65th TSRC, Sept 19th, Lexington, KY
Pres 40.

An evaluation of CEMA and adducts of cysteinylglycine as potential biomarkers of exposure to acrylonitrile, formaldehyde and acetaldehyde from cigarette smoke

Emmanuel MINET[1], Francis Cheung[1], Graham Errington[1], Katharina Sterz[2], Gerhard Scherer[2]

[1]British American Tobacco, Group R&D, Southampton, UK
[2]Analytisch-Biologisches Forschungslabor GmbH, Munich, Germany

Biomarkers of exposure are being developed to evaluate toxicants internal dose

The US National Academy of Sciences' Institute of Medicine has defined a **biomarker of exposure** as: "a constituent or metabolite that is measured in a biological fluid or tissue (internal dose) that has the potential to interact with a biological macromolecule (effective dose)", and ideally is:

1. Specific to the source compound
2. Correlated with exposure dose
3. Easy to obtain
4. Able to be measured accurately

http://www.iom.edu/Reports/2001/Clearing-the-Smoke-The-Science-Base-for-Tobacco-Harm-Reduction.aspx

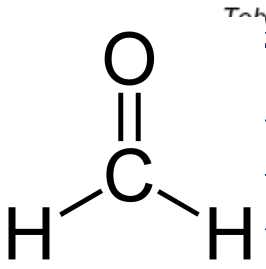
Formaldehyde, acetaldehyde and acrylonitrile are toxicants proposed for mandated lowering or monitoring in the 2008 Burns *et al.* paper

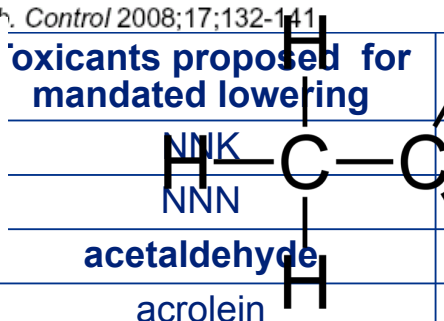
Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal

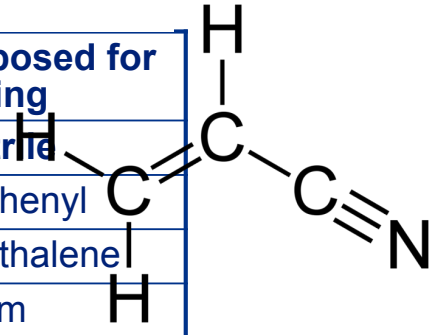
D M Burns, E Dybing, N Gray, S Hecht, C Anderson, T Sanner, R O'Connor, M Djordjevic, C Dresler, P Hainaut, M Jarvis, A Opperhuizen and K Straif

Tob. Control 2008;17;132-141

Formaldehyde Acetaldehyde Acrylonitrile







Toxicants proposed for mandated lowering	Toxicants proposed for monitoring
NNK	**acrylonitrile**
NNN	4-aminobiphenyl
acetaldehyde	2-aminonaphthalene
acrolein	cadmium
benzo[a]pyrene	catechol
1,3-butadiene	crotonaldehyde
carbon monoxide	Hydrogen cyanide
formaldehyde	hydroquinone
	Nitrogen oxide

	μg/cig (mainstream smoke)	*IARC*	*IARC volume, year*
Formaldehyde	1.6 - 75.5 μg	1	88, 2006
Acetaldehyde	32 - 828 μg	2B	71, 1999
Acrylonitrile	0.9 - 19.6 μg	2B	71, 1999

Hecht (2011) Research Opportunities Related to Establishing Standards for Tobacco Products Under the Family Smoking Prevention and Tobacco Control Act. *Nicotine Tob. Res.* e-pub Jan 10

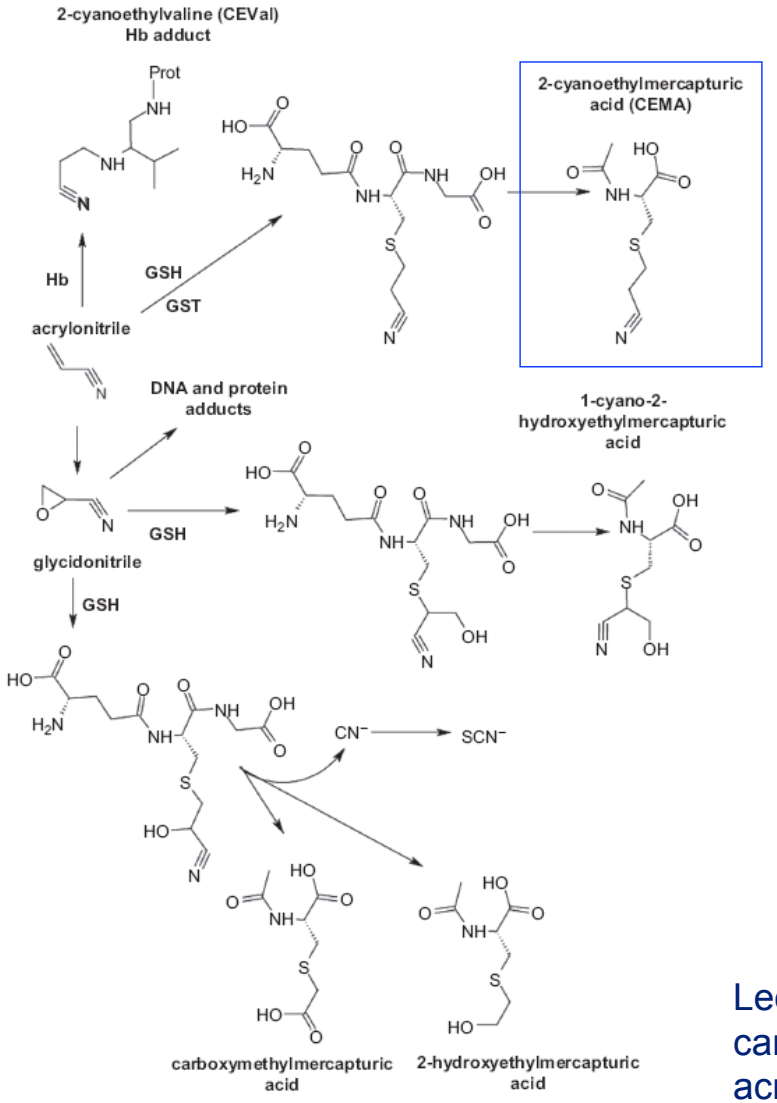
Objective

Biomarkers of exposure have been validated for most of the Burns *et al.* list, however:

•The dose response-relationship for CEMA, a urinary biomarker for acrylonitrile exposure, should be assessed further

•There is currently no suitable urinary biomarker to measure acetaldehyde and formaldehyde exposure and putative biomarkers should be identified.

Simplified metabolic pathway of acrylonitrile, modified from Leonard *et al.*, 1999.



Leonard *et al.* (1999) Mutagenicity, carcinogenicity and teratogenicity of acrylonitrile. *Mut. Res.* 436: 263-83

A clinical study was designed to interrogate the dose-response relationship of CEMA with smoking behavior

LC-MS/MS analysis with API 5000
IS: CEMA-d3
LOD: 0.06ng/ml
LOQ: 0.17ng/ml

Key elements of the clinical study design

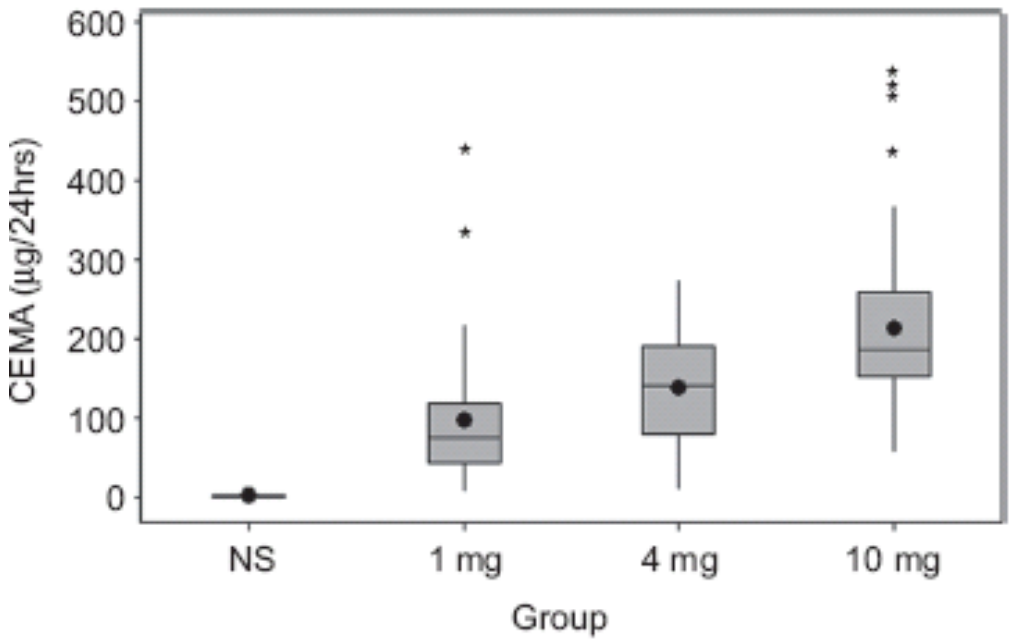
Residential study, 24 h urine
Subjects smoke 15-20 cigs/day
Non-smokers n=50
1 mg smokers n=48
4 mg smokers n=45
10 mg smokers n=47

Table 3. Mainstream acrylonitrile content in smoke of 1, 4, and 10 mg ISO tar products used in this study. Data is reported for the standard ISO and Massachusetts smoking regimes.

ISO Tar yields (mg/cig)	*1*	*4.7*	*10.5*
Acrylonitrile ISO regime (μg/cig)	0.72 ± 0.07	2.89 ± 0.17	7.56 ± 0.37
Massachusetts Tar yields (mg/cig)	*7*	*13.6*	*24*
Acrylonitrile Massachusetts regime (μg/cig)	9.21 ± 0.46	12.36 ± 0.46	18.18 ± 0.4

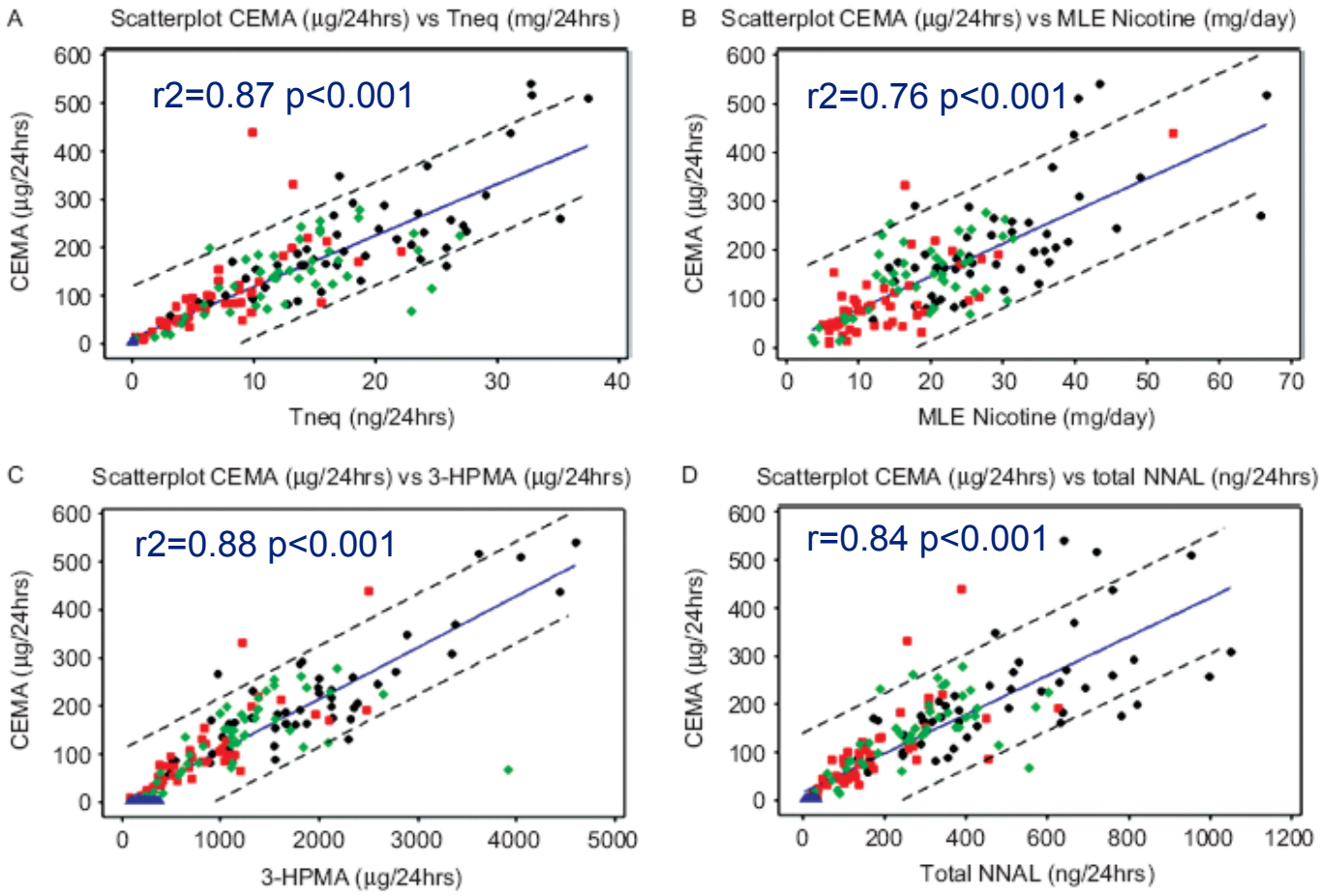
Minet *et al.* (2011) Urinary excretion of the acrylonitrile metabolite 2-cyanoethylmercapturic acid is correlated with a variety of biomarkers of tobacco smoke exposure and consumption. Biomarkers, 16(1): 89-96

Boxplots of urinary CEMA excretion rates by smoking status and ISO tar yields show significant differences between groups



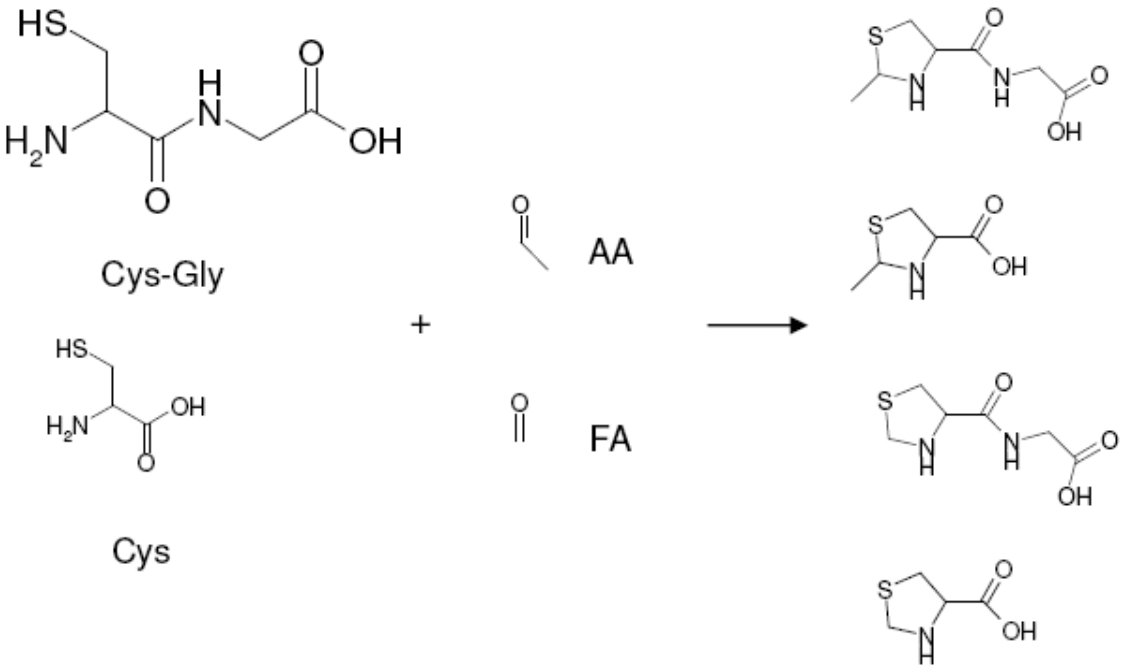
Minet *et al.* (2011)

Scatter plots and Pearson matrix confirms CEMA correlation with other markers of smoking dose



Minet *et al.* (2011)

Two proposed metabolic pathways for the disposition of formaldehyde and acetaldehyde formed by reaction with cysteinylglycine and cysteine

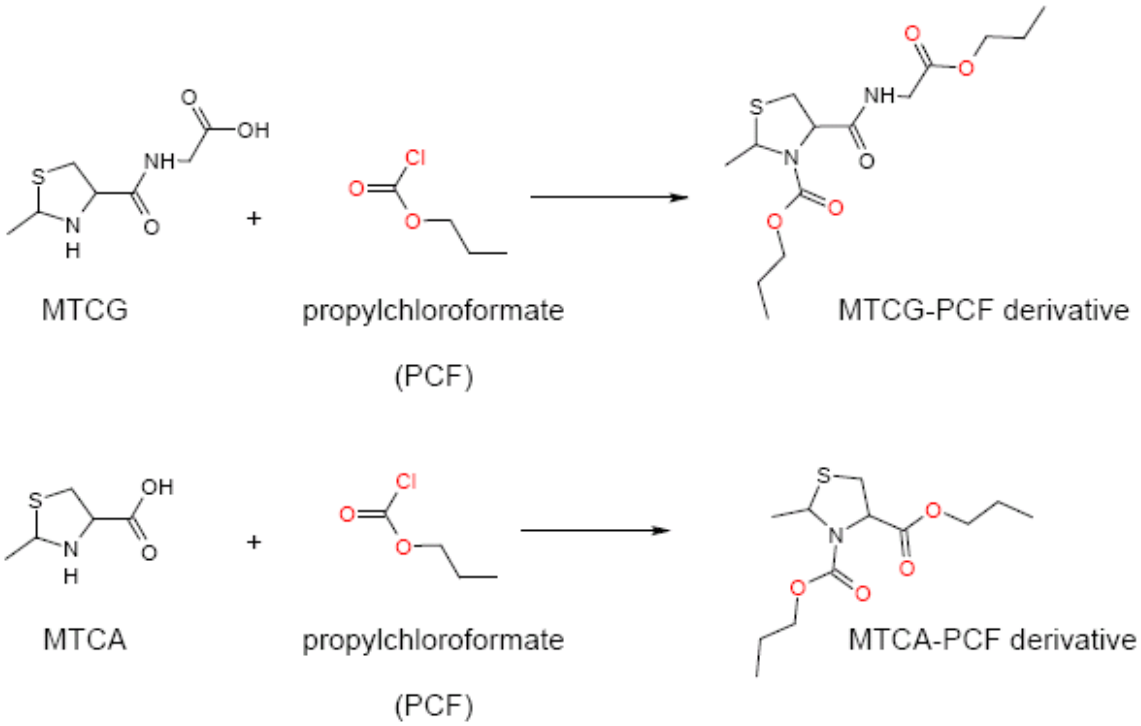


[1] Kera *et al.* (1985) Conjugation of acetaldehyde with cysteinylglycine, the first metabolite in glutathione breakdown by gamma-glutamyltranspeptidase. *Agents Actions*. 17(1): 48-52.

[2] Hemminki (1982) Urinary sulfur containing metabolites after administration of ethanol, acetaldehyde and formaldehyde to rats. *Toxicol Lett.* 11(1-2):1-6.

A derivatization step is necessary to achieve linearity of the LC-MS/MS quantification method

Initial LC-MS/MS method showed non-linearity of the quantification derivatization with pentylchloroformate solved this issue



Clinical samples were analyzed to assess the potential dose-response relationship of acetaldehyde and formaldehyde biomarkers with smoking behavior

LC-MS/MS analysis with API 5000
IS: $^{13}C_2$-MTCG, $^{13}C_2$-MTCA
LOD: 0.2-0.9 ng/ml
LOQ: 0. 5-2.5 ng/ml
(range for MTCG, MTCA, TCG, TCA)

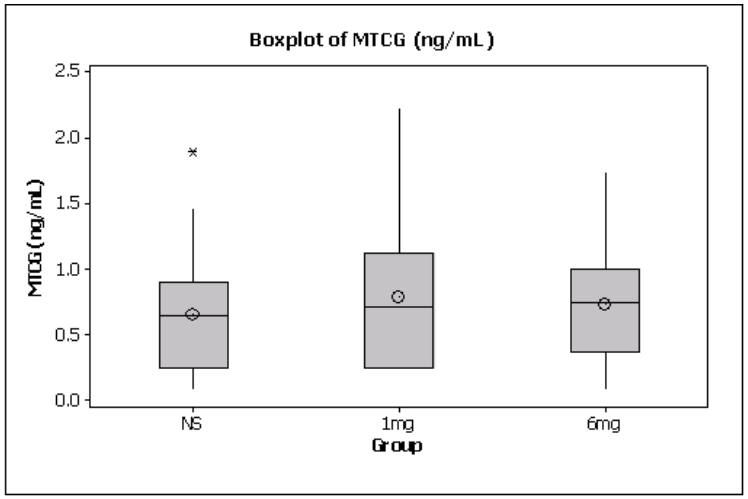
Ambulatory study, 24 h urine
Groups: 6 mg smokers cig: n=49
1 mg smokers cig: n=46
non-smokers: n=50
No alcohol consumption 72 h prior to urine collection

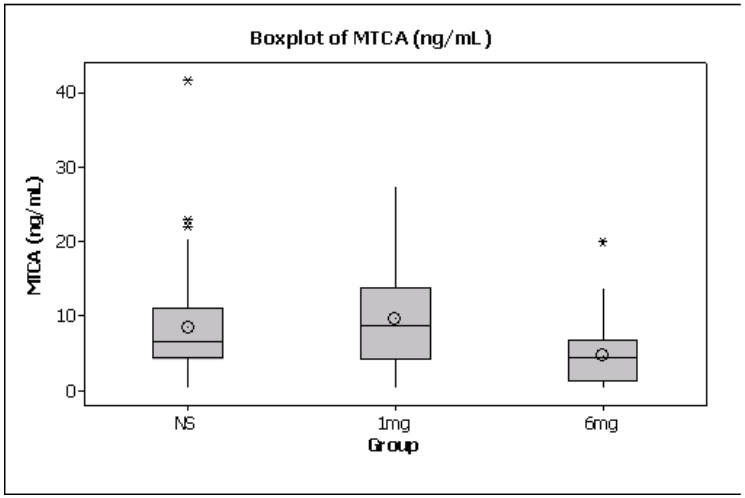
Acetaldehyde and formaldehyde yield

acetaldehyde (μg/cig)				*Formaldehyde (μg/cig)*		
Smoking regime	***6 mg***	***1 mg***		***Smoking regime***	***6 mg***	***1 mg***
GRDI	729	79		GRDI	22	4
WG91	987	442		WG91	54	19
HCI	811	1152		HCI	60	47

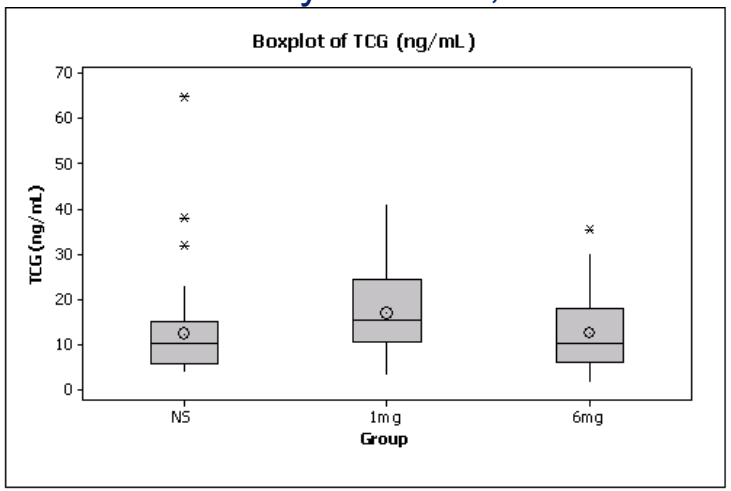
Boxplots of urinary TCG, TCA, MTCG and MTCA excretion rates by smoking status and ISO tar yields show no significant differences between groups

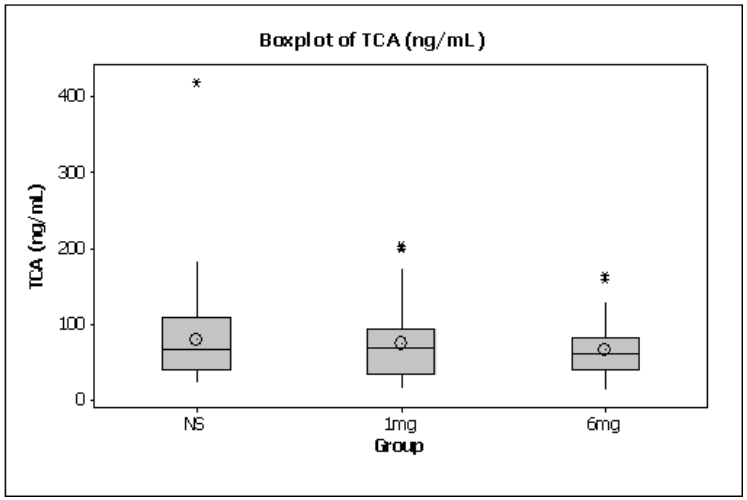
Acetaldehyde: MTCG, MTCA





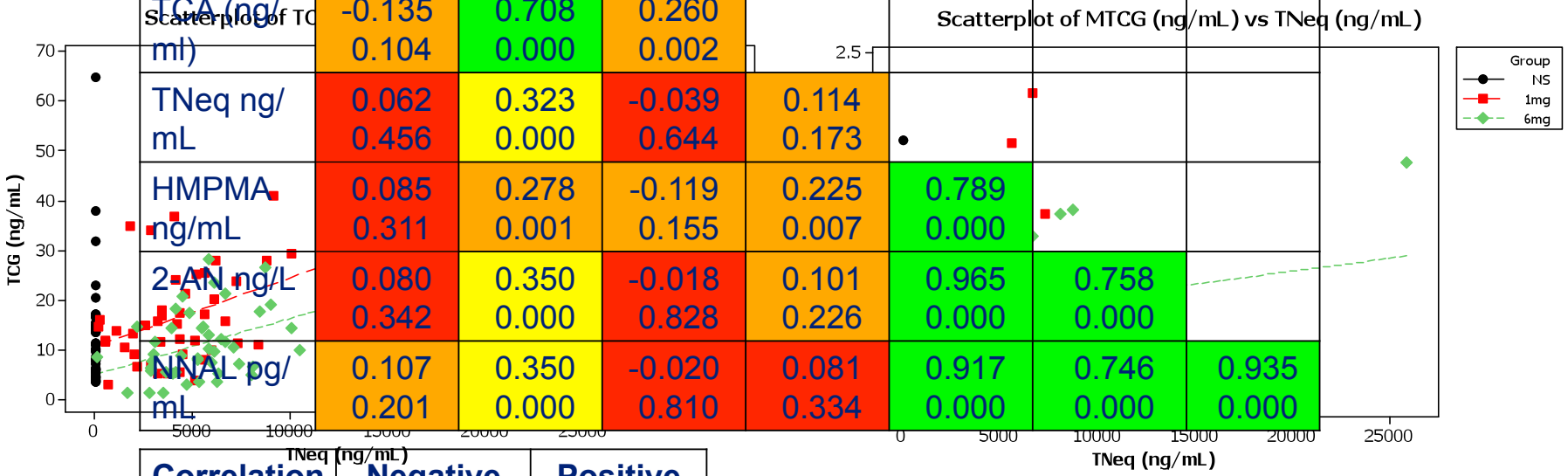
Formaldehyde: TCG, TCA





Scatter plots and Pearson matrices show no correlation with other markers of smoking dose

	MTCG (ng/mL)	TCG (ng/mL)	MTCA (ng/mL)	TCA (ng/mL)	TNeq (ng/mL)	HMPMA (ng/mL)	2-AN (ng/L)
TCG (ng/ml)	-0.001 0.990						
MTCA (ng/ml)	0.109 0.194	0.298 0.000					
TCA (ng/ml)	-0.135 0.104	0.708 0.000	0.260 0.002				
TNeq ng/mL	0.062 0.456	0.323 0.000	-0.039 0.644	0.114 0.173			
HMPMA ng/mL	0.085 0.311	0.278 0.001	-0.119 0.155	0.225 0.007	0.789 0.000		
2-AN ng/L	0.080 0.342	0.350 0.000	-0.018 0.828	0.101 0.226	0.965 0.000	0.758 0.000	
NNAL pg/mL	0.107 0.201	0.350 0.000	-0.020 0.810	0.081 0.334	0.917 0.000	0.746 0.000	0.935 0.000



Correlation	Negative	Positive
None	−0.09 to 0.0	0.0 to 0.09
Small	−0.3 to −0.1	0.1 to 0.3
Medium	−0.5 to −0.3	0.3 to 0.5
Large	−1.0 to	0.5 to 1.0

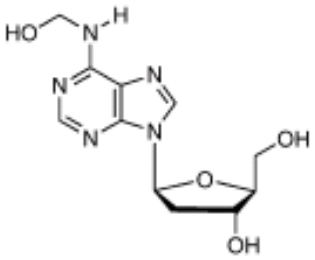
Conclusion:

CEMA demonstrated a good dose-response relationship with nicotine exposure and with a panel of smoke toxicant biomarkers

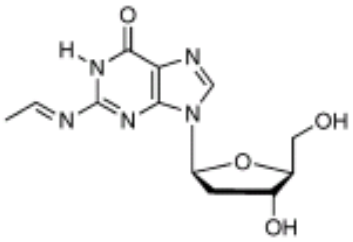
In contrast the putative biomarkers for acetaldehyde and formaldehyde exposure were not well correlated with nicotine exposure

We found a high urinary background for MTCG and TCG which interfere with the establishment of a reliable correlation in the context of tobacco smoking

Alternative biomarkers for acetaldehyde and formaldehyde exposure include N^6-hydroxymethyldeoxyadenosine and N^2-ethyldene-deoxyguanosine



N^6-hydroxymethyl-dAdo



N^2-ethyldene-dGuo

Cancer Res. 2009 September 15; 69(18): 7170–7174. doi:10.1158/0008-5472.CAN-09-1571.

Clear Differences in Levels of a Formaldehyde-DNA Adduct in Leukocytes of Smokers and Non-smokers

Mingyao Wang, **Guang Cheng**, **Silvia Balbo**, **Steven G. Carmella**, **Peter W. Villalta**, and **Stephen S. Hecht***
Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455

Chem Res Toxicol. 2007 January ; 20(1): 108–113.

Smokers

Mean ± S.D.	179 ± 205
	(29/32 positive)

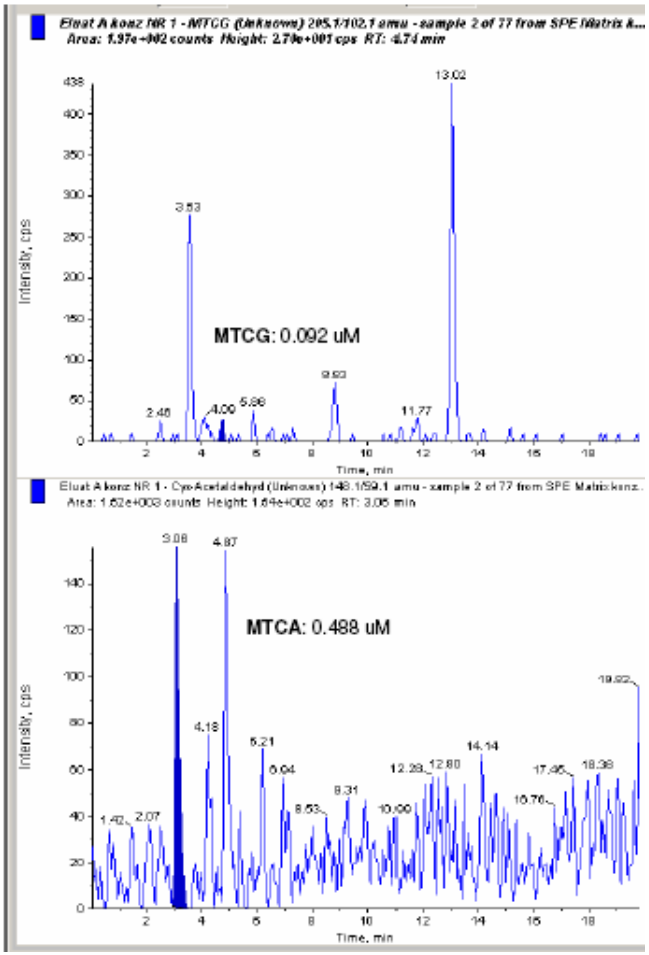
Non-smokers

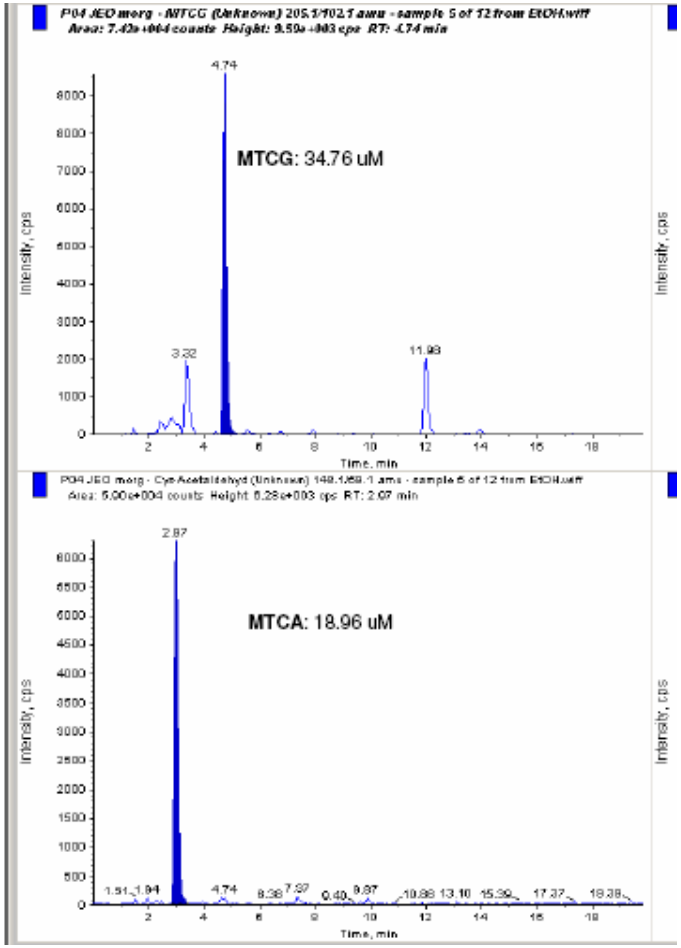
Mean ± S.D.	15.5 ± 33.8
	(7/30 positive)

Quantitation of an Acetaldehyde Adduct in Human Leukocyte DNA and the Effect of Smoking Cessation

Li Chen, **Mingyao Wang**, **Peter W. Villalta**, **Xianghua Luo**, **Rachel Feuer**, **Joni Jensen**, **Dorothy K. Hatsukami**, and **Stephen S. Hecht***
The Cancer Center and Transdisciplinary Tobacco Use Research Center, University of Minnesota, Minneapolis, Minnesota 55455

MTCG could potentially be a better biomarker of acetaldehyde exposure through alcohol consumption





Acknowledgements

BAT clinical study team

Jim Shepperd

Alison Eldridge

This presentation is available on bat-science.com