAL-glucuronides	Urine	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
Total 1-Hydroxypyrene (1-OHP)	Urine	Pyrene
Gas-Vapor Phase		
3-Hydroxypropylmercapturic acid (3-HPMA)	Urine	Acrolein
S-Phenylmercapturic acid (*S*-PMA)	Urine	Benzene
Monohydroxybutenylmercapturic acid (MHBMA) Dihydroxybutylmercapturic acid (DHBMA)	Urine	1,3-Butadiene

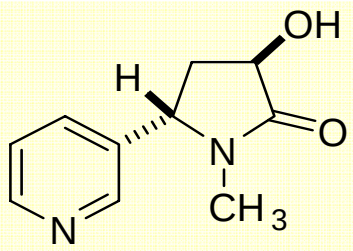
Biomarker of Exposure: Nicotine Equivalents



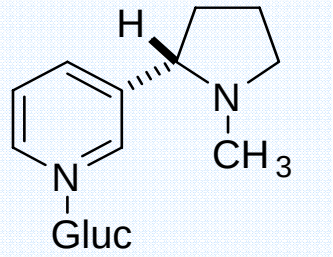
Nicotine



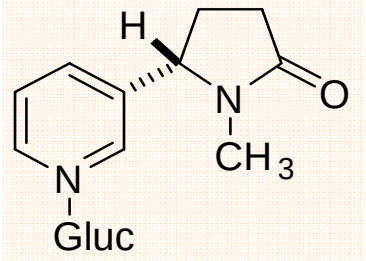
Cotinine



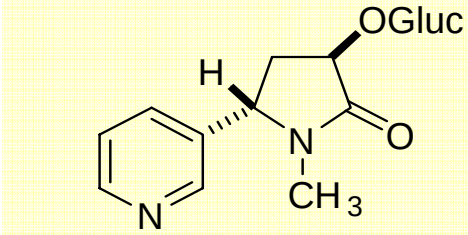
trans-3'-hydroxycotinine



Nicotine-N-Gluc



Cotinine-Gluc



trans-3'-hydroxycotinine-Gluc

Nicotine Equivalents = molar sum of nicotine and 5 major metabolites

(mg/24h) = [**Total Nicotine** *(mg/24h)* **/162.2** *mg/mmol*

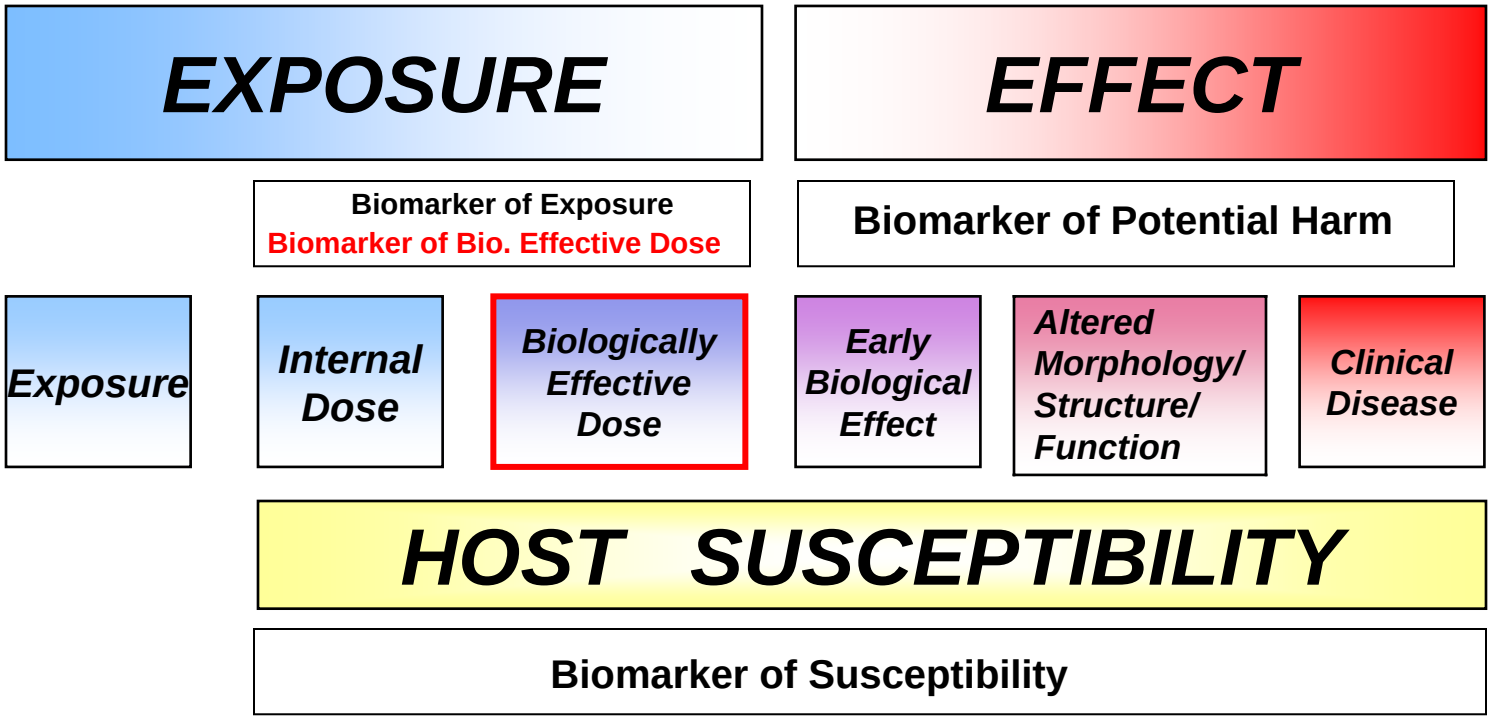
\+ **Total cotinine** *(mg/24h)* **/ 176.2** *mg/mmol*

\+ **Total *trans*-3'-hydroxycotinine** *(mg/24h)* **/ 192.22** *mg/mol*]

x 162.23 *mg/mmol*

LSRO Reduced Risk Review, Core Committee Meeting: October 19, 2005

Clinical Exposure Evaluation



National Research Council Committee on Biomarkers, 1987

Biomarker of Biologically Effective Dose

"The amount that a tobacco constituent or metabolite binds to or alters a macromolecule"

"Estimates of the BED might be performed in surrogate tissues"

Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction (The Institute of Medicine, 2001, p. 150)

Biomarkers of Biologically Effective Dose

Biomarker	Matrix	Smoke Constituent
Particulate Phase		
4-Aminobiphenyl hemoglobin (4-ABP-Hb) adducts	Red blood cells	4-Aminobiphenyl
Gas-Vapor Phase		
Carboxyhemoglobin (COHb)	Whole blood	Carbon monoxide

IOM Regulatory Principle 4

"Manufacturers should be permitted to market tobacco-related products with exposure-reduction or risk-reduction claims only after prior agency approval based on scientific evidence

(a) that the product substantially reduces exposure to one or more tobacco toxicants and

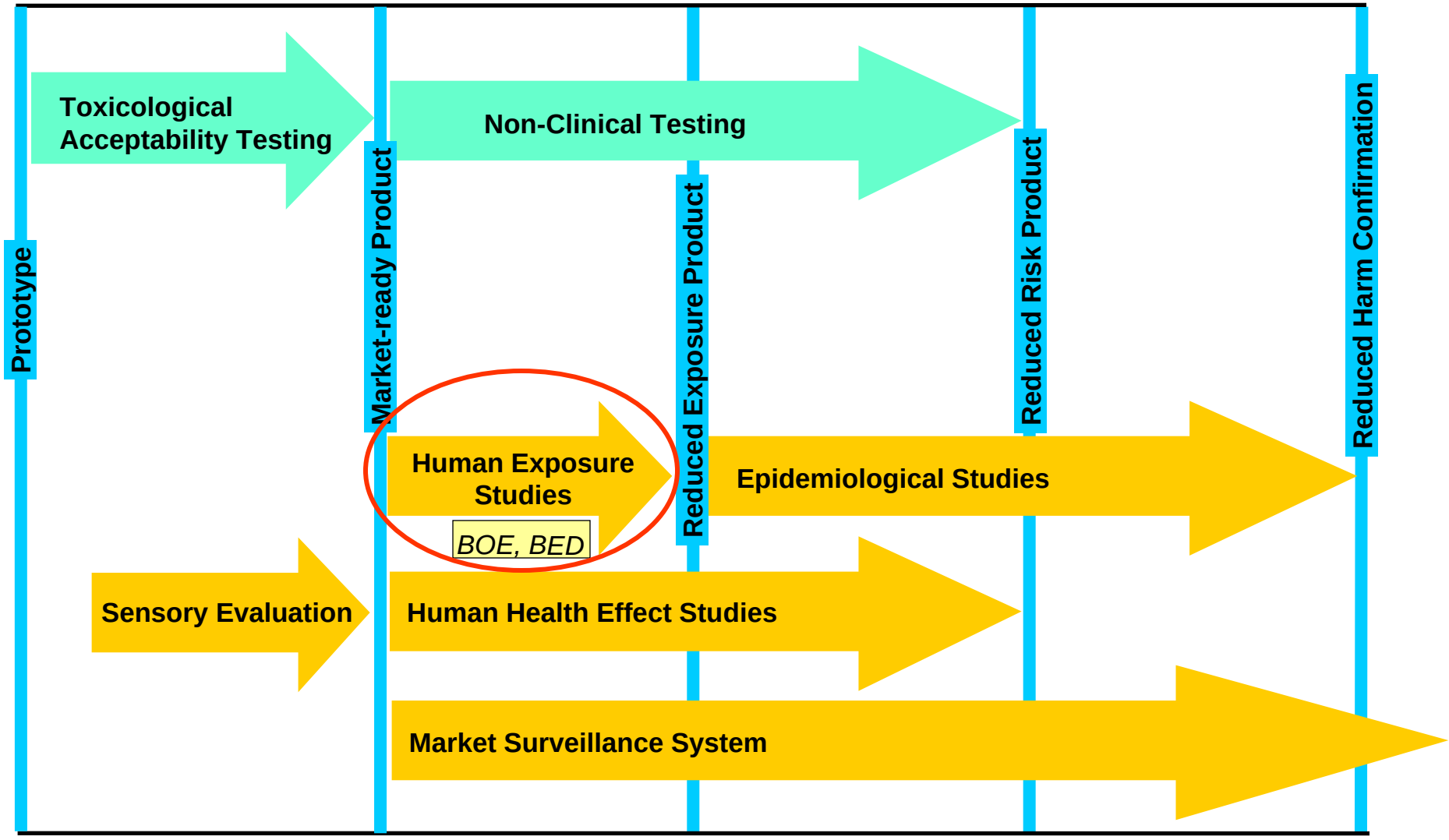
(b) if a risk reduction claim is made, that the product can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects

as compared with whatever benchmark product the agency requires to be stated in the labeling.

The 'substantial reduction' in exposure should be sufficiently large that measurable reduction in morbidity and/or mortality (in subsequent clinical or epidemiological studies) would be anticipated, as judged by independent scientific experts."

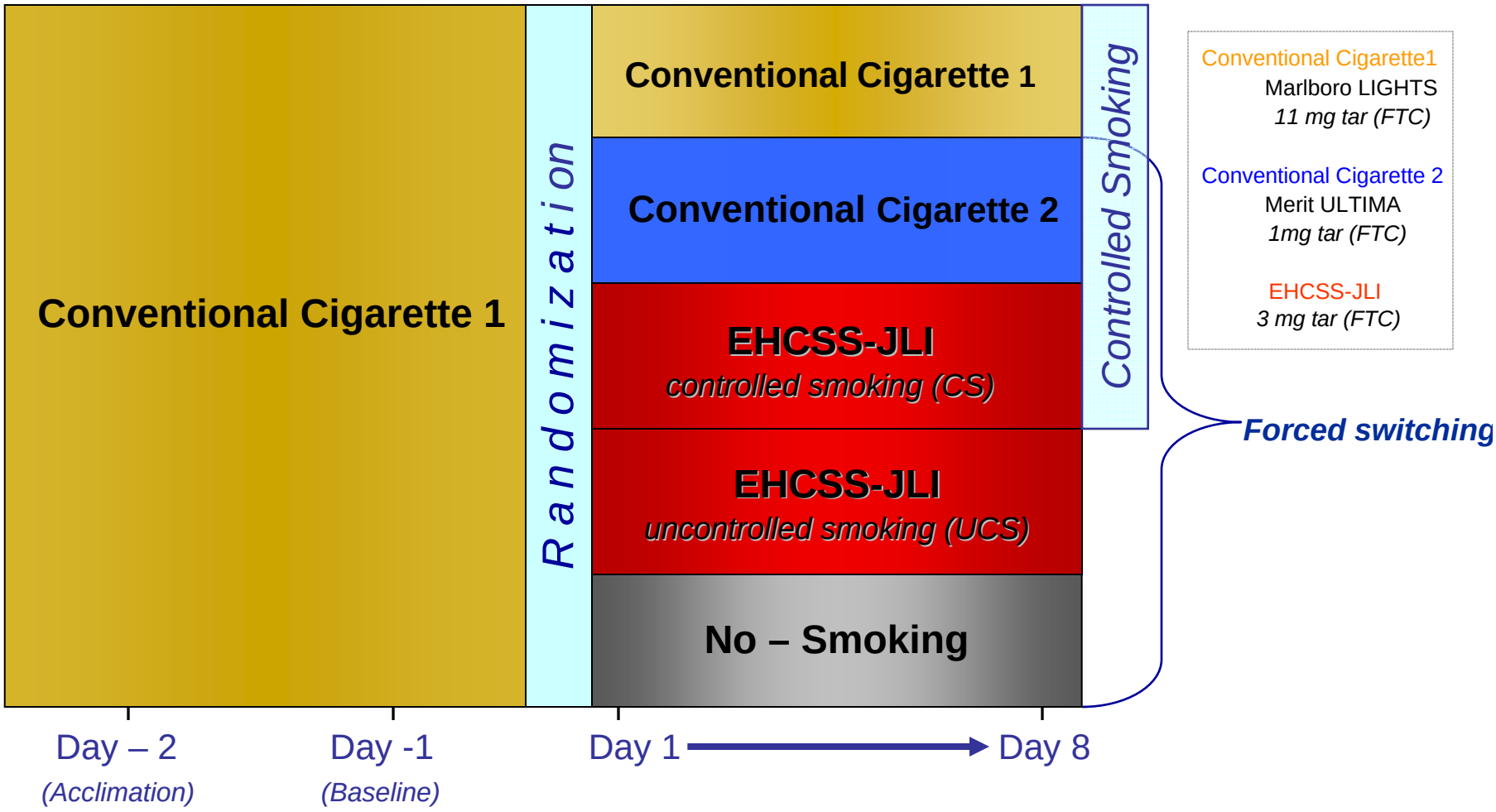
Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction (The Institute of Medicine, 2001, p. 10)

Harm Reduction Evaluation Process



Clinical Study Design: Short-Term Exposure

Randomized, controlled, open-label, parallel-group



Controlled Smoking

- Cigarette smoking is limited to one product with no minimum daily number.
- On the Acclimation Day, the maximum daily number is limited to 20% more than the subject's usual maximum daily number according to the subject's smoking history.
- The maximum daily number on subsequent study days is limited to the number actually smoked on the Acclimation Day.
- On each day, smoking opportunities are offered at equal intervals (~every 32 minutes) between 07:00 and 23:00 only.
- On each day after the Acclimation Day, the total daily cigarettes smoked are evenly divided over the day (07:00 to 23:00).

Roethig, et al. *J Clin Pharmacol* 2005;45:133-145.

Uncontrolled Smoking

- *A smoking schedule used in controlled, confined, short-term exposure studies in order to generate information about possible compensatory smoking behavior, including number of cigarettes smoked.*

- Cigarette smoking is limited to one product with no minimum daily number.
- Cigarettes may be smoked at any time between 07:00 and 23:00.
- The maximum daily number is limited to 60 cigarettes regardless of the subject's smoking history.

Cigarette Consumption *(cigs/24h) (mean±SD)*

Study Day	EHCSS-JLI *CS*	EHCSS-JLI *UCS*	Conventional Cig 1	Conventional Cig 2	No Smoking
Baseline*	**19.2** (3.5)	**18.3** (4.3)	**18.7** (4.4)	**17.5** (4.7)	**19.9** (4.1)
1	17.8 (4.1)	18.1 (8.0)	18.4 (3.8)	16.9 (5.2)	
2	17.7 (4.0)	18.3 (9.0)	19.4 (4.0)	17.3 (4.4)	
3	19.1 (4.5)	22.2 (10.2)	19.4 (3.7)	18.0 (5.0)	
4	18.2 (5.0)	20.5 (10.6)	19.2 (3.9)	18.0 (4.9)	
5	19.5 (3.9)	24.2 (13.3)	19.1 (4.4)	17.6 (4.7)	
6	19.9 (3.7)	23.1 (12.6)	19.1 (3.6)	17.5 (4.4)	
7	19.7 (3.9)	26.4 (9.6)	19.9 (4.4)	17.7 (5.0)	
8	**19.7** (3.8)	**27.6** (13.8)	**19.1** (3.8)	**17.0** (4.6)	

*At **Baseline**, all subjects smoked **Conventional Cigarette 1**.

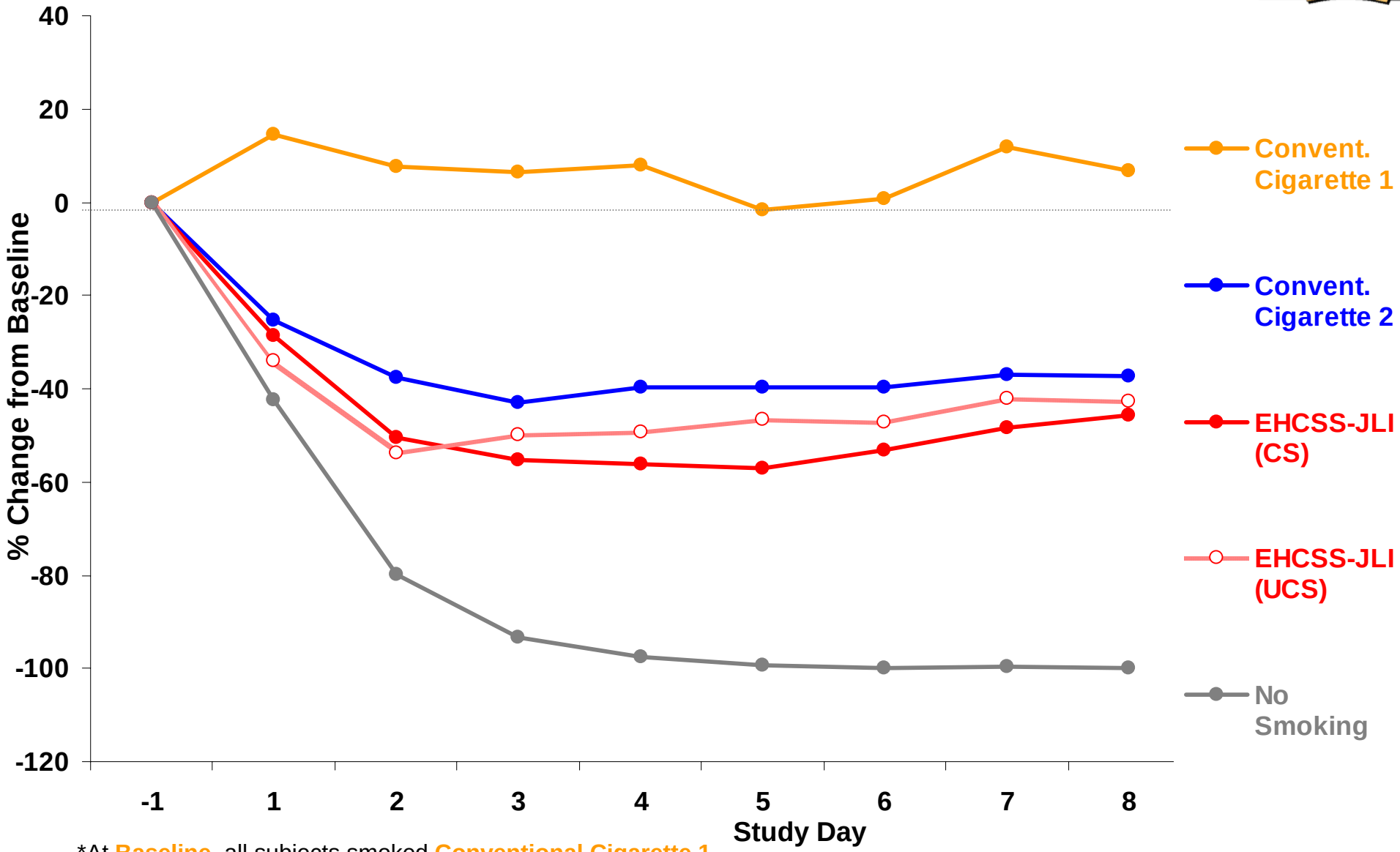
Mean % Change from Baseline to Day 8

Biomarker	units	EHCSS-JLI CS	EHCSS-JLI UCS	Convent. Cig 2	Convent. Cig 1	No Smoking
Nicotine Equivs	mg/24h	-44	-45	-33	6	-100
	*p**		*0.13*	*<0.0159*	*<0.0001*	
Plasma Cotinine	ng/mL	-53	-46	-51	13	-100
	*p**		*0.87*	*<0.0001*	*<0.0001*	
Total NNAL	ng/24h	-51	-64	-16	34	-76
	*p**		*0.10*	*0.0003*	*<0.0001*	
Total 1-OHP	ug/24h	-63	-72	-44	6	-74
	*p**		*0.49*	*0.16*	*<0.0001*	
Urine mutagenicity	rev/24h	-44	-40	-24	7	-56
	*p**		*0.58*	*0.0305*	*<0.0001*	
COHb $AUC_{(7\text{-}23h)}$	%*h	-86	-89	-31	6	-92
	*p**		*0.92*	*<0.0001*	*<0.0001*	
3-HPMA	ug/24h	-56	-47	-25	5	-83
	*p**		*0.21*	*<0.0001*	*<0.0001*	
S-PMA	ug/24h	-84	-84	-19	14	-94

* At **Baseline**, all subjects smoked **Conventional Cigarette 1**.
CS= Controlled Smoking, **UCS**= Uncontrolled Smoking

* *p*-value for difference between **EHCSS-JLI (CS)** and this comparator group in LS mean % change from **Baseline** to Day 8. For mutagenicity, square root transformed means were used.

24-Hour Urine Nicotine Equivalents *(mg/24h) (mean)*



*At **Baseline**, all subjects smoked **Conventional Cigarette 1**.

CS= Controlled Smoking, **UCS**= Uncontrolled Smoking

Biomarkers at Day 8 After Adjusting for the Residual Effect

Biomarker	Day 8 **EHCSS-JLI** *(CS)* **Mean % Change** from **Baseline***	Day 8 **EHCSS-JLI** *(CS)* **Mean % Difference** v. Day 8 **Convent. Cig 1**	Day 8 **EHCSS-JLI** *(CS)* **Mean % Difference** v. Day 8 **Convent. Cig 2**
COHb AUC $_{(7\text{-}23h)}$ *(%*h)*	-95	-95	-92
3-HPMA *(ug/24h)*	-68	-67	-42
***S*-PMA** *(ug/24h)*	-93	-93	-88
Total NNAL *(ng/24h)*	-75	-78	-54
Total 1-OHP *(ug/24h)*	-94	-94	-89
Urine Mutagenicity** *(rev/24*	-84	-87	-70

* At **Baseline**, all subjects smoked **Convent. Cigarette 1**.

** Median used for mutagenicity instead of mean.

Residual effect = **No-Smoking** group mean at Day 8

= the effect due to carryover *(i.e., from pre-study tobacco product use)* and confounding influences *(e.g., environmental or dietary exposure)* which is best estimated in the **No-Smoking** group at Day 8

*Smoking Topography** OVER ALL Days 1-8** *(mean, SD)*

Parameter	Convent. Cig 1	Convent. Cig 2	EHCSS-JLI CS	EHCSS-JLI UCS
Number of Puffs Per Cig	12	12	8	8
SD	*2*	*2*	*0*	*1*
Mean Puff Volume *(mL)*	47	59	86	75
SD	*13*	*13*	*25*	*26*
Mean Puff Duration *(s)*	1.4	1.6	2.8	2.3
SD	*0.4*	*0.5*	*0.8*	*1.0*
Mean IPI *(s)*	25	21	50	37

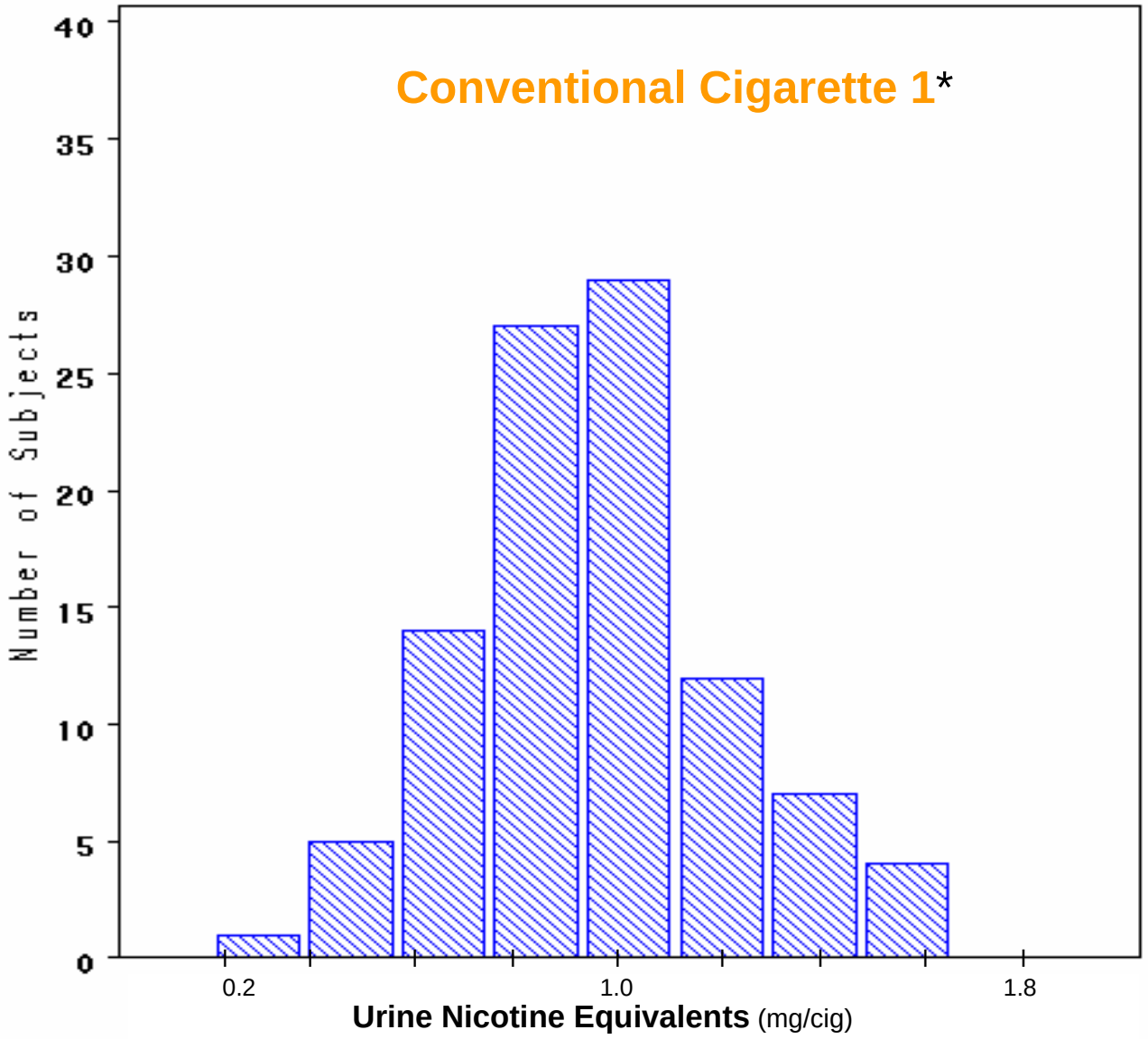
* Absolute values for smoking topography data obtained by the CReSSmicro™ portable topography device should be interpreted with caution. Limited biologic validation has been done. This device may have limitations in accuracy and precision particularly at very high and very low puff volumes.

**Excludes top and bottom 10% to eliminate outliers due to data errors and artifacts.

24-Hour Urine Nicotine Equivalents Adjusted By Number of Cigarettes Smoked (mg/cig)

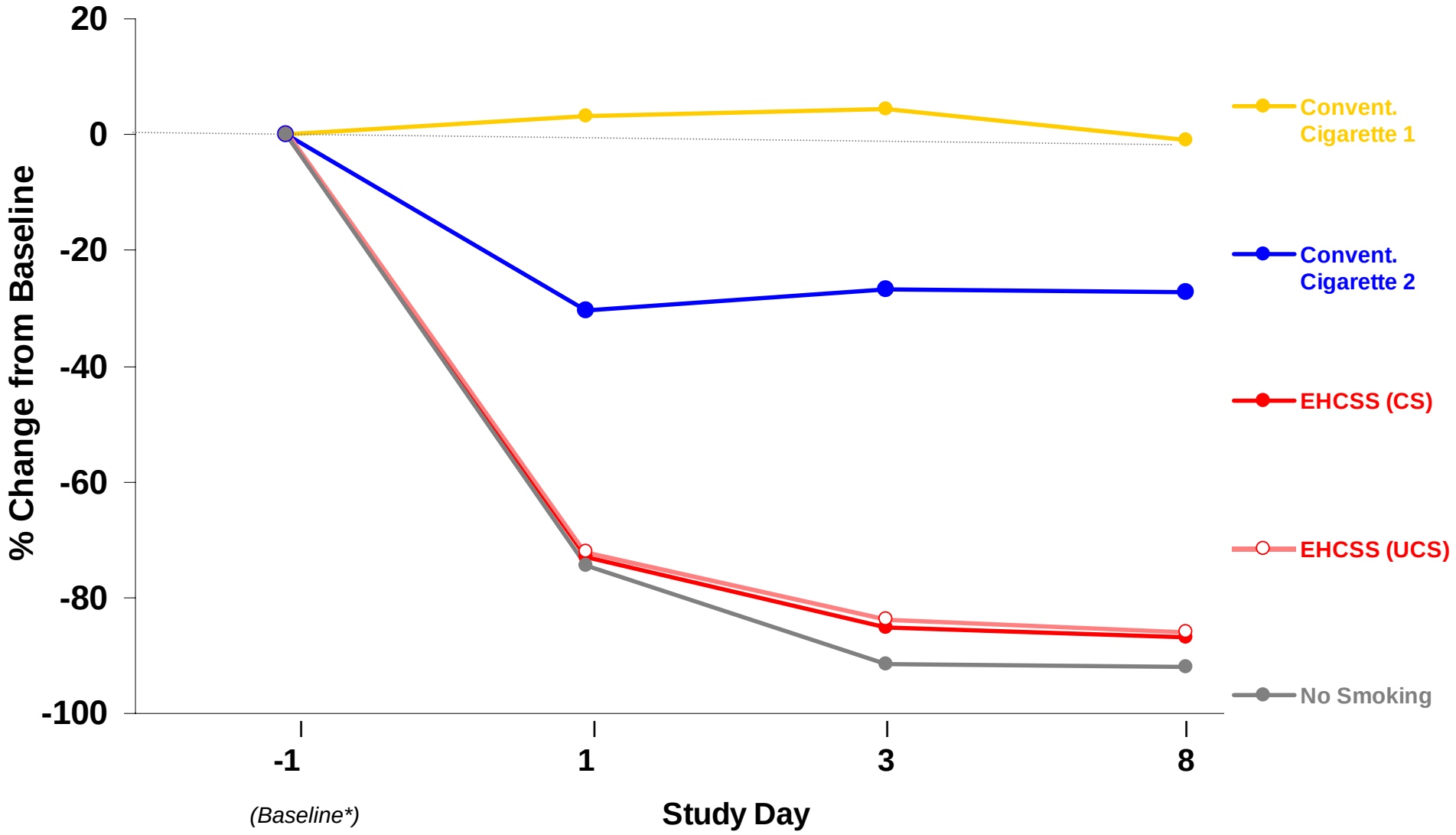
		Convent. Cig 1	
Study Day	**N**	*mean*	*SD*
Baseline	20	**1.01**	0.27
1	20	**1.15**	0.39
2	20	**1.04**	0.53
3	20	**1.03**	0.38
4	20	**1.04**	0.26
5	20	**0.96**	0.29
6	19	**1.00**	0.25
7	19	**1.08**	0.31
8	19	**1.07**	0.27
Overall	157	**1.05**	0.34

Urine Nicotine Equivalents per Cigarette *(mg/cig)*



*At **Baseline**, all subjects smoked **Conventional Cigarette 1** (N = 99).

Carboxyhemoglobin $AUC_{7\text{-}23h}$ *(%*h) (mean)*



*At **Baseline**, all subjects smoked **Conventional Cigarette 1**.

CS= Controlled Smoking, **UCS**= Uncontrolled Smoking

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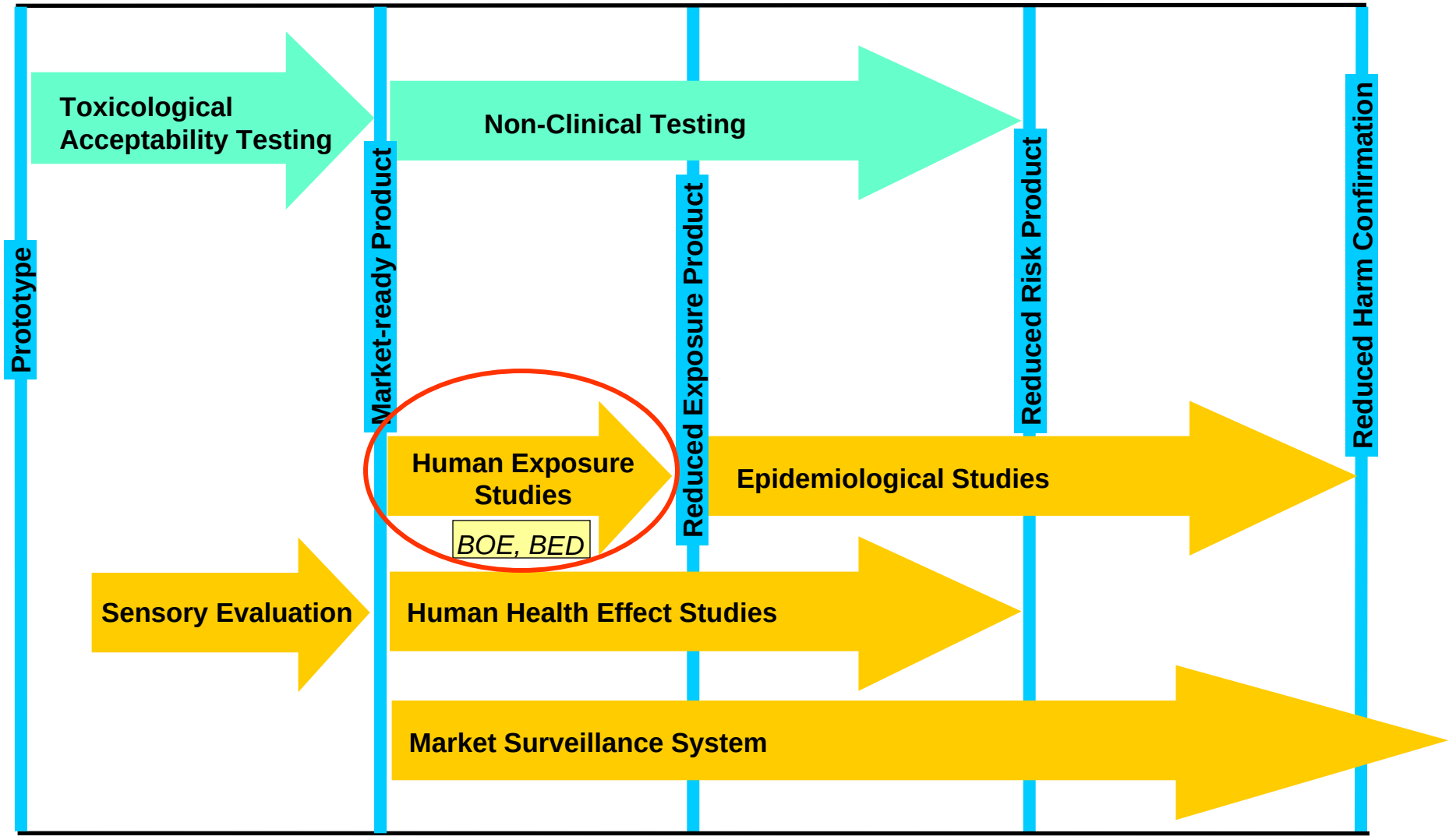
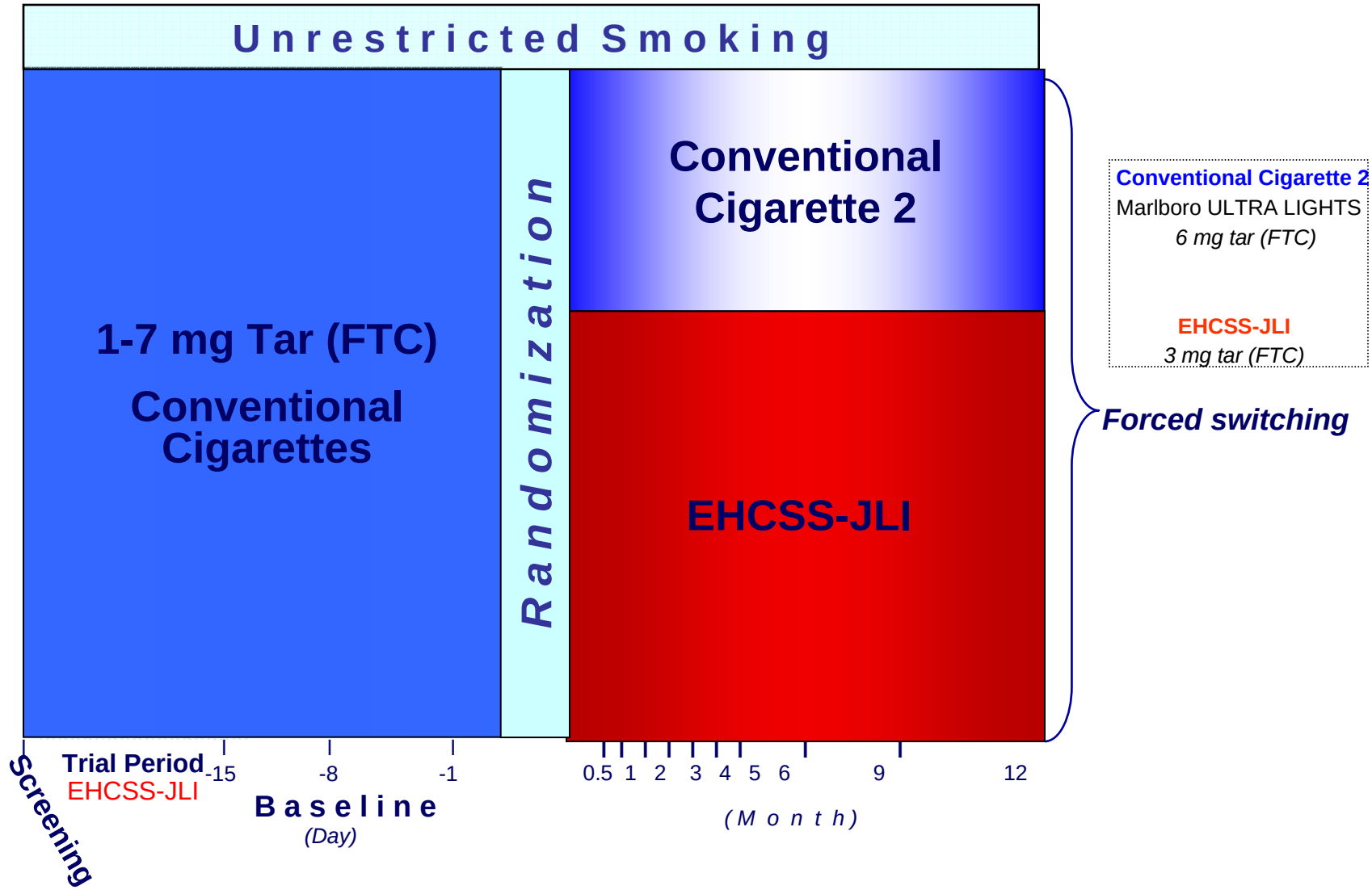
Harm Reduction Evaluation Process



Clinical Study Design: Long-Term Exposure

Randomized, controlled, open-label, parallel-group



Unrestricted Smoking

- Cigarette smoking is limited to one product.
- Cigarette smoking in a subject's normal life setting with no restrictions on time of smoking or number of cigarettes smoked.

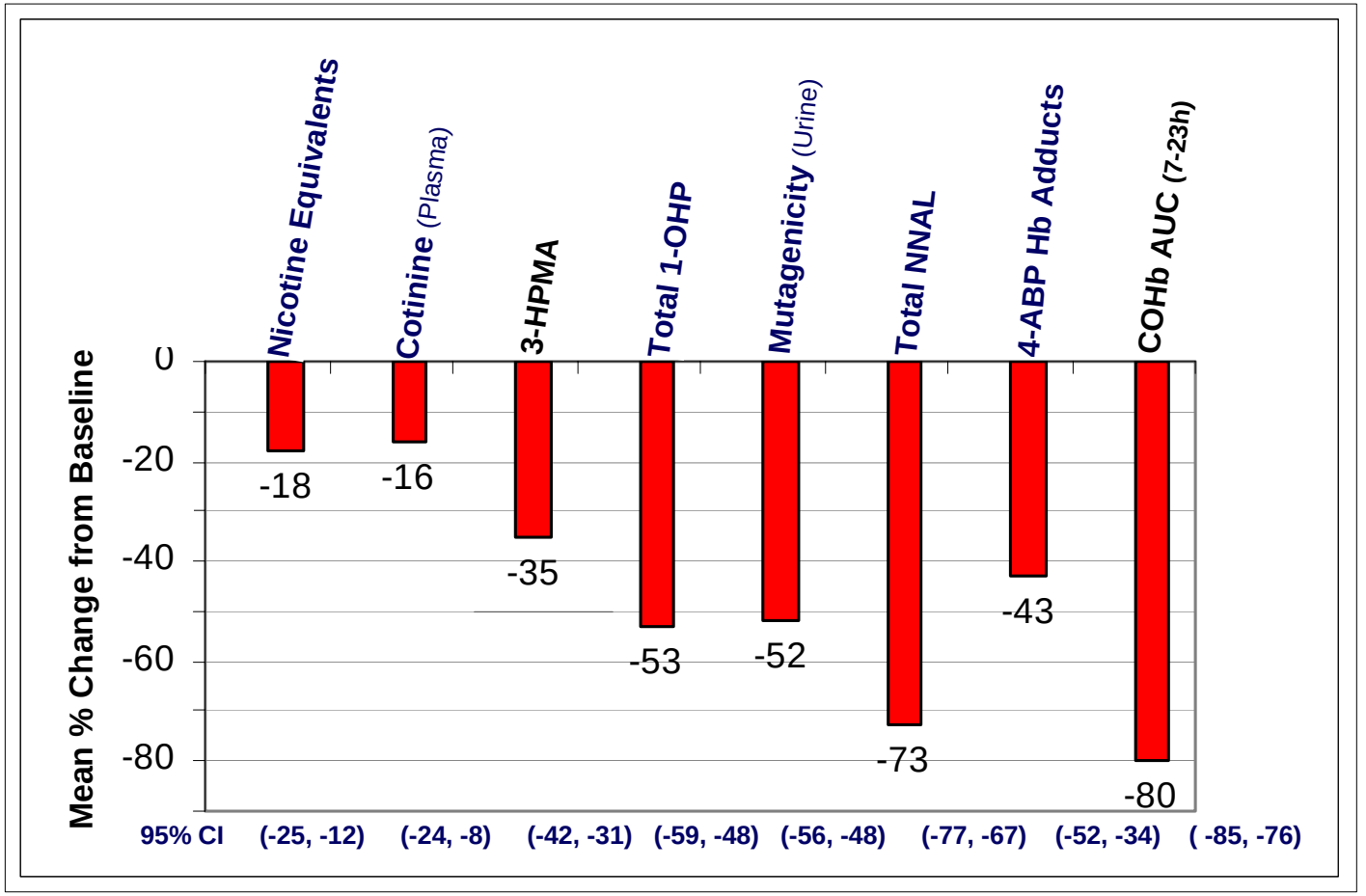
Cigarette Consumption *(cigs/24h) (mean±SD)*

	Mean ± SD Change from Baseline* Overall *(Months 0.5 thro 12)*		
	EHCSS	**Convent. Cig 2**	**p-value**
Baseline*	**24** ± 10	**23** ± 7	0.9875
Overall	**46** ± 25	**29** ± 13	
Overall % Change from Baseline	**95** ± 95	**27** ± 48	0.0001

*At **Baseline**, all subjects smoked **conventional cigarettes with 1-7 mg tar (FTC).**

Biomarkers of Exposure

Mean* % Change from Baseline**
Over 12 Months of Smoking EHCSS-JLI



* Least squares mean

** At Baseline, all subjects smoked conventional cigarettes with 1 to 7 mg tar (FTC).

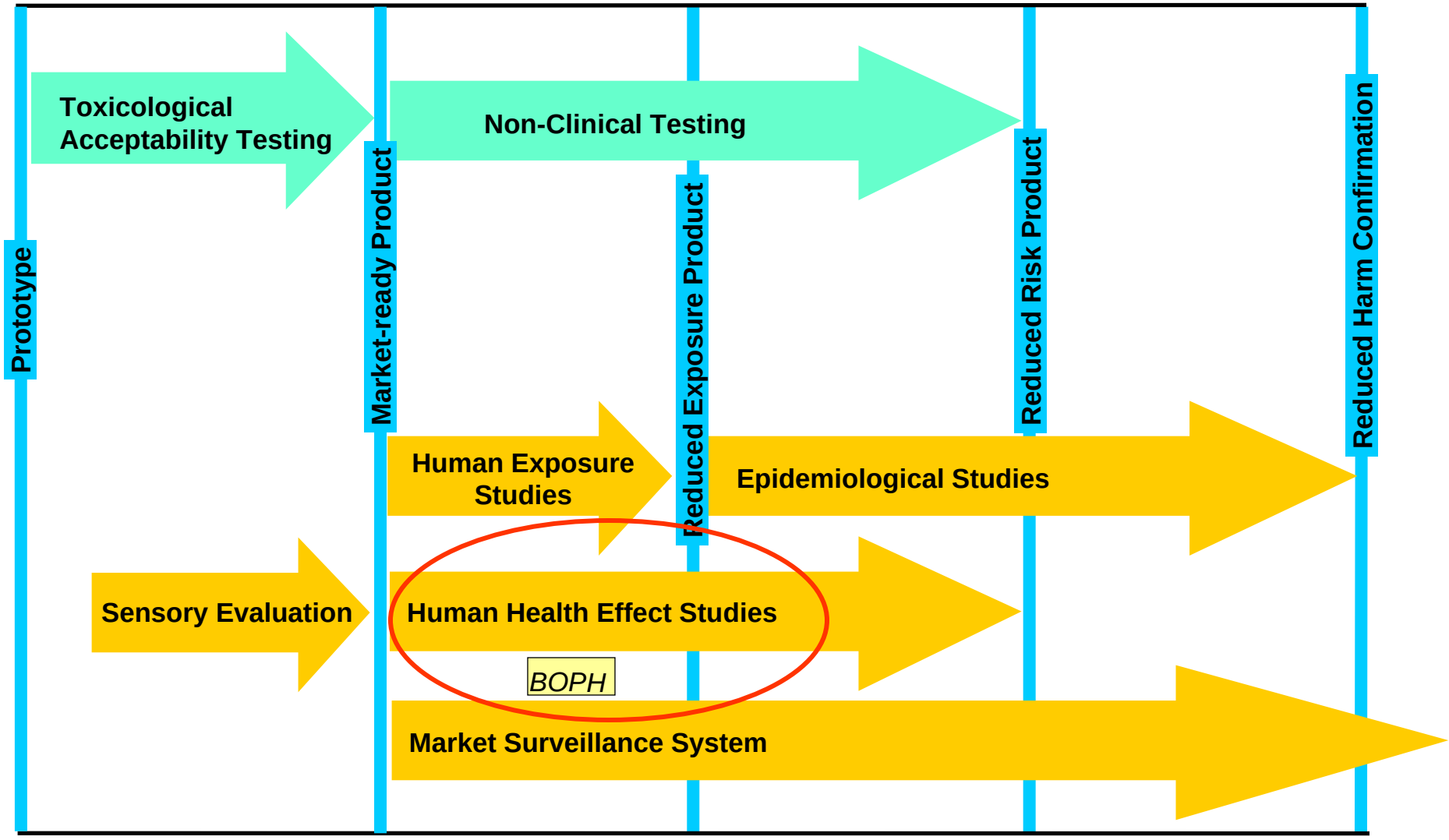
Biomarkers of Exposure

Biomarker	Bio-Matrix	Units	Smoke Constituent	Diff* in LS mean % change from BL** (EHCSS-JLI v. Convent. Cig 2)	95% confidence interval
Nicotine equivalents	Urine	mg/24h	Nicotine	**-18**	(-29, -7)
Total 1-OH-pyrene	Urine	mg/24h	PAHs	**-25**	(-34, -15)
3-HPMA	Urine	mg/24h	Acrolein	**-34**	(-44, -25)
Mutagenicity	Urine	revertants/ 24h	Mutagenic substances	**-41**	(-49, -34)
Total NNAL	Urine	ng/24h	TSNAs	**-71**	(-80, -63)
$COHb\ AUC_{(7\text{-}23h)}$	Blood	%sat*h	CO	**-84**	(-92, -77)
4-ABP Hb adducts	Blood	pg/g Hb	4-ABP	**-57**	(-71, -42)

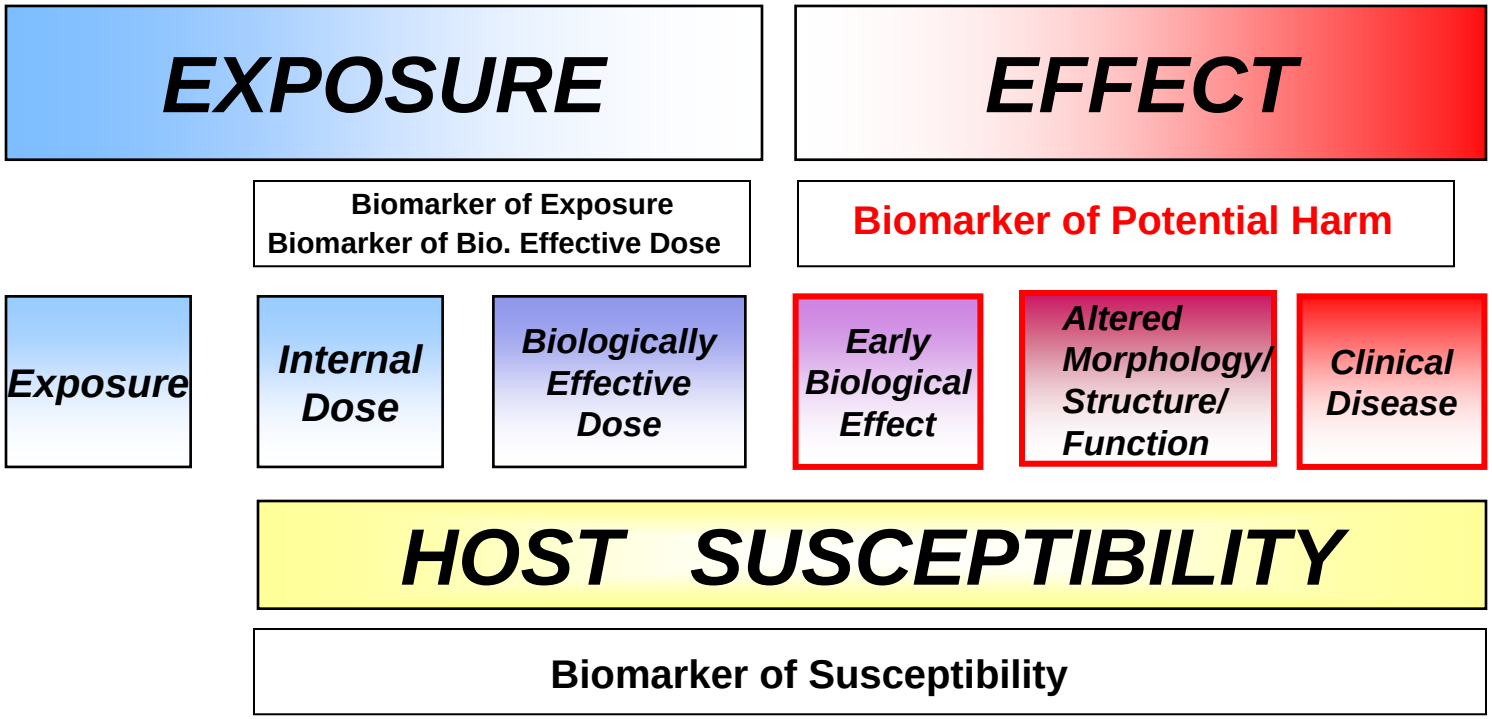
$p \leq 0.001$

** At **Baseline**, all subjects smoked **conventional cigarettes with 1-7 mg tar (FTC).**

Harm Reduction Evaluation Process



Clinical Effect Evaluation



National Research Council Committee on Biomarkers, 1987

Biomarker of Potential Harm

"A measurement of an effect due to exposure"

"These include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with harm"

"Also includes 'preclinical changes"

Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction (The Institute of Medicine, 2001, p. 150)

Biomarker of Potential Harm: Ideal Qualities

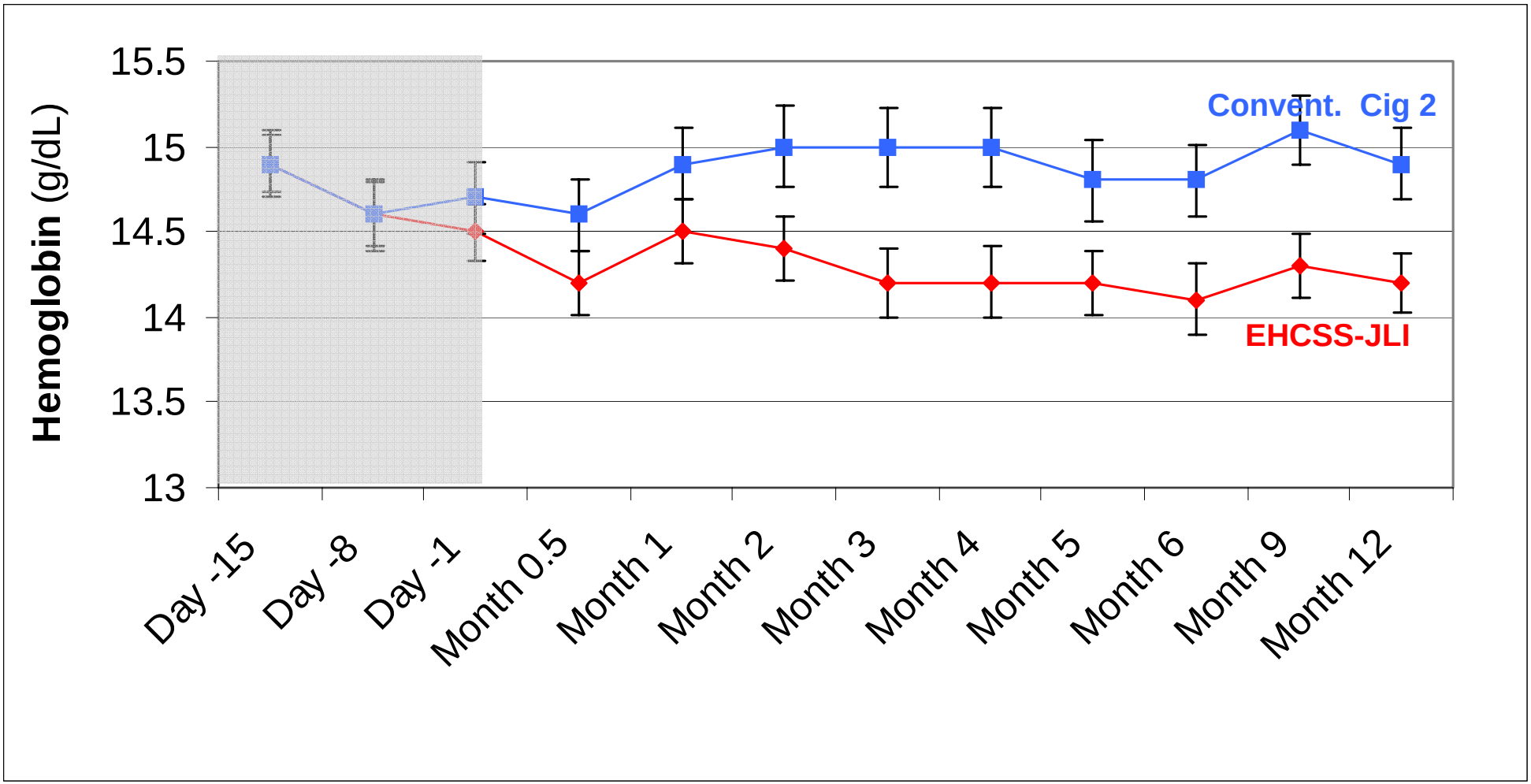
- Non-invasive or minimally invasive sampling
- Reliable, accurate, validated, simple, rapid analytical method
- Differentially expressed
- Directly associated with adverse health effect/disease; proportional to extent of disease
- Predictive value

Biomarkers of Potential Harm

Biomarker	Matrix	Health Effect
Red Blood Cell Parameters	Blood	Red blood cell mass
White Blood Cell Count	Blood	Inflammation
HDL- and LDL-Cholesterol	Serum	Atherosclerosis
Triglycerides	Serum	Atherosclerosis
Fibrinogen	Plasma	Cardiovascular disease
hs C-Reactive Protein	Serum	Inflammation
11-Dehydrothromboxane B_2	Urine	Platelet activation
von Willebrand Factor Antigen	Plasma	Endothelial cell damage
Microalbumin	Urine	Endothelial cell damage
Total Bilirubin	Serum	Depletion of antioxidant capacity
8-*epi*-Prostaglandin $F_{2\alpha}$	Urine	Lipid peroxidation
IL-8	Sputum	Inflammation
Myeloperoxidase	Sputum	Inflammation
FEV_1 and FVC (% of predicted)	N/A	Chronic Obstructive Pulmonary Disease

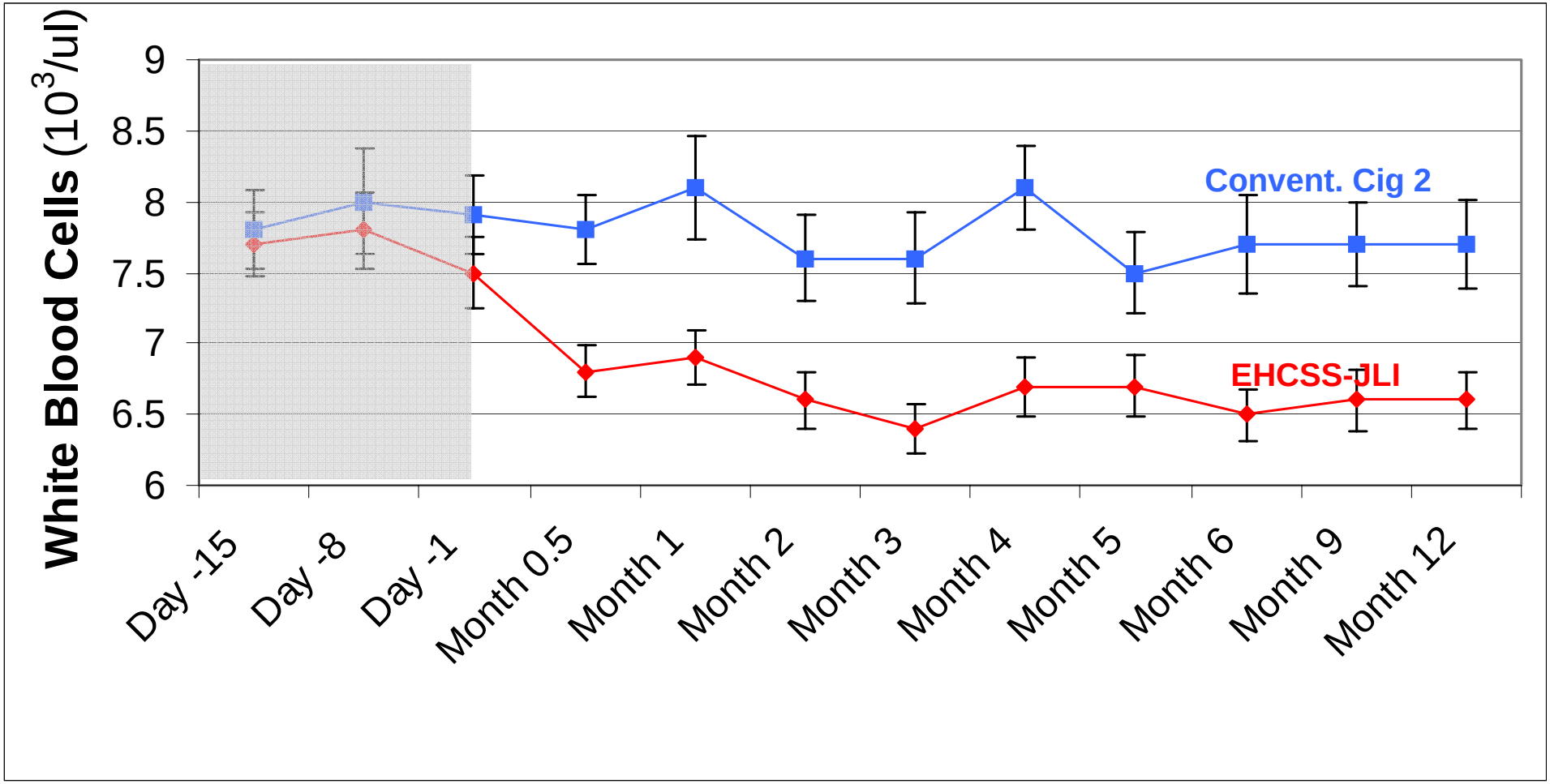
Biomarker of Potential Harm:

Red Blood Cell Hemoglobin *(mean ± SEM)*



Biomarker of Potential Harm:

White Blood Cells *(mean ± SEM)*



Biomarkers of Potential Harm

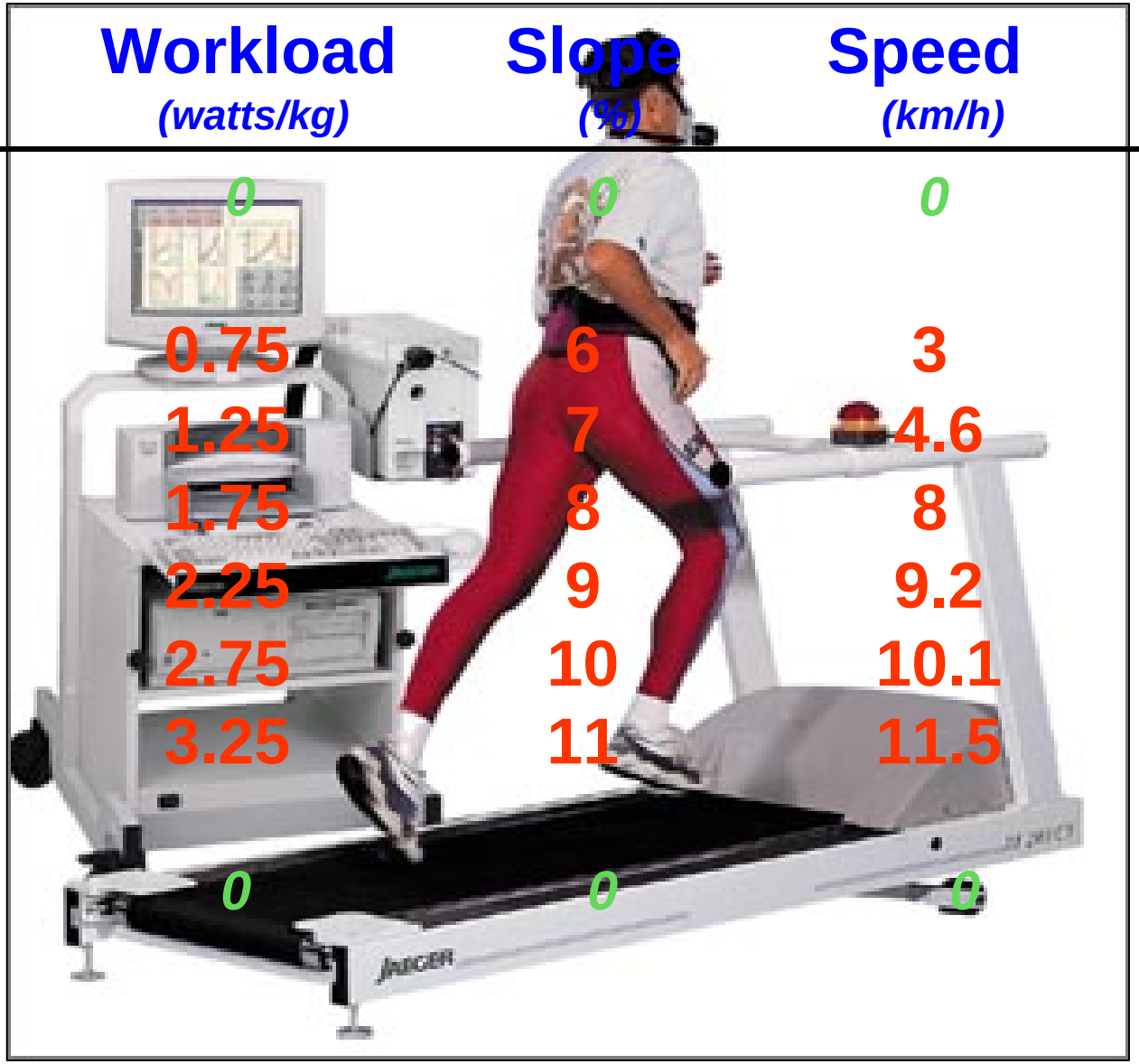
Biomarker	Bio-Matrix	Units	Diff* in LS mean change from BL** (EHCSS-JLI v. Convent. Cig 2)	95% confidence interval
Hemoglobin	Blood	g/dL	**-0.41**	(-0.64, -0.17)
Hematocrit	Blood	%	**-1.32**	(-1.86, -0.77)
White Blood Cells	Blood	cells/uL	**-590**	(-990, -200)
HDL-Cholesterol	Serum	mg/dL	**+4**	(+1, +7)
11-Dehydrothromboxane B_2	Urine	ng/24h	**-383**	(-734, -132)

* $p \leq 0.01$

** At **Baseline**, all subjects smoked **conventional cigarettes with 1-7 mg tar (FTC).**

Functional Test: Cardiopulmonary Exercise Test

Time (min)	Workload (watts/kg)	Slope (%)	Speed (km/h)	Phase
-6	0	0	0	Rest
0	0.75	6	3	Exercise
3	1.25	7	4.6	
6	1.75	8	8	
9	2.25	9	9.2	
12	2.75	10	10.1	
15	3.25	11	11.5	
18-23	0	0	0	Recovery



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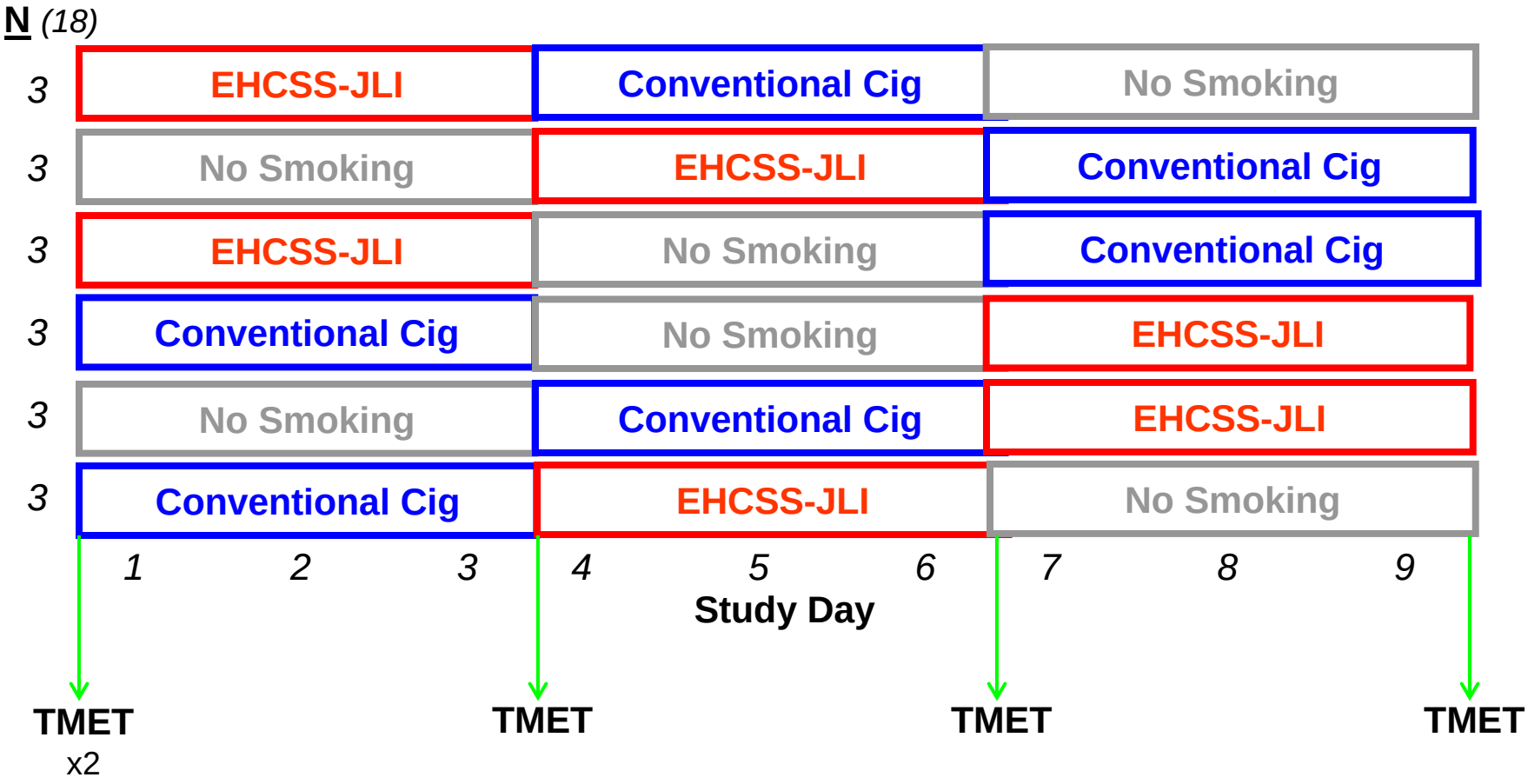
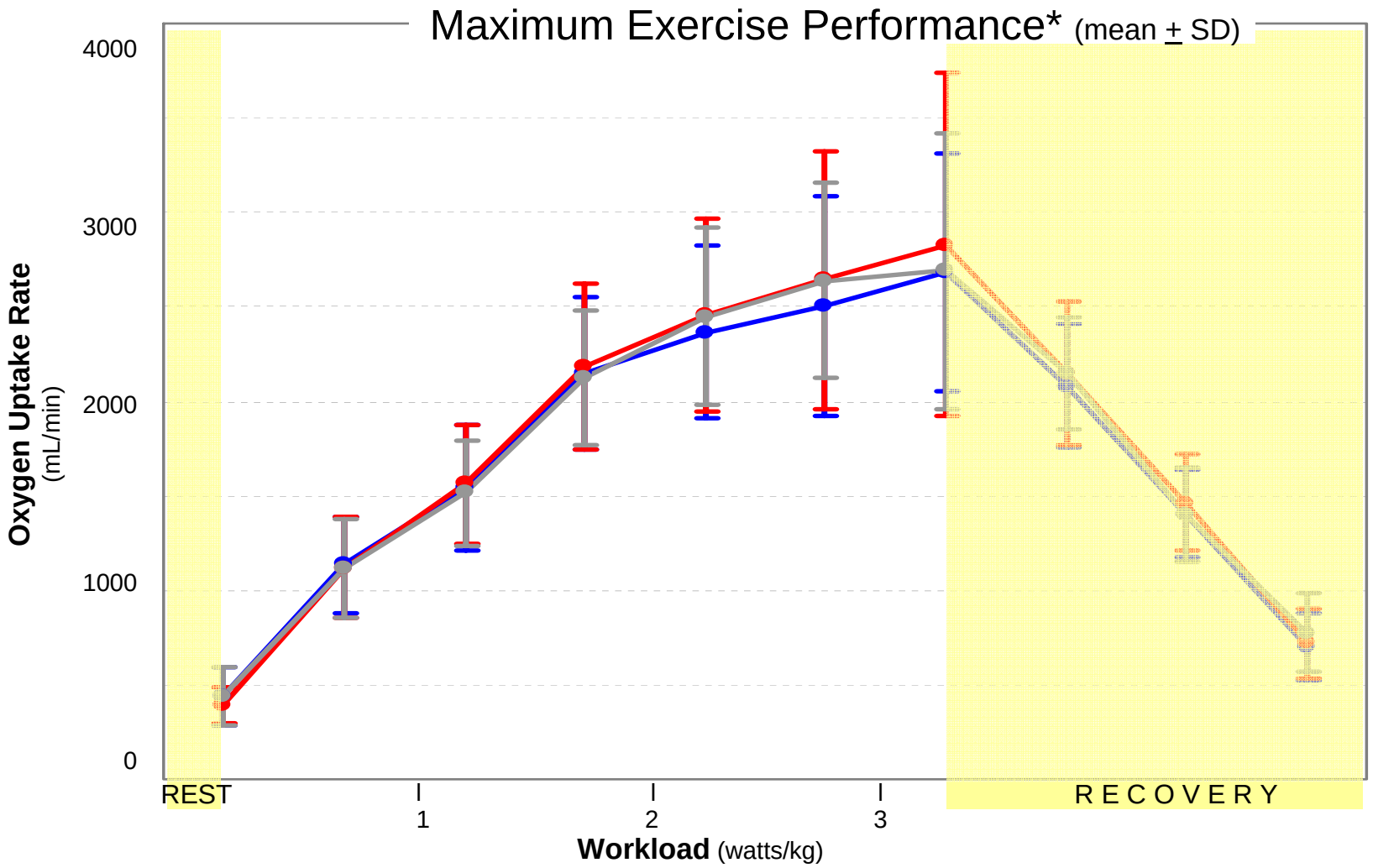
Functional Test: Cardiopulmonary Exercise Test



Conventional Cig = Marlboro LIGHTS *(11 mg tar [FTC])*

TMET = **T**read**M**ill **E**xercise **T**est

Functional Test: Cardiopulmonary Exercise Test



Convent. Cig

EHCSS-JLI

No Smoking

At **Baseline**, all subjects smoked **Conventional Cig.**

p=0.003 for **EHCSS** v. **Conventional Cig.**
p=0.07 for **EHCSS** v. **no smoking**