Biomarkers of Industrial and Environmental Exposure to 1,3-Butadiene

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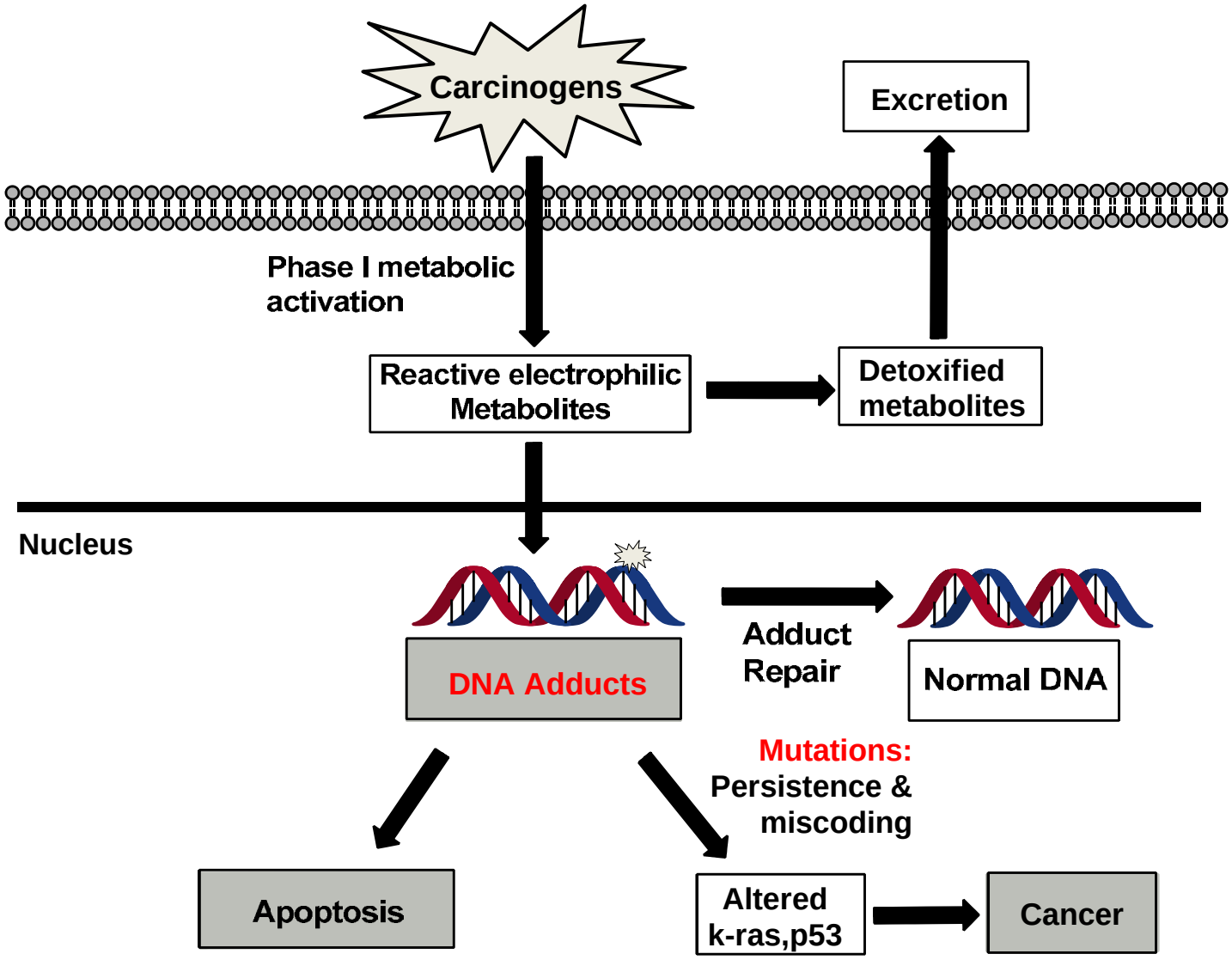
Slides Prepared for the 2014 Symposium on Understanding the Health Risks of Lower Olefins

November 5, 2014

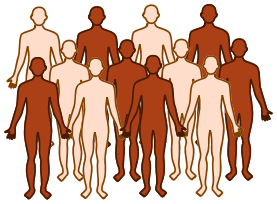
Central dogma of chemical carcinogenesis



Adapted from Tsunehiro Oyama et al. *Frontiers in Bioscience* , 9, 1967-1976, 2004, Hecht, S. S. J. Natl. Cancer Inst. 91, 1194-1210

Sources of human exposure to BD

Occupational exposure
(0.5-1.0 ppm)



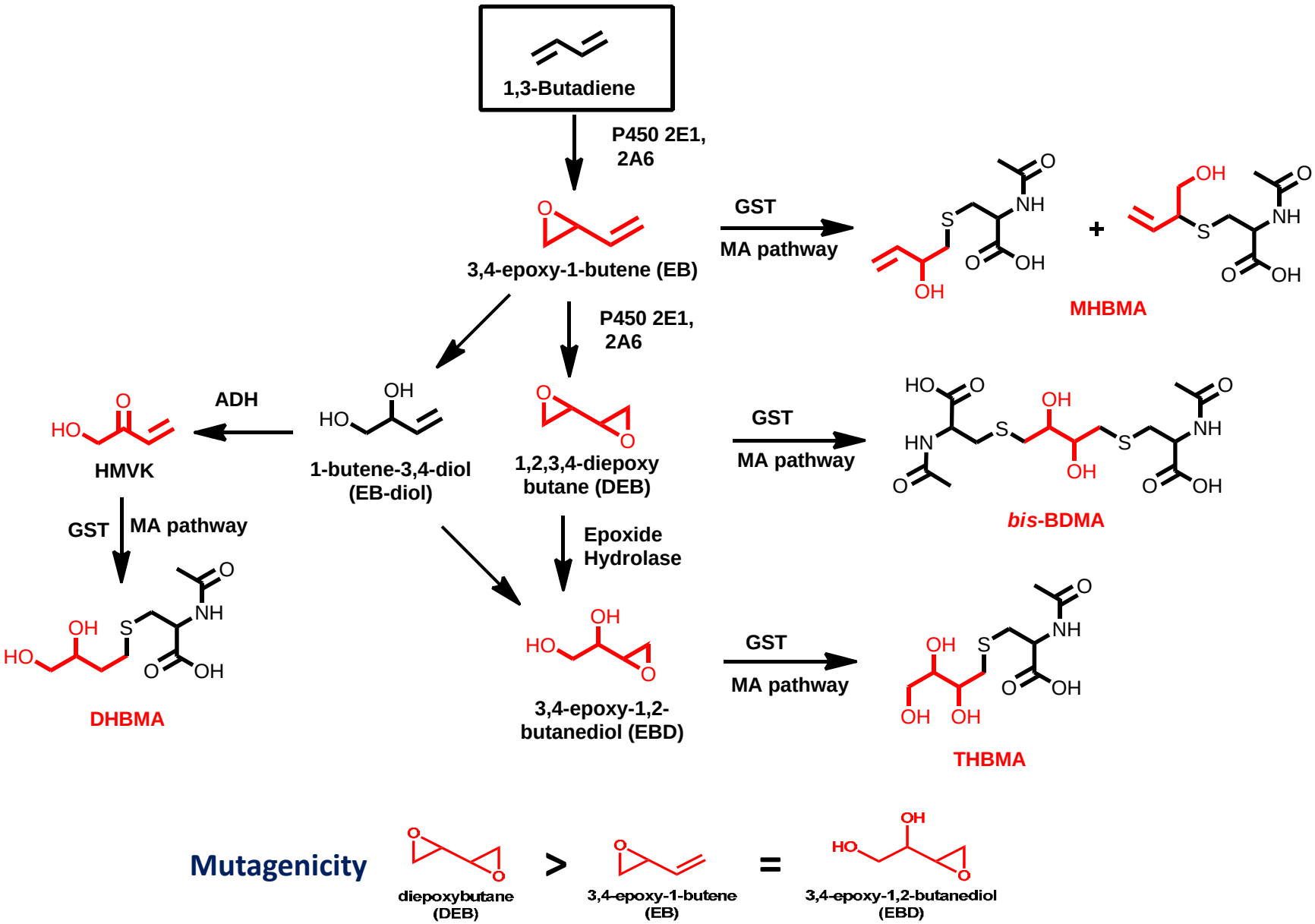
1,3-butadiene (BD)

~ 0.15 ppb
Wood burning
forest fires

~20-75 µg
per cigarette

Automobile exhaust
~0.1-10 ppb in urban air

Metabolism of 1,3-Butadiene



Mutagenicity

S Kotapati et al. Chem. Res. Toxicol. 2011, 24, 1516–1526; Van Sittert et al. Toxicol. Sci. 2000, 56, 189–202; S Kotapati et al. Carcinogenesis. 2014;35(6):1371-8.

Interspecies differences in sensitivity to BD-mediated cancer

- Laboratory mice develop tumors following exposure to 6.25 ppm BD, while rats require ~ 200-fold higher concentrations.

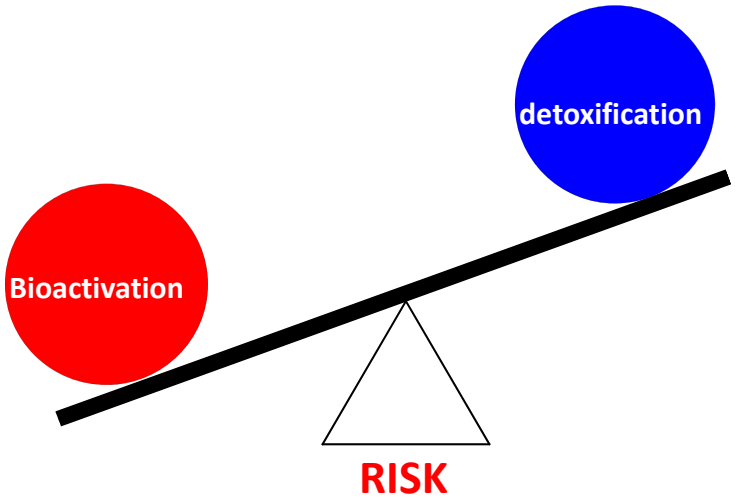
- This may be explained by a more efficient formation of DEB in mice:
  - Higher amounts of DEB are detected in blood of BD-exposed mice.

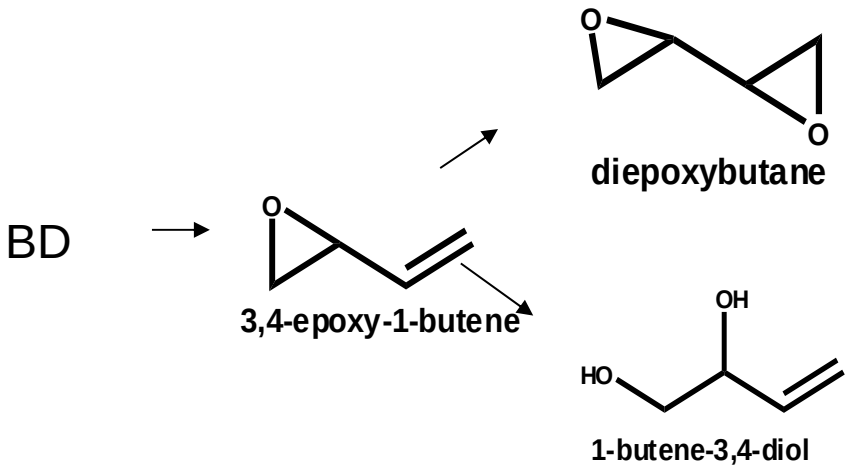
    Filser et al. *Chem. Biol. Interact. 166*, 93-103 (2007)
  - Greater amounts of DEB-globin adducts are found in mice.

    Boysen et al. *Chem. Biol. Interact. 166*, 84-92 (2007)

What determines individual susceptibility to BD?

Individuals and ethnic groups may differ in respect to metabolic activation and deactivation of BD, leading to an differences in formation of DNA-reactive BD intermediates/ modified risk.





Genetic polymorphisms in BD Metabolizing Genes

Protein	Variant	Changes to Genotype/Phenotype
GSTT1 [12-14]	rs11550605	A>C → Thr104Pro; decreased protein expression
	rs199521920	C>T in exon 2 → Asp43Asn; decreased protein expression
		C>T in exon 2 → Thr65Met; decreased protein expression
CYP2E1 [15-16]	rs6413432	A>T in intron 6; introduces Dra1 site
	rs3813867	G>C in 5' upstream region; introduces Pst1 site
	rs2031920	C>T in 5' upstream region; removes RsaI site
EPHX1 [8]	rs1051740	T>C in exon 3 → Tyr113His; decreased protein activity
	rs2234922	A>G in exon 4 → His139Arg; increased protein activity

Ethnic differences in incidence of genetic polymorphisms in xenobiotic-metabolizing gene (percentages)

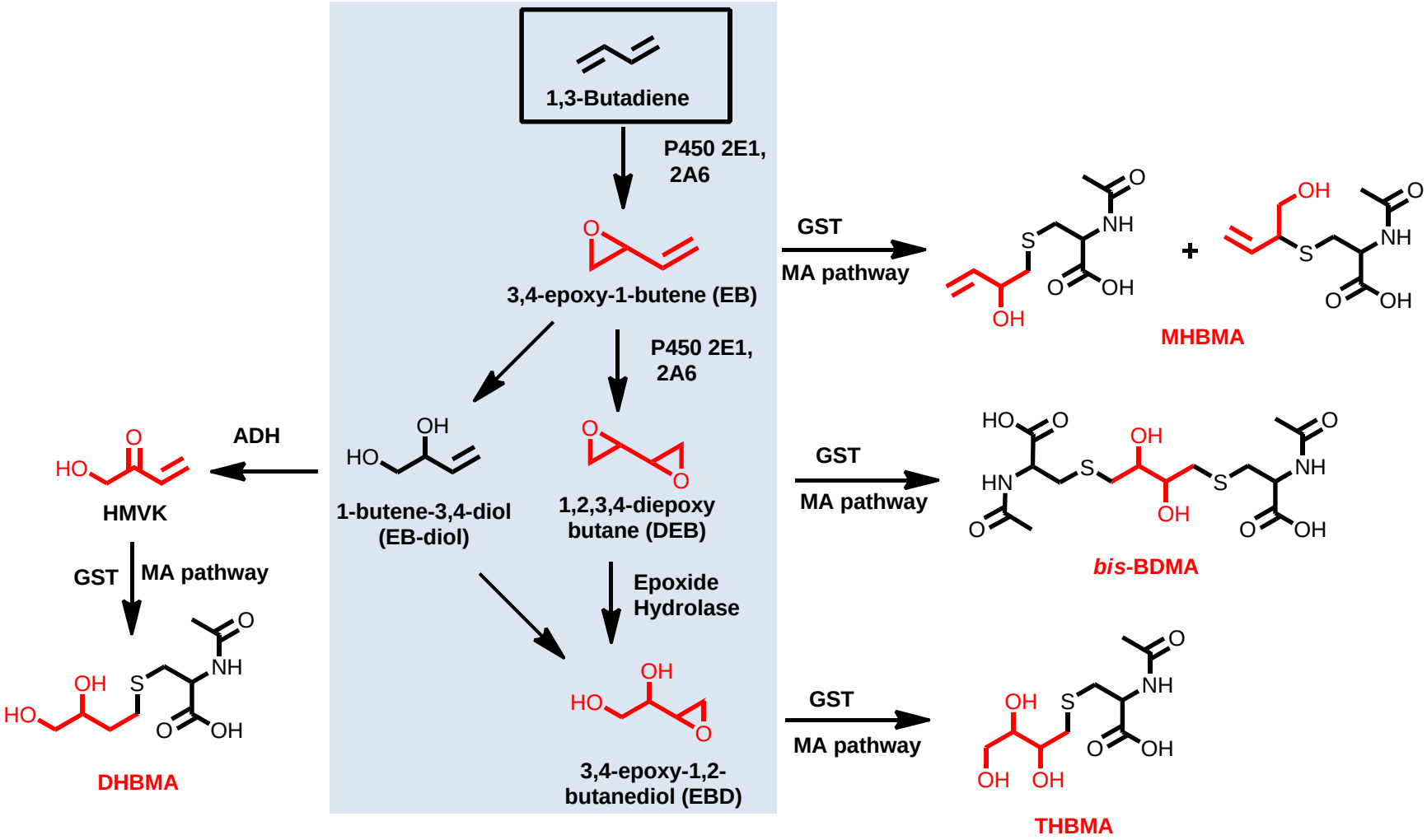
	GSTM1-1 Null	GSTT1-1 Null	Slow EH	Fast EH	Low CYP2E1 Activity	Low CYP2A6 Activity
European American	52 [7]	14.7 [7]	27.9 [8]	19.1 [8]	1 [7]	21.5 [9]
African American	27 [7]	21.8 [7]	20.8 [8]	28.9 [8]	4 [7]	2.5 [9]
Japanese	48.6 [7]	44.3 [11]	44 [10]	14 [10]	19.3 [7]	48 [9]

Wormhoudt, L. W. et al. *Crit. Rev. Toxicol.* **1999**, 29, 59-124.
London, S. J. et al. *Lung Cancer.* **2000**, 28, 147-155.
Fernandez-Salguero, P. et al. *Am. J. Hum. Gent.* **1995**, 57, 651-660.
Yoshikawa, M. et al. *Int. J. Mol. Med.* **2000**, 5, 49-53.

Goals of this work

- Develop biomarkers of human exposure to BD (urinary metabolites, DNA adducts)
- Evaluate BD exposure in general population, smokers, and occupationally exposed workers
- Investigate ethnic and individual variability in BD metabolism/DNA adduct formation.

Urinary Metabolites of 1,3-Butadiene



GST: Glutathione-S-transferase,
ADH: Alcohol dehydrogenase

Metabolic Ratio = MHBMA/(MHBMA + DHBMA)

- **Inversely proportional to epoxide hydrolase activity**
- **Higher ratio suggests higher risk**

S Kotapati et al. Chem. Res. Toxicol. 2011, 24, 1516–1526; Van Sittert et al. Toxicol. Sci. 2000, 56, 189–202; S Kotapati et al. Carcinogenesis. 2014;35(6):1371-8.

Mass Spectrometry Based Quantitation of Urinary BD-Mercapturic Acids

Urine Aliquot (100 µL)

↓

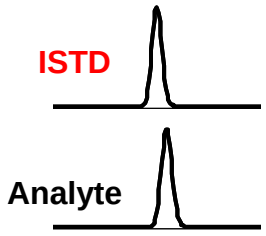
Add internal standard
(60 ng each of deuterium labeled MA)

↓

Solid Phase Extraction
(Isolute ENV+ 1ml/50 mg)

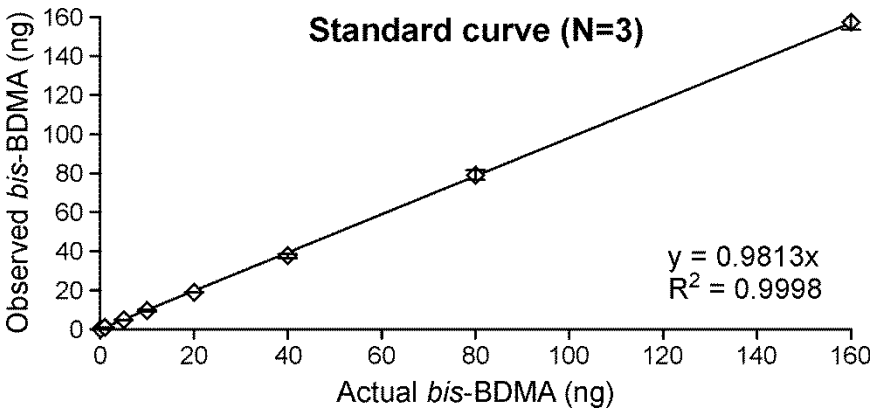
↓

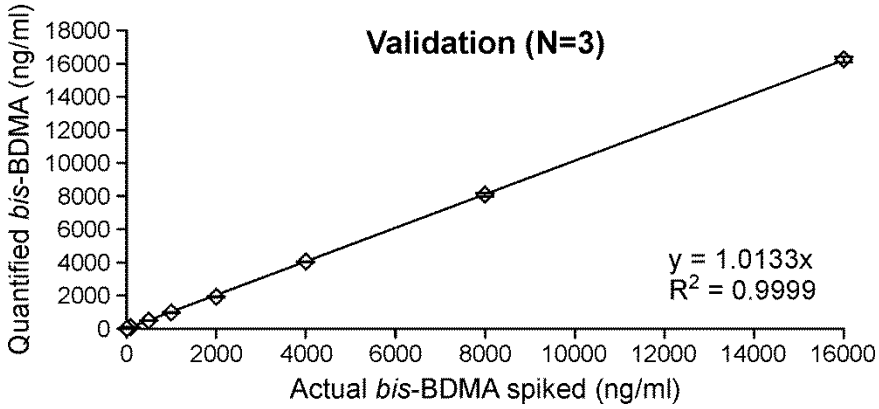
HPLC- ESI$^-$- MS/MS analysis



MHBMA and DHBMA: Varian Pursuit 3 Diphenyl column 2.1 x 150
THBMA and *bis*-BDMA: SIELC Primesep D 2.1 x 100

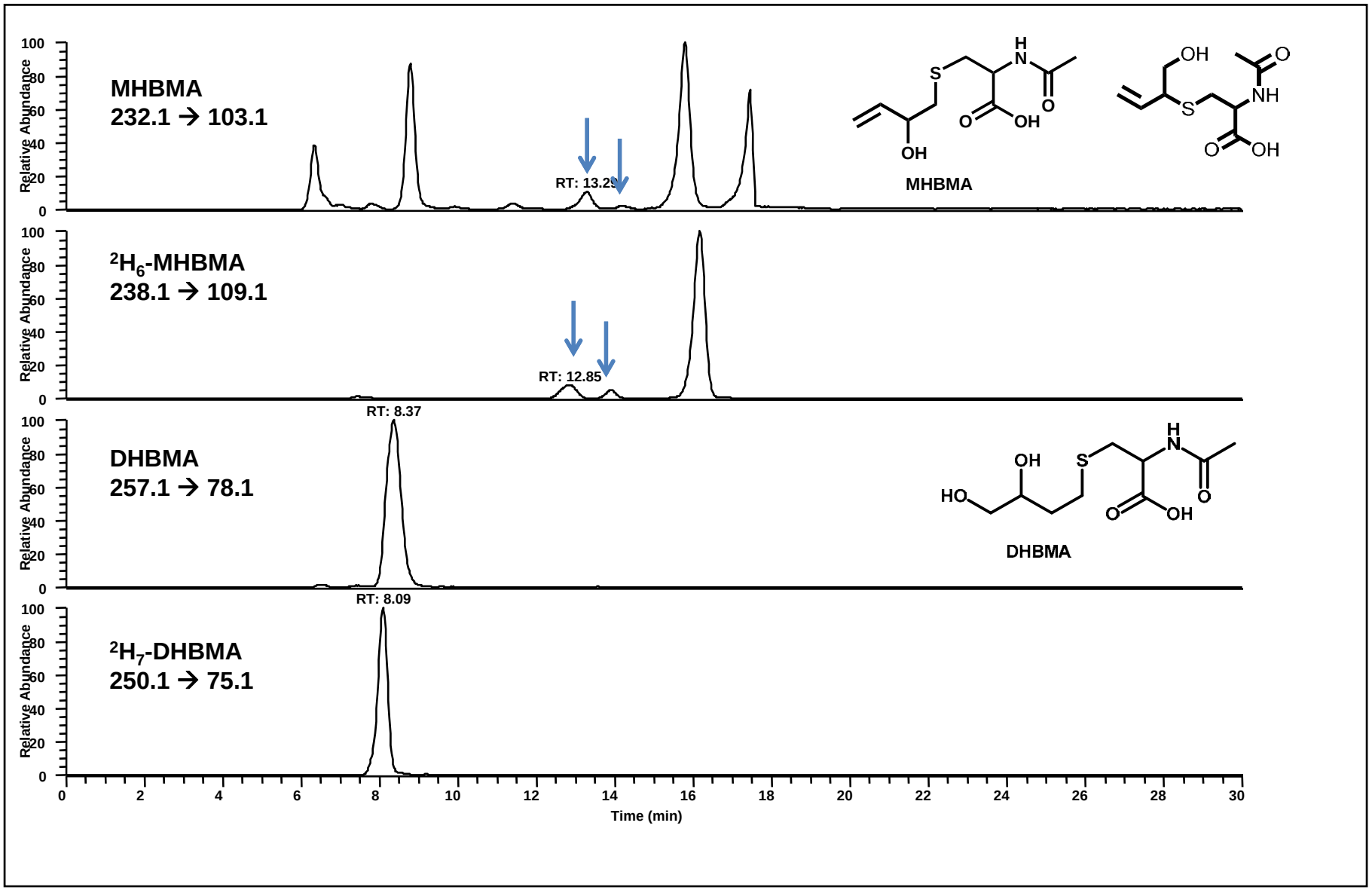
HPLC-MS/MS method for MHBMA and DHBMA



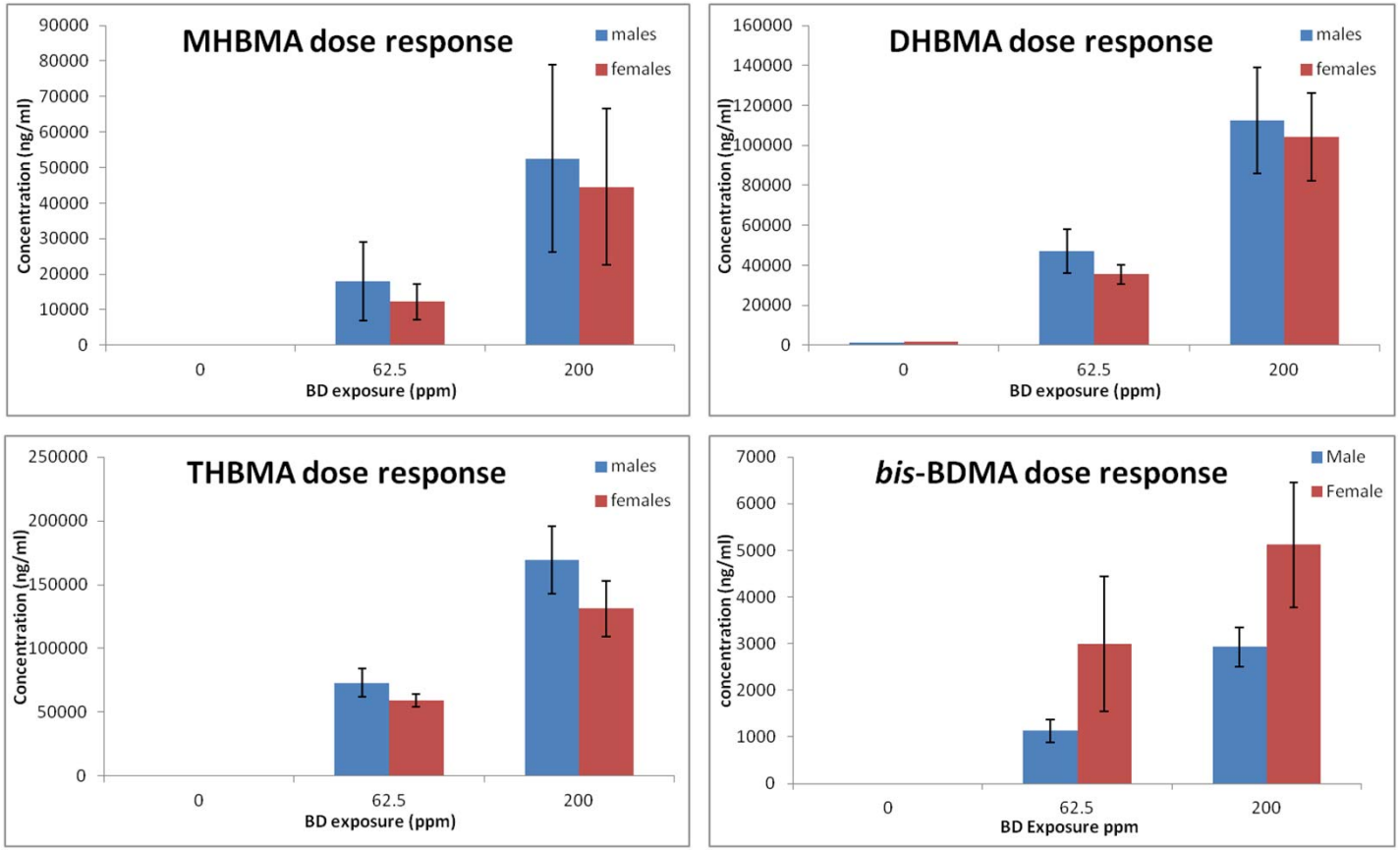


Range (ng/ml)	LOD (ng/ml)	LOQ (ng/ml)	Intra-day precision (%)	Inter-day Precision (%)	Accuracy (%)	Extraction recovery (%)
5-16,000	1	5	0.88	1.17	98.04	46

Representative HPLC-ESI-MS/MS Traces for MHBMA and DHBMA in Human Urine



The levels of all BD-mercapturic acids in rat urine increase linearly with BD exposure

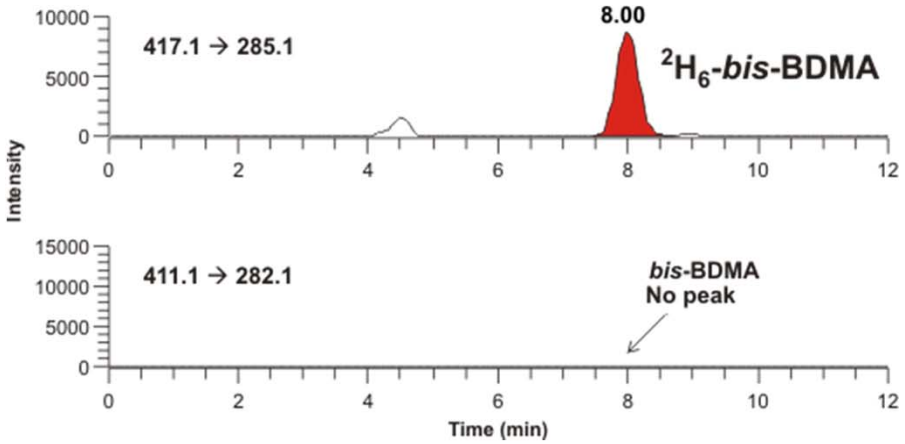


Rat urine samples provided by Dr. Vernon Walker *(University of Vermont)*

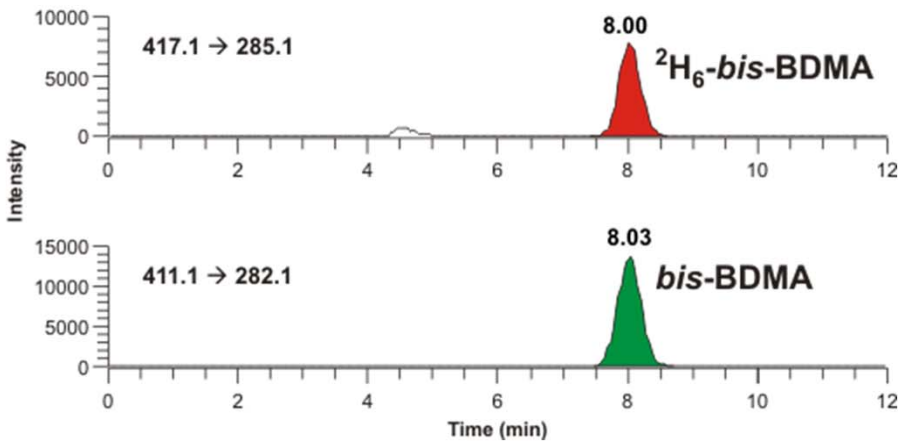
Kotapati, S. et al. *Carcinogenesis* 35(6):1371-8 (2014)

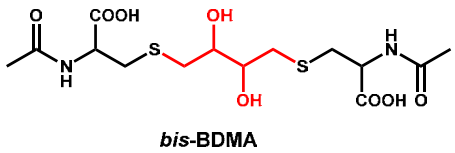
DEB-derived *bis*-BDMA was detected in the urine of F344 rats exposed to BD, but not in humans

Control rat



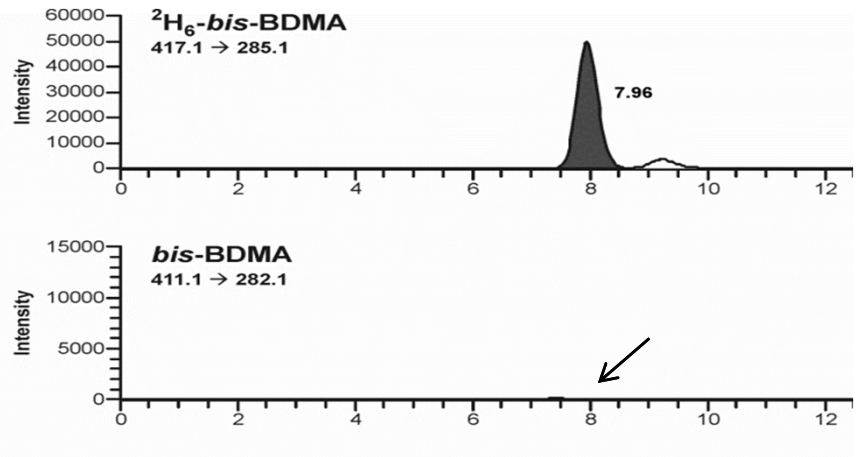
Rat exposed to 62.5 ppm BD





bis-BDMA

Occupationally exposed worker (2 ppm)



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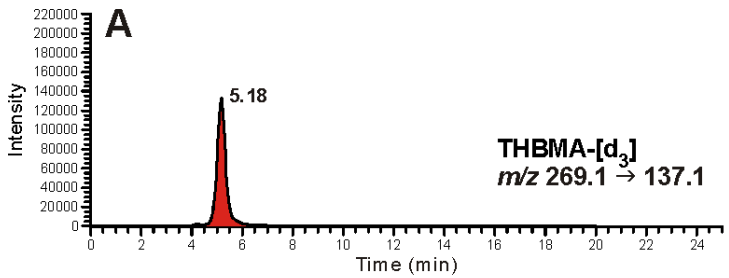
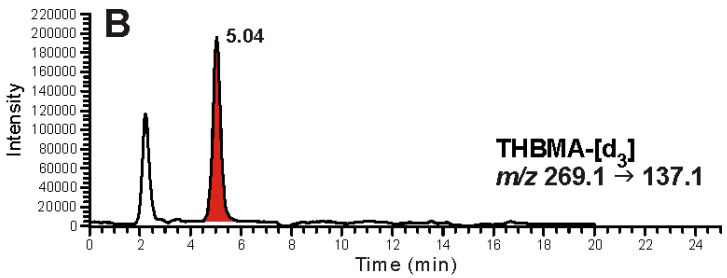
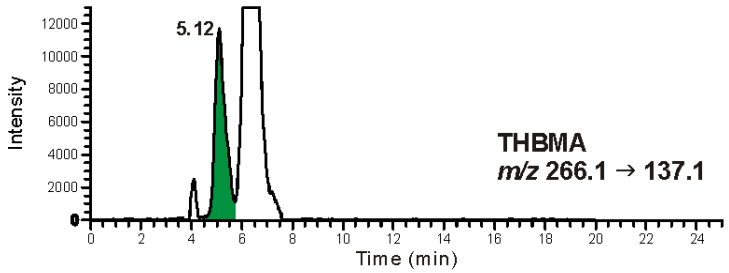
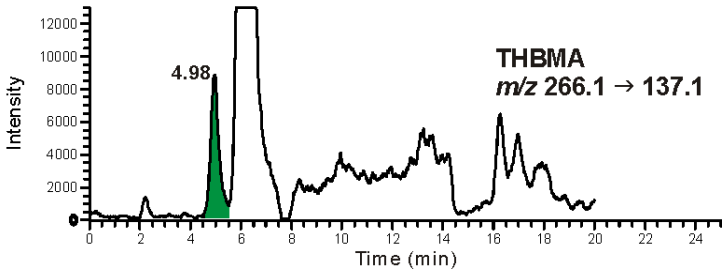
First detection of THBMA in humans

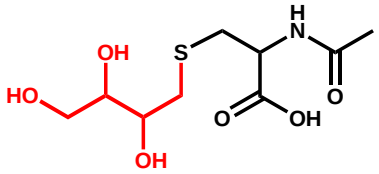


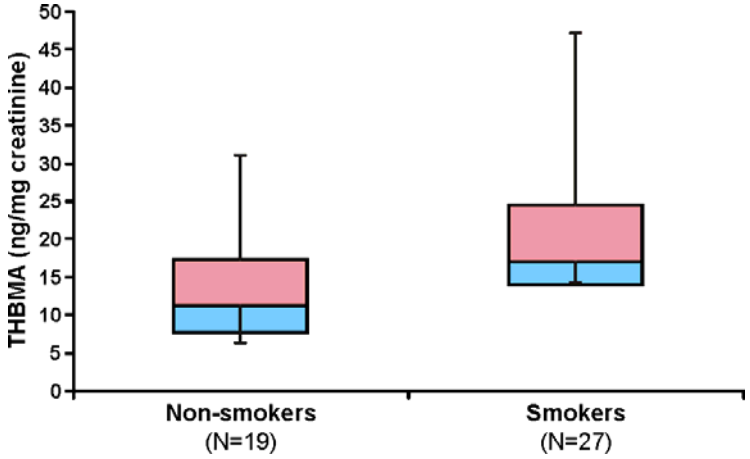


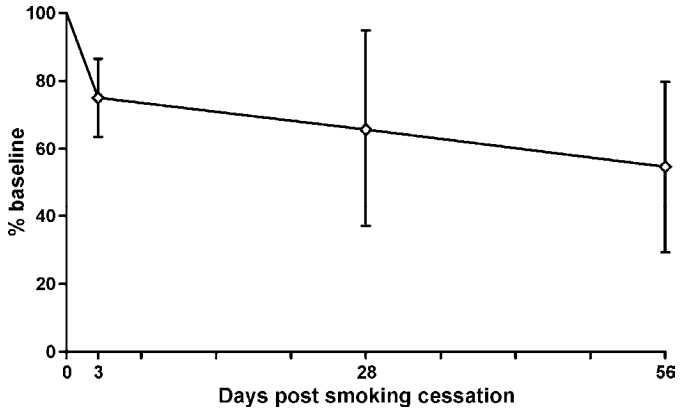




SMOKER **NON-SMOKER**

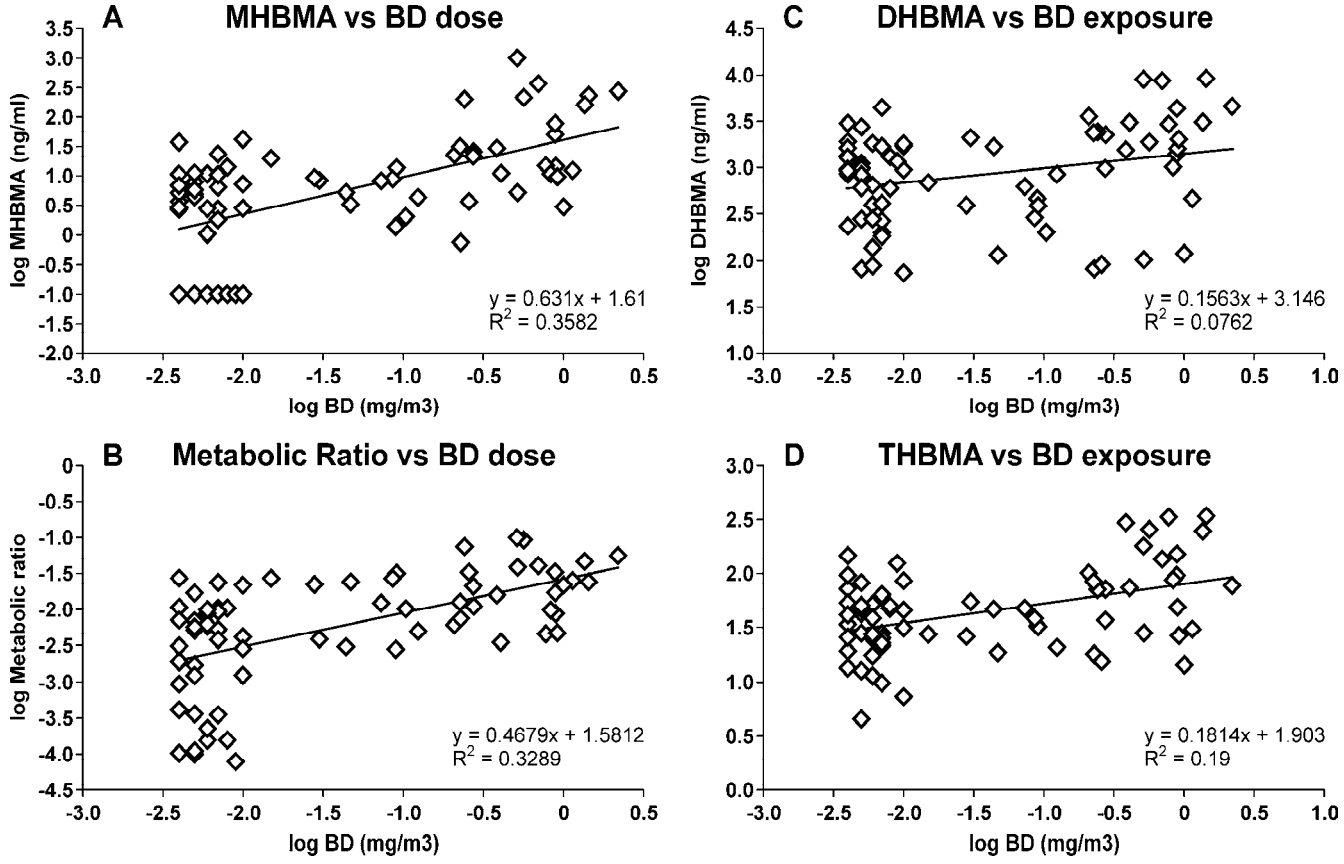






Kotapati, S. *et. al.* **Chem Res Toxicol.** 2011, 24, 1516–1526.

Association of urinary BD-mercapturic acids with exposure



MHBMA is associated with BD exposure, but the correlation is weak for DHBMA and THBMA

Kotapati, S. et al. *Carcinogenesis* 35(6):1371-8 (2014)

BD-urinary acids in urine of occupationally exposed workers

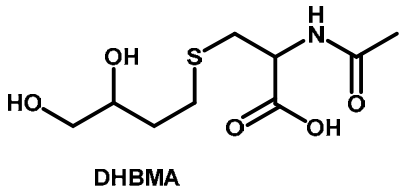
Czech cohort (Albertini et al. Chemico-Biological Int. 166 (2007) 63-77)

			Concentrations (ng/ml)			
	N	BD exposure (mg/m^3)	MHBMA	DHBMA	THBMA	Metabolic Ratio
Males						
Controls	21	0.007 ± 0.005	9.9 ± 11	1480 ± 968	58 ± 33	0.007 ± 0.008
Exposed	16	0.68 ± 0.41	96 ± 111	3136 ± 2560	139 ± 104	0.027 ± 0.026
Females						
Controls	19	0.007 ± 0.005	3.1 ± 4.8	561.2 ± 531.5	24.2 ± 16.6	0.006 ± 0.007
Exposed	16	0.32 ± 0.34	8.3 ± 8.1	716.1 ± 830.7	47.4 ± 70.9	0.017 ± 0.012

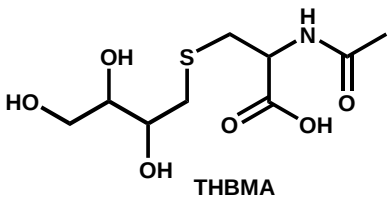
- Significantly higher concentrations of BD-mercapturic acids in urine of exposed workers
- Greater increase in males vs females
- Large amounts of BD-mercapturic acids detected in unexposed controls
- No bis-BDMA detected in human urine

Kotapati, S. et al. *Carcinogenesis* 35(6):1371-8 (2014)

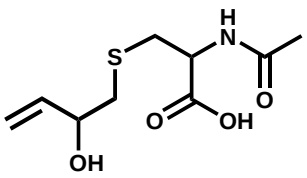
Relative concentrations of BD-mercapturic acids in urine of BD-exposed rats and in human smoker urine



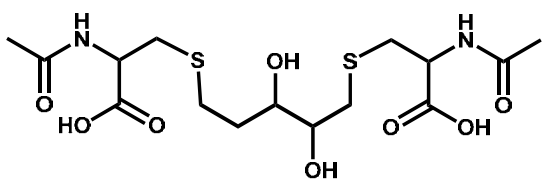
DHBMA



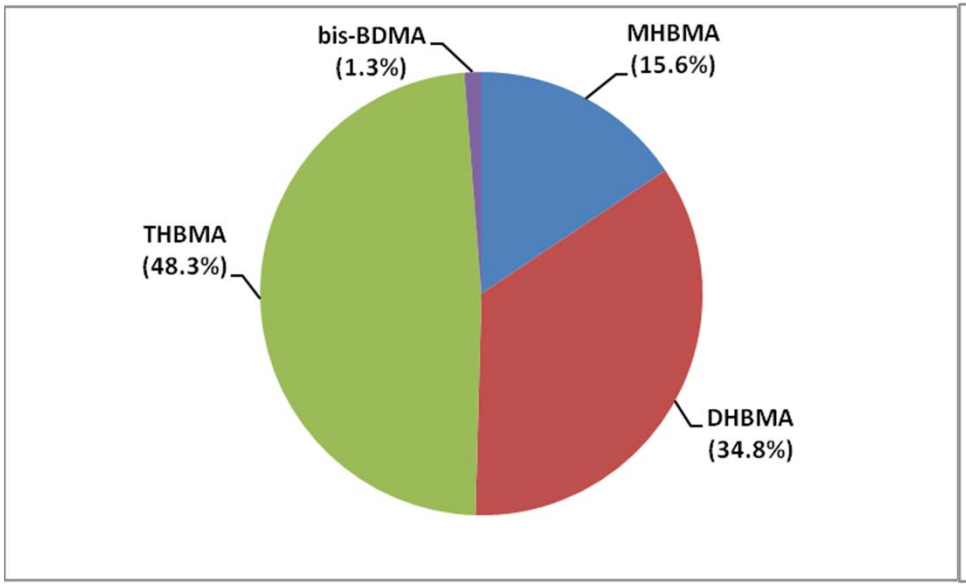
THBMA



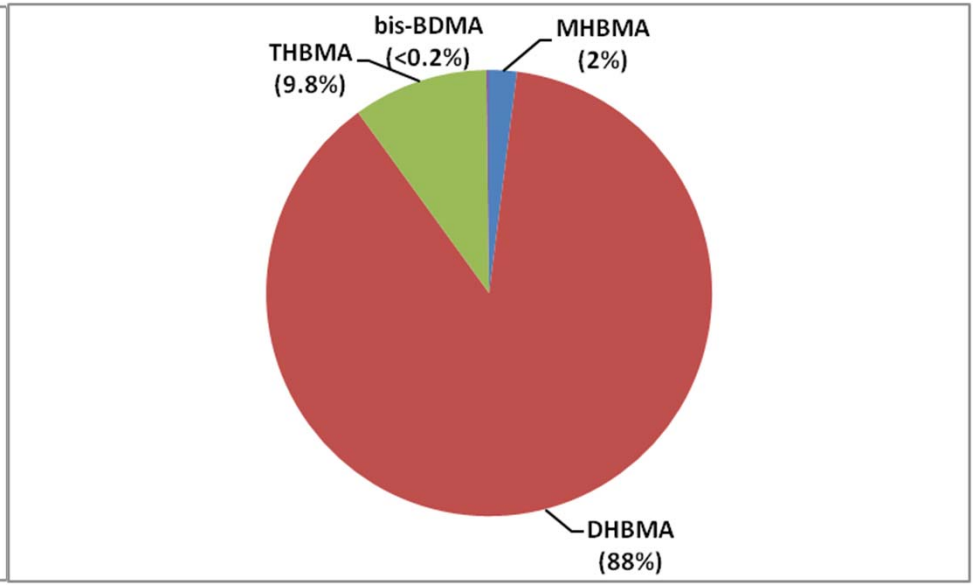
MHBMA



bis-BDMA



Rats (62.5 ppm BD)



Humans (1- 2 ppm BD)

- Humans excrete a lot of DHBMA
- bis-BDMA is undetectable in human urine

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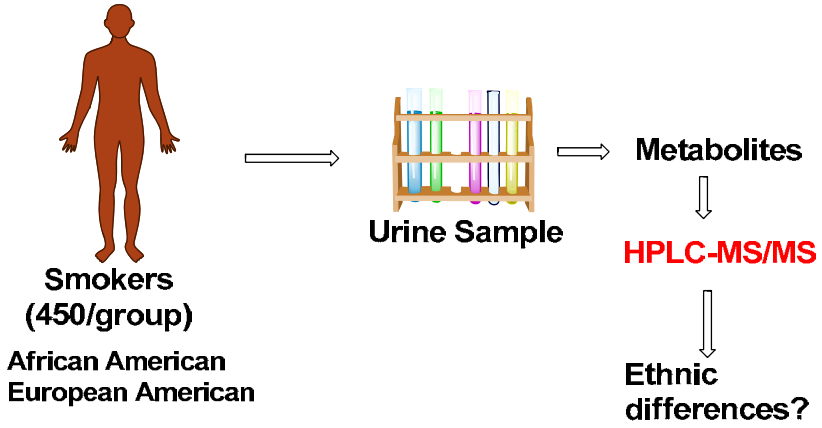
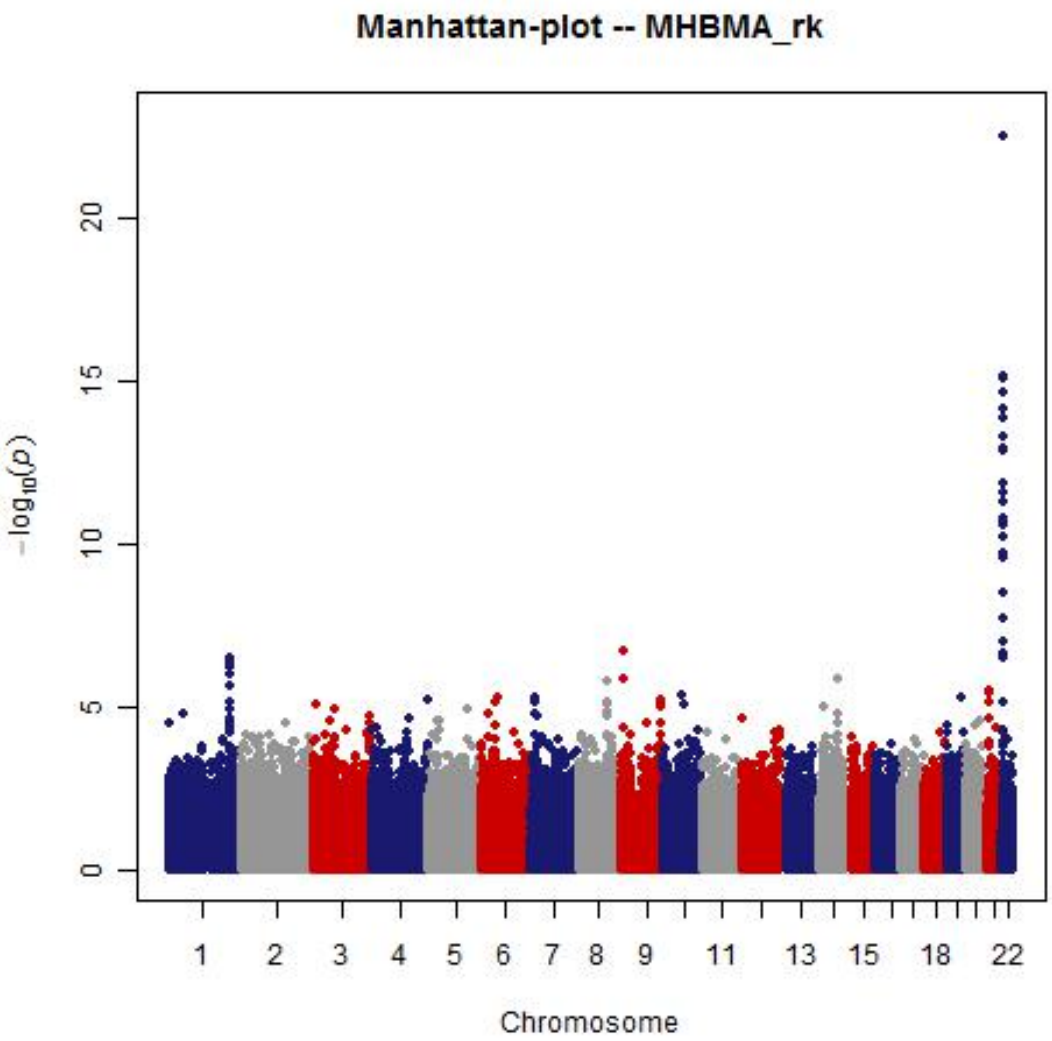
Ethnic differences in excretion of urinary BD-mercapturic acids



MHBMA			
Group	N	Mean ± SD (nmol/mg)	P-value
Afr. Am.	346	10.87 ± 8.3	0.0002
Jap. Am.	380	9.64 ± 19.8	< 0.0001
Eur. Am.	426	14.3 ± 12.6	-

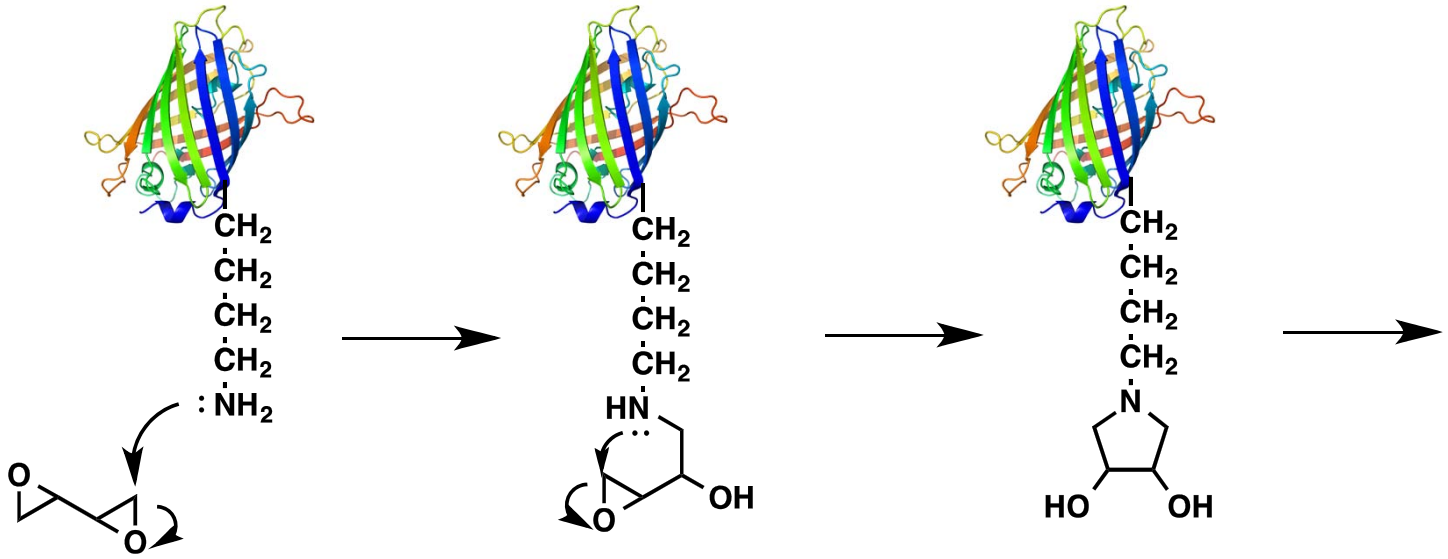
DHBMA			
Group	N	Mean ± SD (nmol/mg)	P-value
Afr. Am.	346	611.85 ± 436.6	0.0142
Jap. Am.	380	806 ± 974.6	0.7425
Eur. Am.	426	725.22 ± 703.5	-

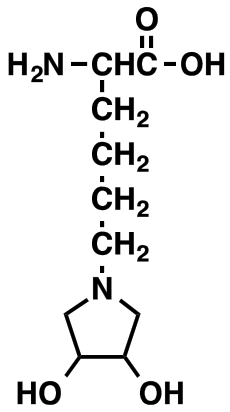
GWAS Shows an Association Between MHBMA Excretion and SNPs on Chromosome 22



19 SNPs with statistical significance on chromosome 22,
mostly glutathione S-transferases
GSTT1 = glutathione S-transferase theta 1
GSTT2 = glutathione S-transferase theta 2

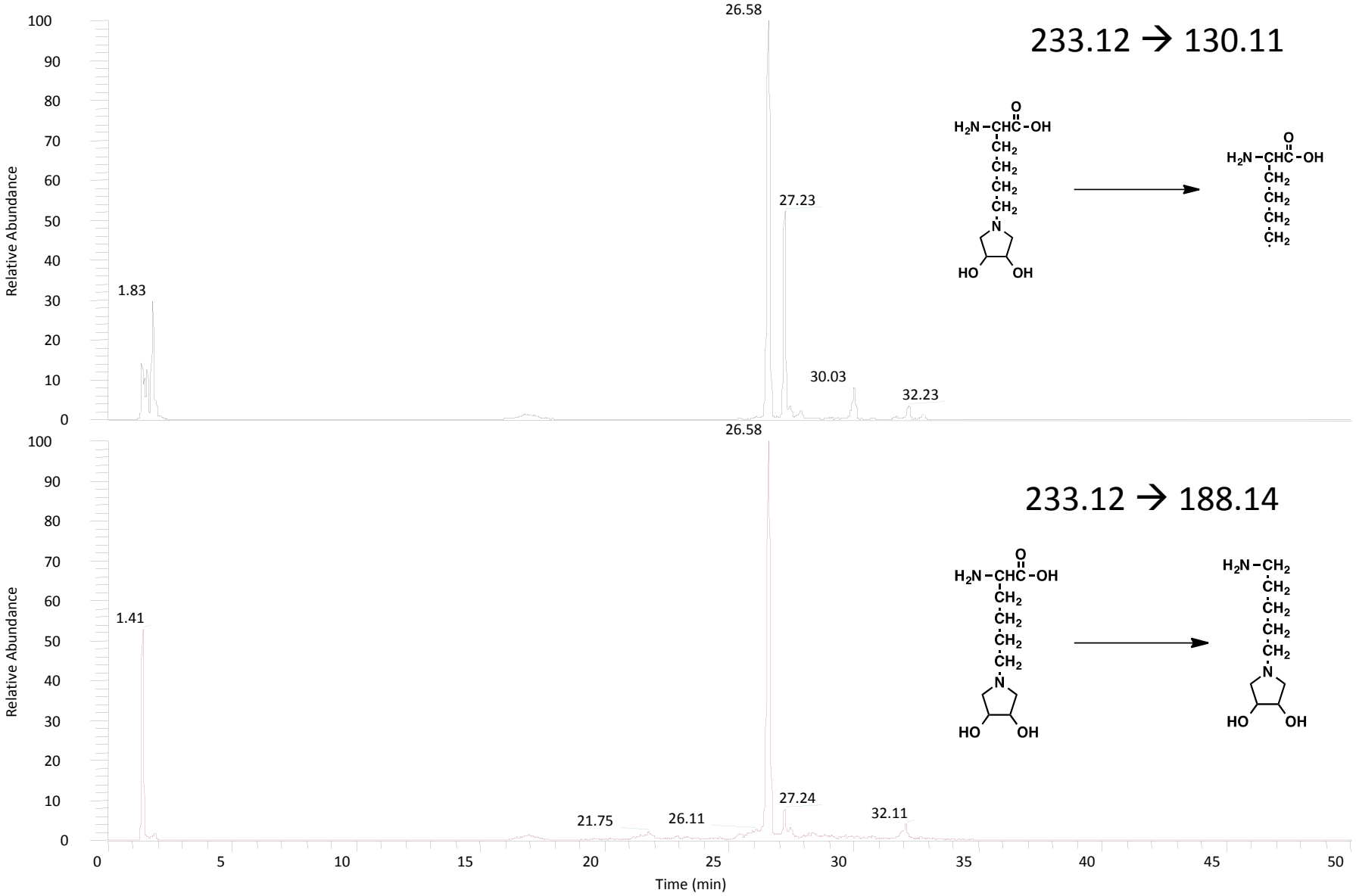
DHB-Lys: a Novel DEB-Specific Urinary Biomarker



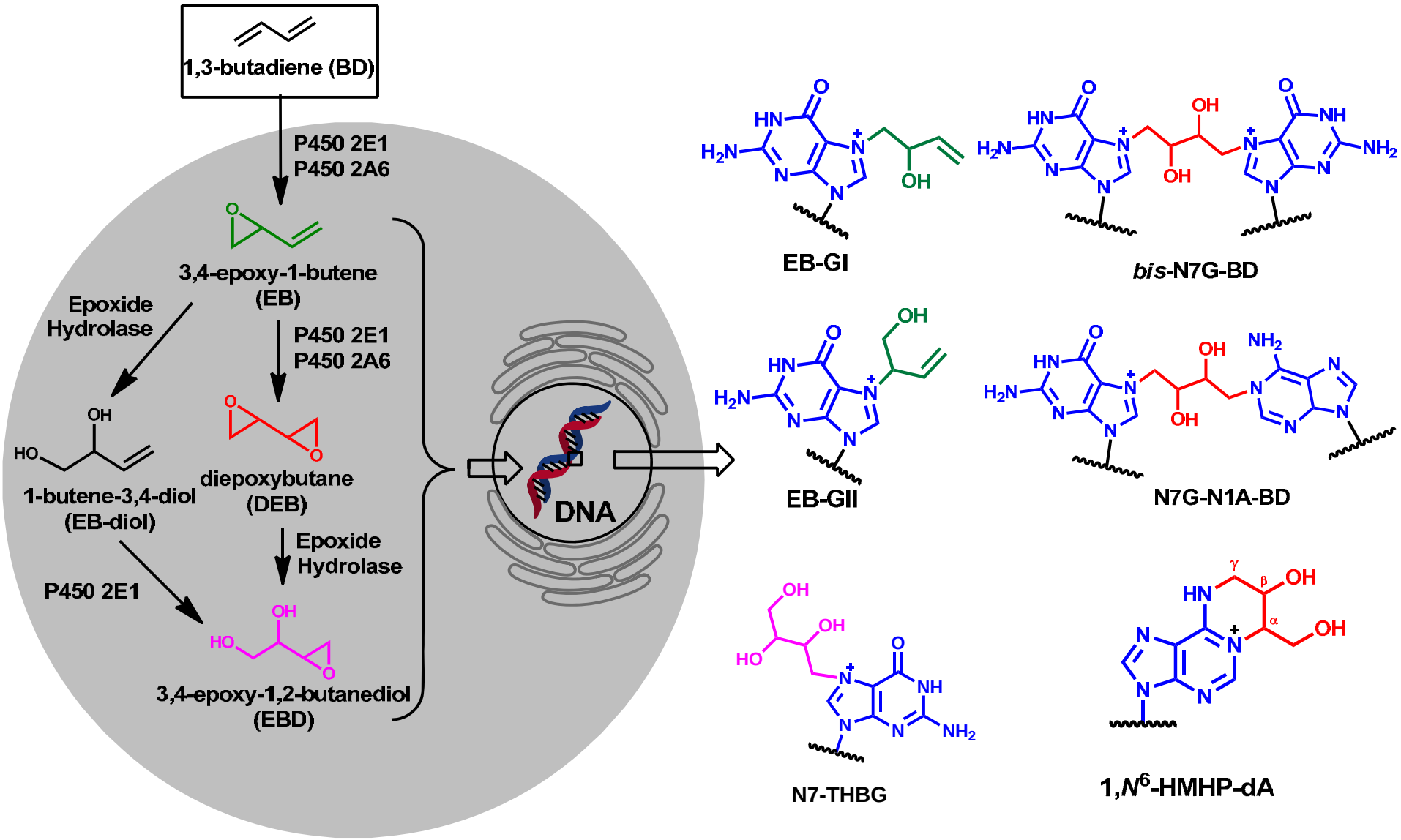


N^6,N^6-(2,3-dihydroxybutan-1,4-diyl)-lysine
(DHB-Lys)

Detection of DHB-Lys in Smoker's Urine



1,3-Butadiene- Induced DNA adducts: Biomarkers of risk

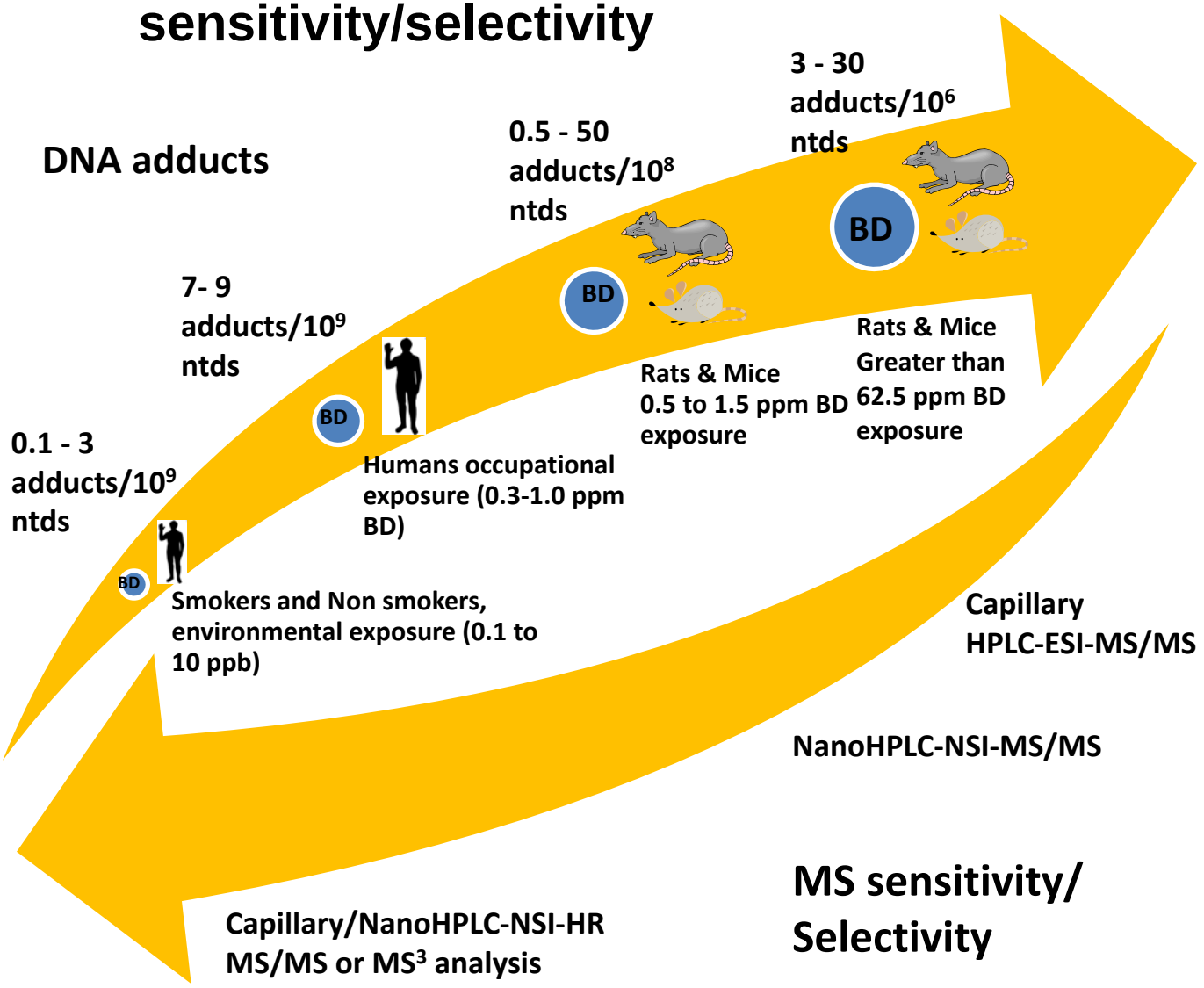


Exp Pathol. 1989;37(1-4):108-13; Atmospheric Environment 2006 40, 170–181;Chem Biol Interact 2007 166(1-3):44-51;
Chem. Res. Toxicol. 2007, 20, 839-847; Chem. Res. Toxicol. 2008, 21, 1163–1170; Chem. Res. Toxicol. 2010, 23, 808–812;
Chem. Res. Toxicol. 2013, 26, 1486−1497.

Studies of BD DNA adducts *in vivo*: Demands for increased MS sensitivity/selectivity

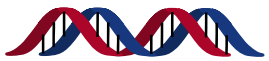
Analytical challenges:

- Limited DNA sample
- Artifacts and contamination
- Accurate quantitation is required to detect small differences between groups
- Endogenous exposome



Tretyakova et al. Chem Res Toxicol. 2012;25(10):2007-35

Sample preparation procedure for the quantitation of N7-guanine DNA adducts



DNA in solution

Blood WBCs/buffycoat,various tissue cells

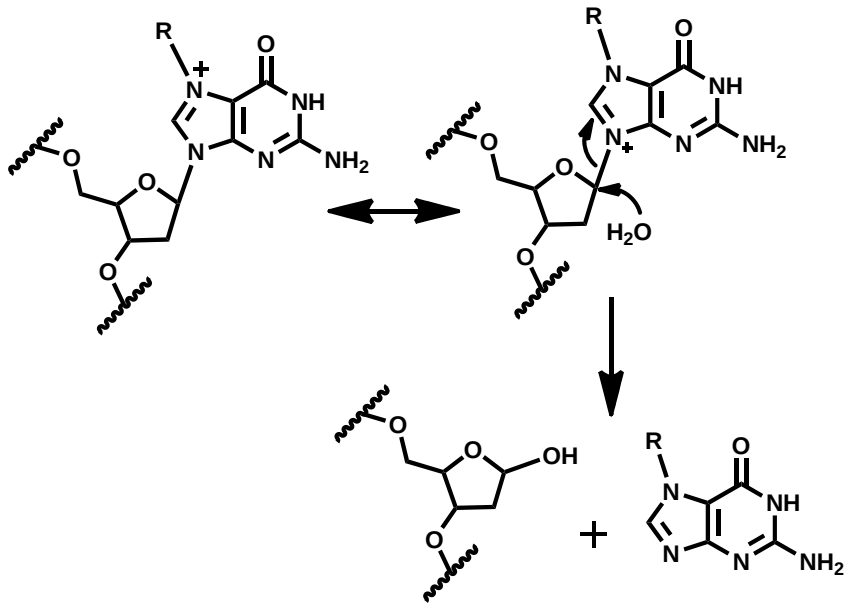
Stable isotope labeled internal standard

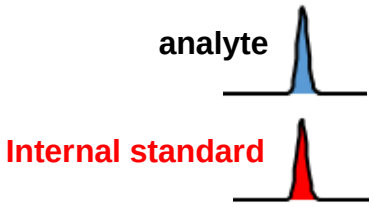
Neutral Thermal Hydrolysis and Ultra-filtration

DNA adduct enrichment

Solid phase Extraction (SPE) and/or Offline HPLC fractionation

Isotope dilution MS analysis



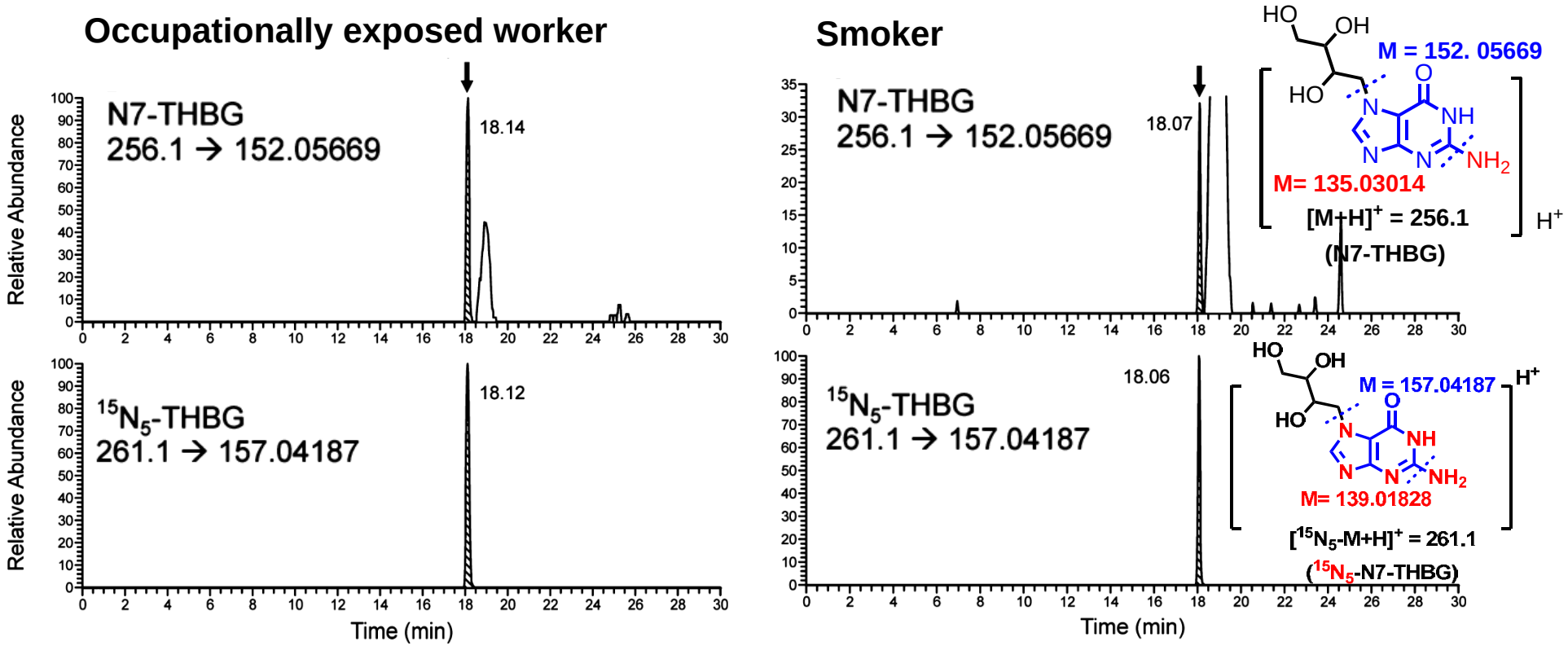


$$\text{Amount}_{\text{analyte}} = \frac{\text{Area}_{\text{analyte}}}{\text{Area}_{\text{Internal Standard}}} \times \text{Amount}_{\text{Internal Standard}}$$

Tretyakova et al. Chem Res Toxicol. 2012;25(10):2007-35

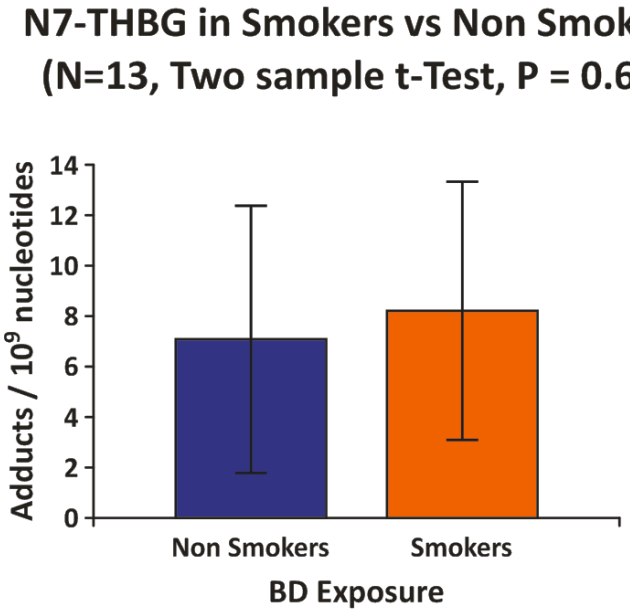
HPLC-ESI$^+$-HRMS/MS analysis of N7-THBG in leukocyte DNA of a smoker and an occupationally BD exposed worker

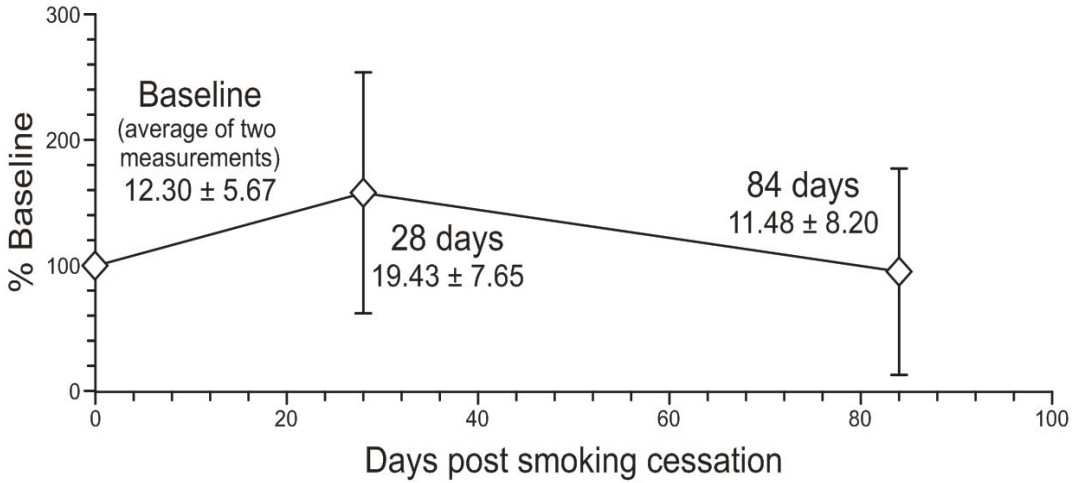
Sample matrix	LOQ	Range	Accuracy	Intra/interday precision	DEB treated HT1080 cells (1-100 µM)
Control DNA from HT1080 cells	1.0 fmol/150 µg of DNA (2 adducts/10^9 nucleotides)	1.0 - 50 fmol (Y =1.059 X, R^2= 0.9989)	92.3 ± 6.7 (N = 5)	%CV < 13%	y = 9.4252x + 2.4797 R^2 = 1 **(Endogenous levels: 2.03 fmol/150 µg DNA)**

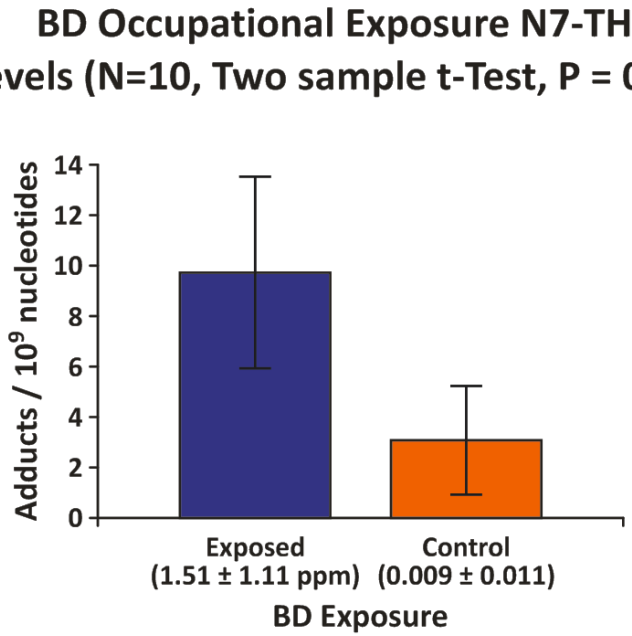


Sangaraju, D et al. Chem Res Toxicol. 2013 Oct 21;26(10):1486-97

N7-THBG concentrations in leukocyte DNA of BD exposed humans

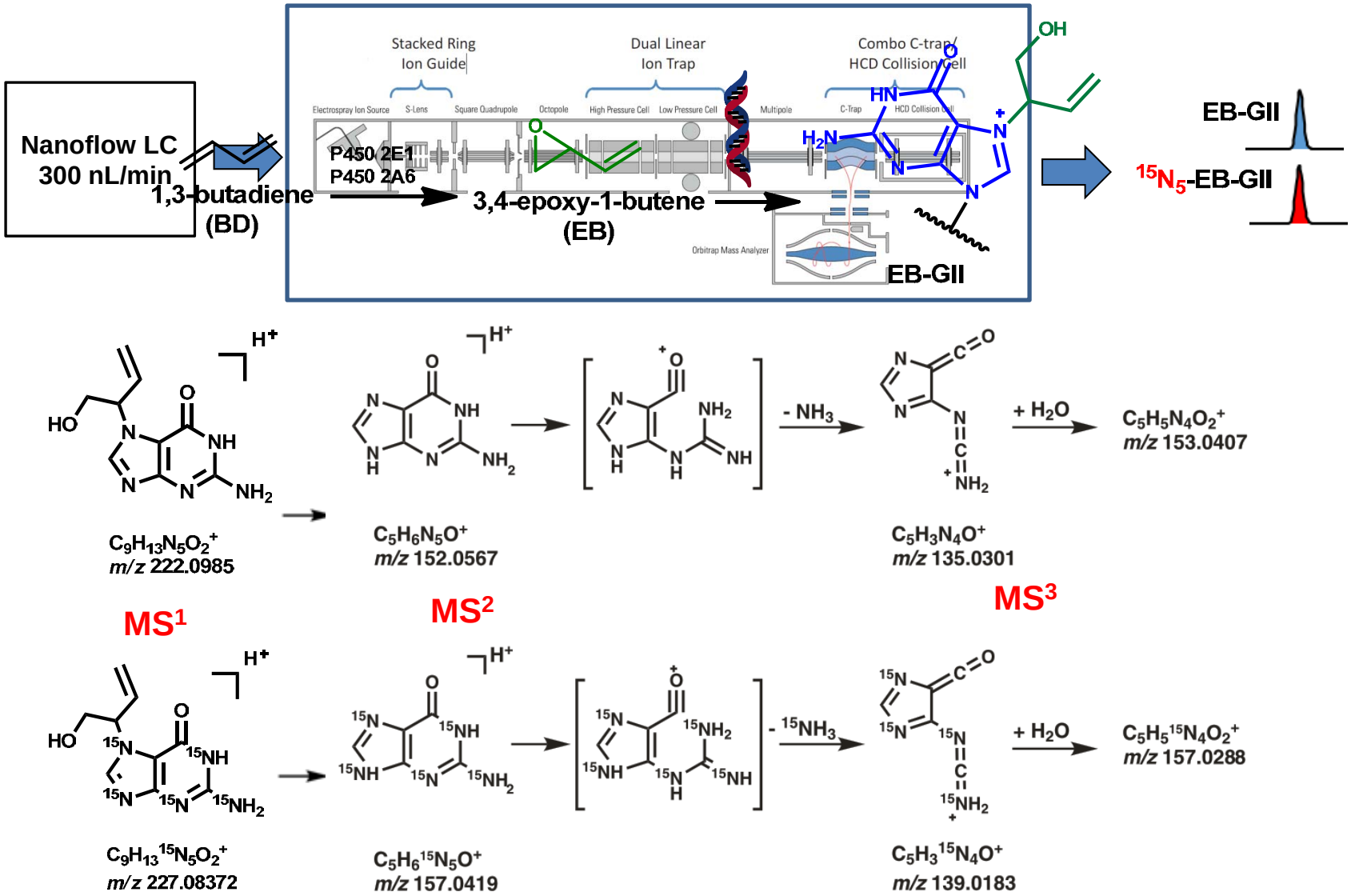






Sangaraju, D et al. Chem Res Toxicol. 2013 Oct 21;26(10):1486-97

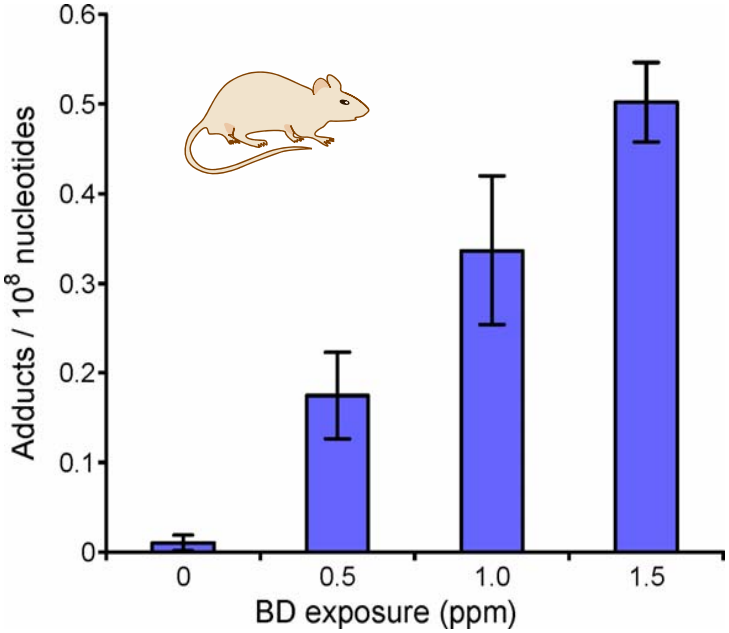
Isotope dilution NanoLC/ESI$^+$-HRMS^3 analysis of EB-GII adducts



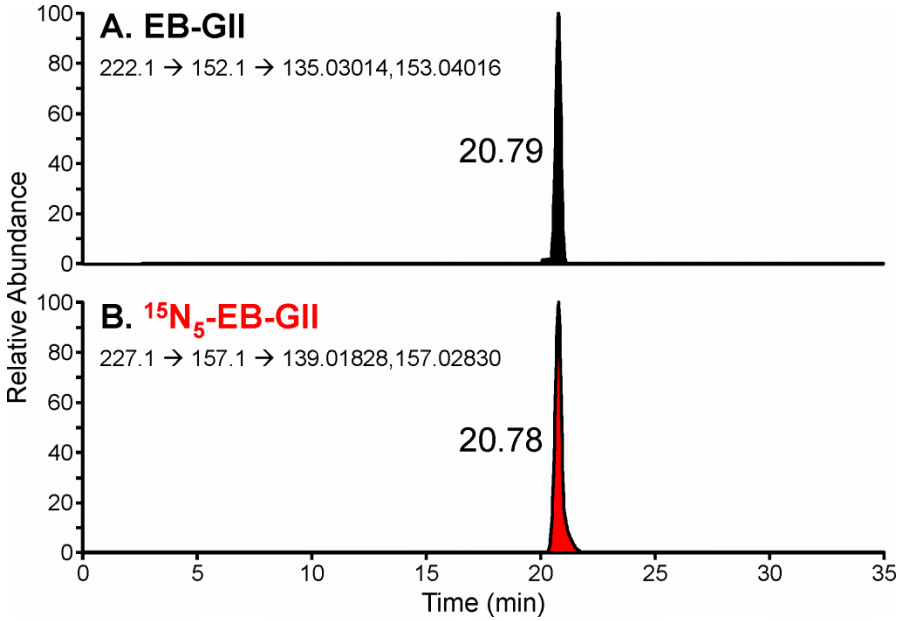
Sangaraju, D et al. J Am Soc Mass Spectrom. 2014 Jul;25(7):1124-35.

EB-GII quantitation in liver of BD-exposed rats

Sample matrix	LOD	LOQ	Range	Accuracy	Intra/interday precision
Nonsmoker Blood DNA	0.02 fmol/150 µg DNA	0.2 fmol/150 µg of DNA (0.4 adducts/10^9 nucleotides)	0.2 - 10 fmol (Y = 0.905 X, R^2 = 0.995)	92.9 ± 7.1 (N=9)	%CV < 8%



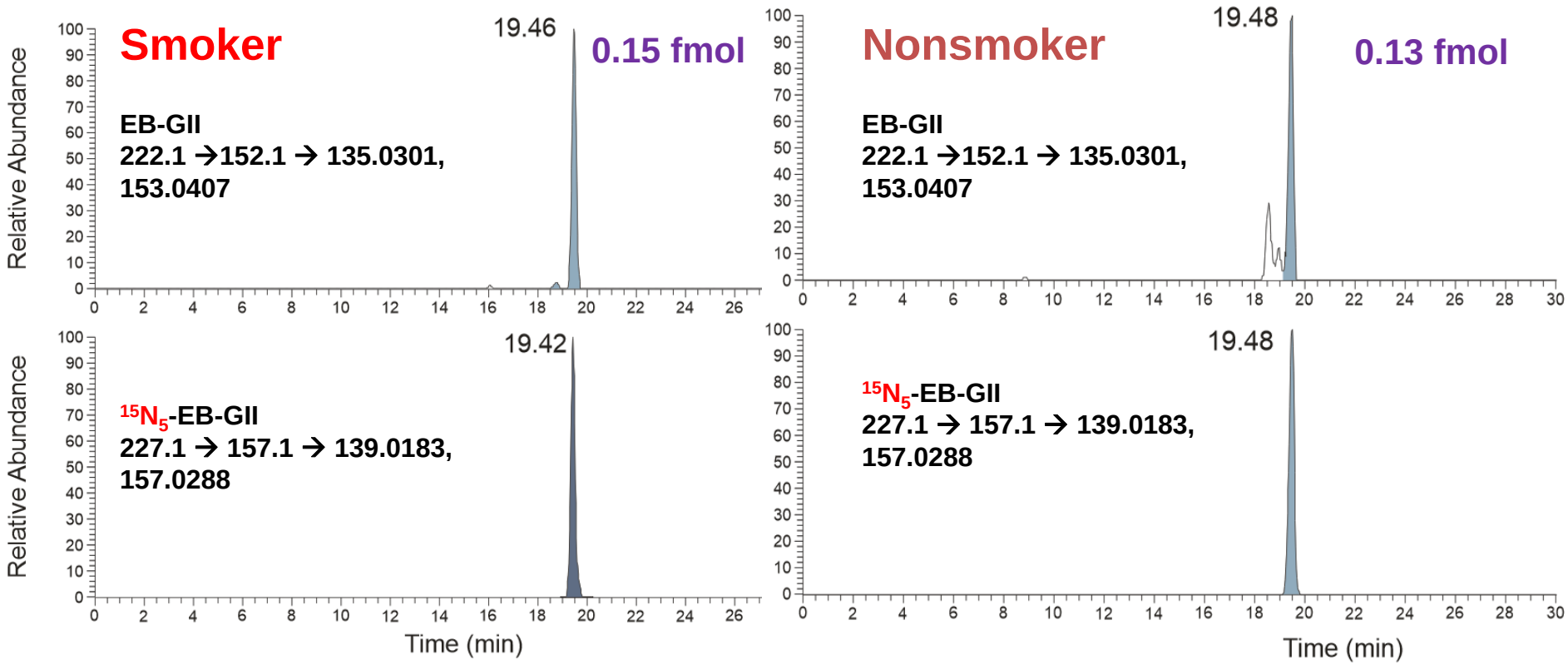
EB-GII formation in liver tissue DNA of F344 rats exposed to BD (0.5, 1, 1.5 ppm) for 2 weeks (5 days per week)



EB-GII adducts in liver DNA of a F344 rat exposed to 1.0 ppm BD by inhalation for 2 weeks

Sangaraju, D et al. J Am Soc Mass Spectrom. 2014 Jul;25(7):1124-35.

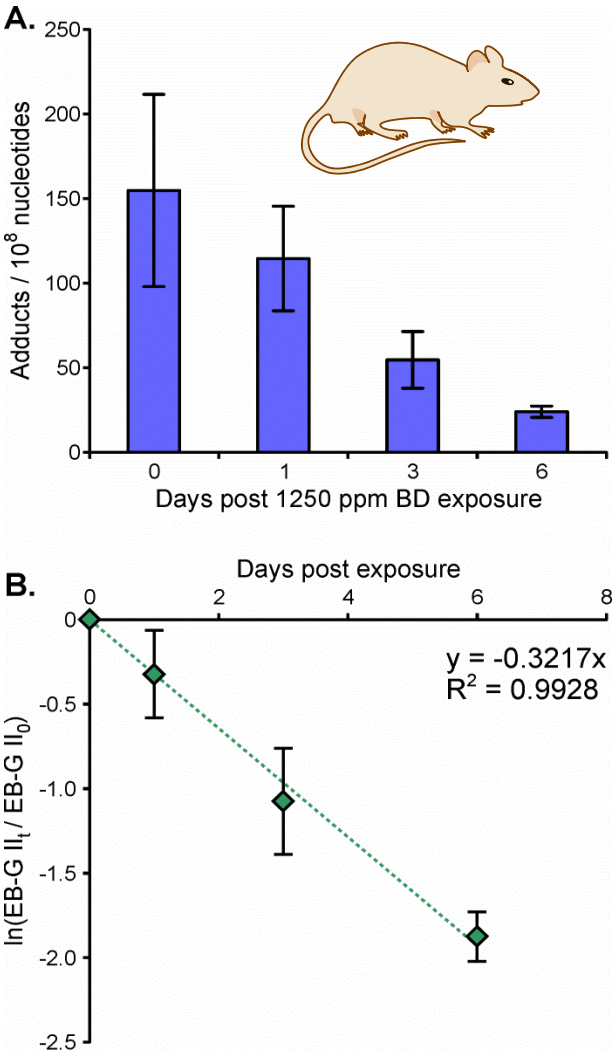
Attempted quantitation of EB-GII in blood leukocyte DNA of smokers and nonsmokers

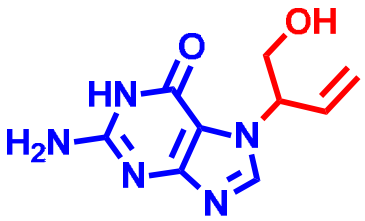


EB-GII levels are either equal or below the Limit of quantitation of the method (0.2 fmol in 150µg of DNA)!

Sangaraju, D et al. J Am Soc Mass Spectrom. 2014 Jul;25(7):1124-35.

EB-GII *in vivo* half life in liver tissue DNA of F344 rats exposed to 1250 ppm BD





EB-GII

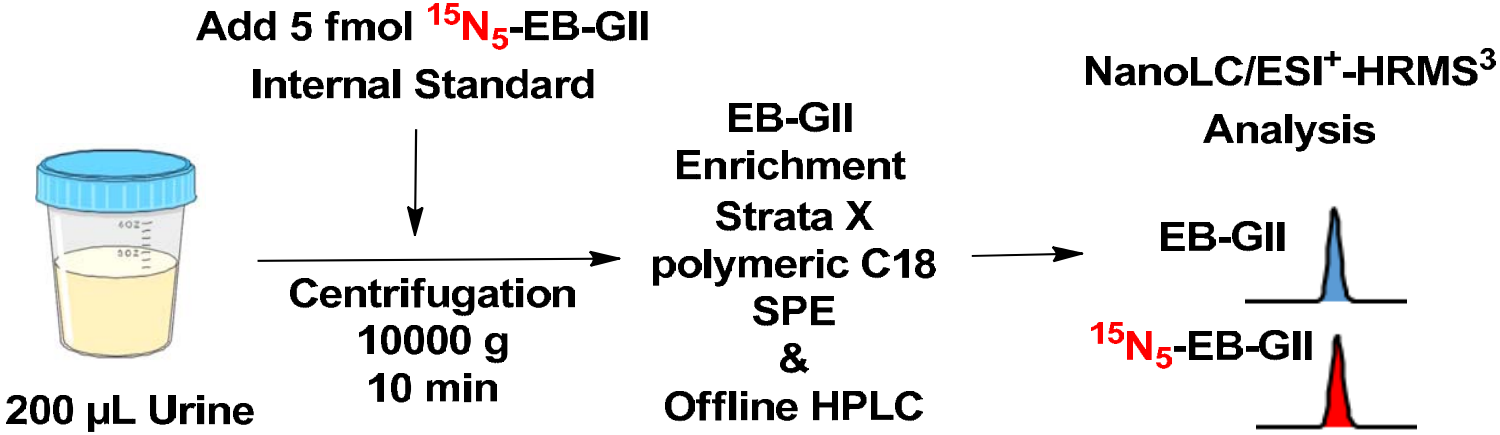
$t_{½}$= 2.20 ± 0.12 days

Hydrolysis/DNA adduct repair

Urinary excretion!

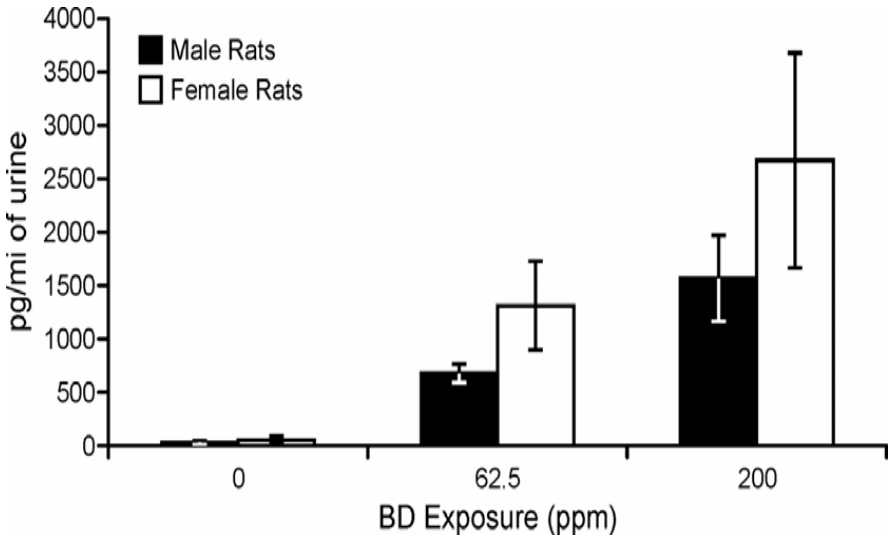
Sangaraju, D et al. J Am Soc Mass Spectrom. 2014 Jul;25(7):1124-35.

Quantitation of urinary EB-GII adducts

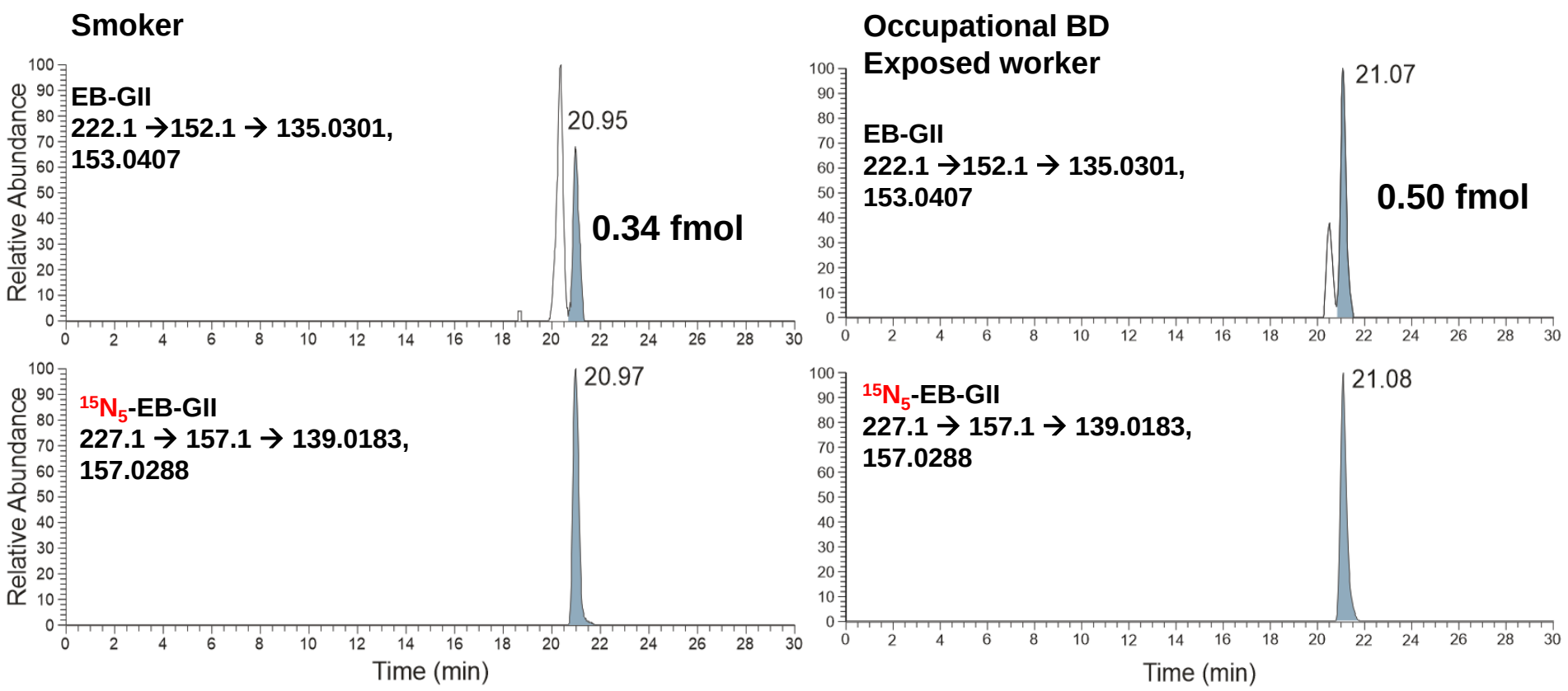


Method validation results	
Sample matrix	Nonsmoker urine (200 µL)
LOD	0.05 fmol
LOQ	0.1 fmol
Range	0.1 - 10 fmol (Y = 1.0042 X, R^2= 0.999)
Accuracy	109.8 ± 10.4 (N=5)
Intra/interday precision	%CV < 13%

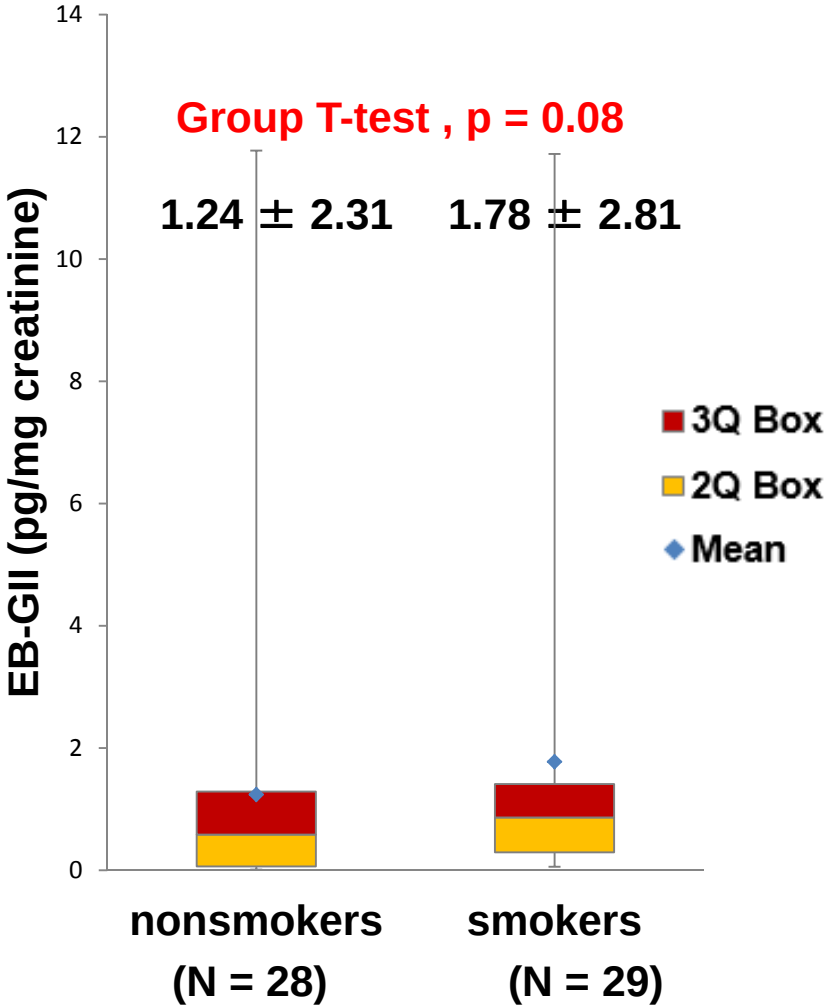
EBG-II urinary levels in male and female F344 rats exposed to 0, 62.5 and 200 ppm

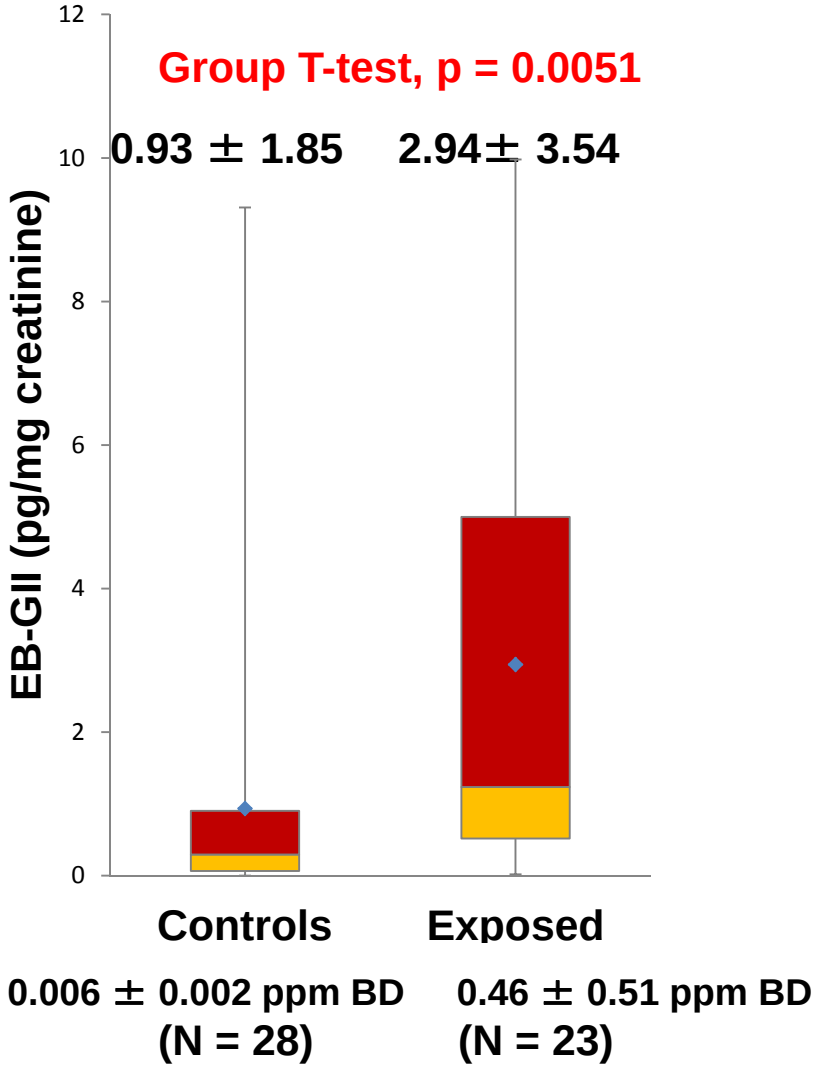


NanoLC/ESI^{+}-HRMS^{3} analysis of EB-GII in urine of smokers and occupationally exposed workers

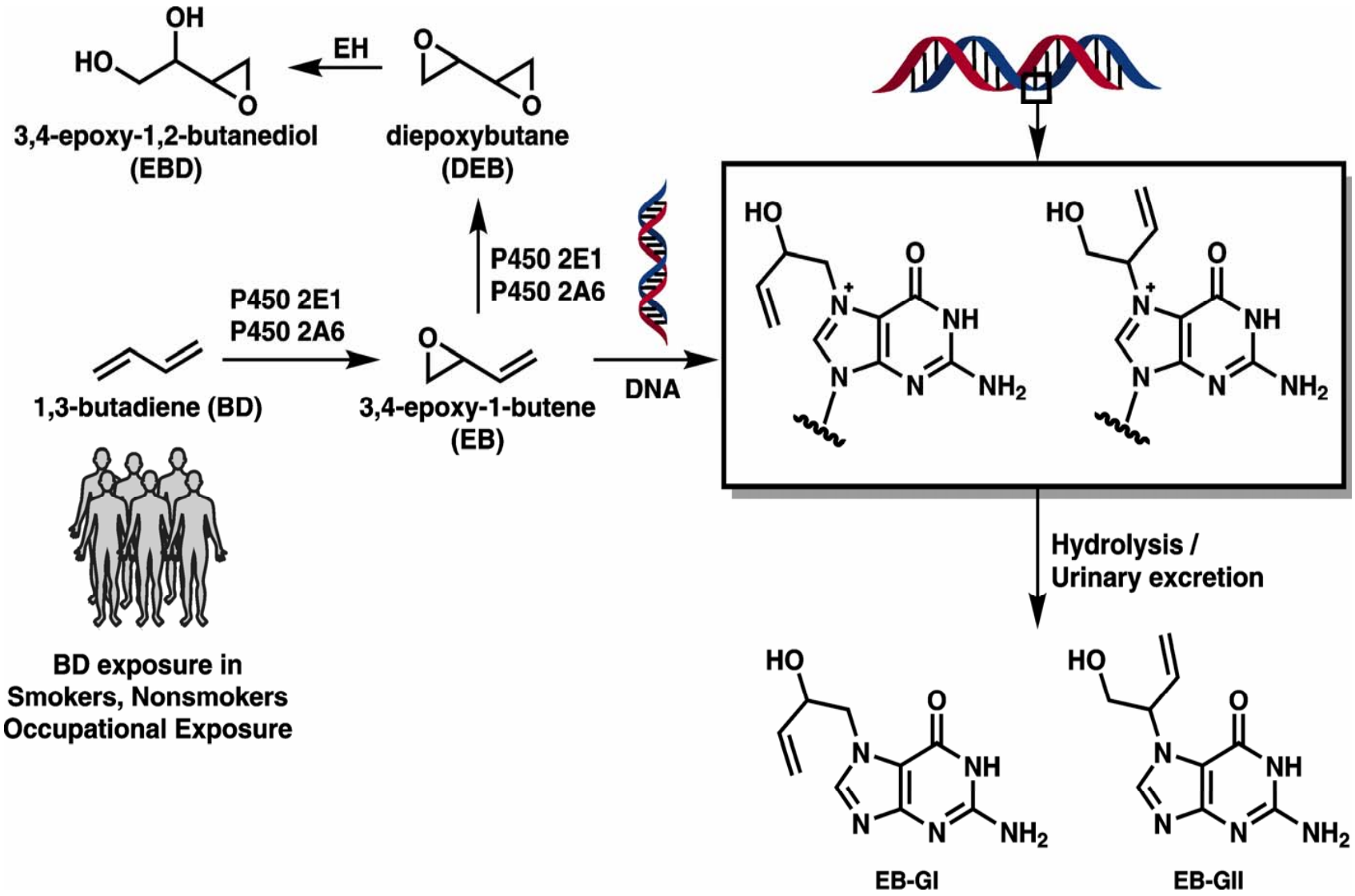


Urinary EB-GII concentrations in occupational BD exposed workers vs administrative controls, nonsmokers vs smokers





Ethnic/racial differences in the formation of butadiene-DNA adducts upon exposure to BD



Racial/ethnic differences in urinary EB-GII excretion

Hypothesis:

Genetic polymorphisms

↓

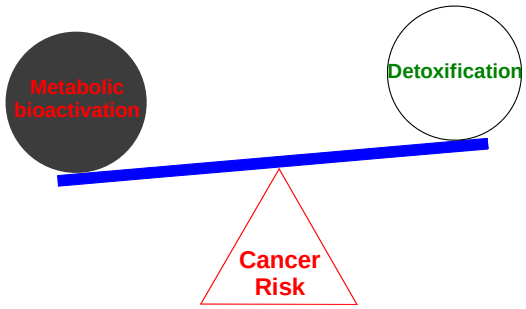
Increased bioactivation

↓

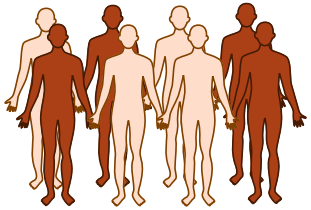
Higher levels of DNA adducts

↓

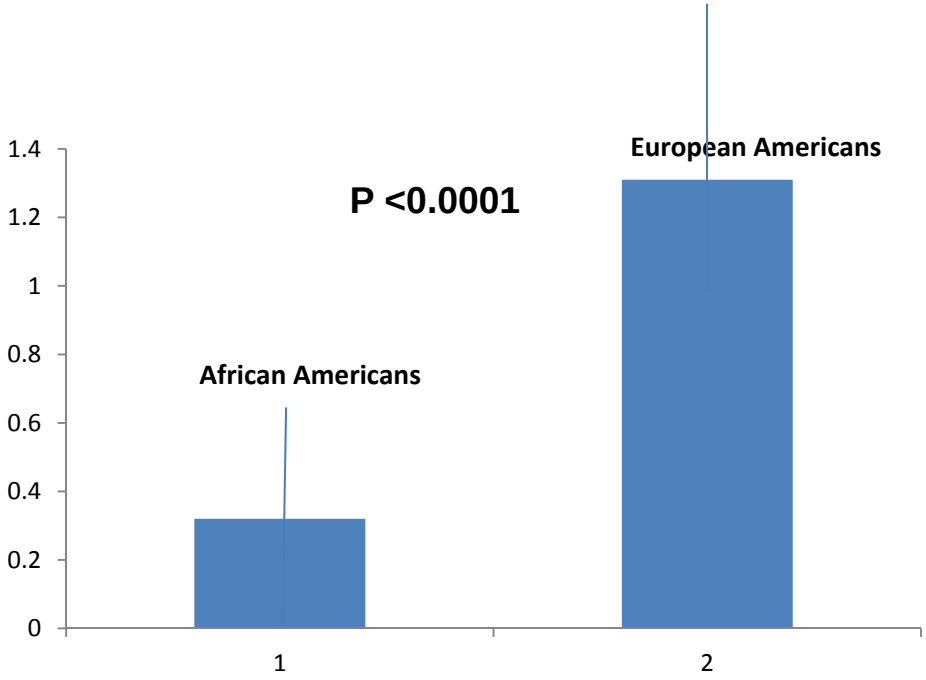
Higher cancer risk



Smokers



African American
European American
(80/group)



Genotyping in progress to identify the origins of ethnic differences

Haiman, C. A. *et. al. N. Engl. J. Med*. (2006), 354, 333-342.

Conclusions

1. Several new biomarkers of exposure to BD have been developed and applied to smokers, occupationally exposed workers, and controls.
2. Urinary BD-mercapturic acids and BD-DNA adducts are associated with exposure.
3. Interspecies and ethnic/interindividual differences in BD metabolism have been revealed.
4. “Unexposed” individuals contain significant numbers of BD-DNA adducts and excrete BD-mercapturic acids.

Future Directions

1. **Evaluate DHB-Lys as a novel DEB-specific urinary biomarker.**

2. **Identify the origins of endogenous THBMA, DHBMA, THB-Gua, and EB-Gua.**

3. **Quantify DEB-derived metabolites and DNA adducts in rats, mice, and humans exposed to sub-ppm concentrations of BD.**

Acknowledgements

Students: Melissa Goggin, Srikanth Kotapati, Dewakar Sangaraju, Emily Boldry

Animal Tissues: James A. Swenberg (UNC), Vernon Walker (Univ. of Vermont)

Human urine and blood samples: Univ. of Minnesota Biorepository, American Chemistry Council Olefins Panel and Richard J. Albertini (University of Vermont)

Special thanks to: Kimberly Wise (American Chemistry Council Olefins Panel) Richard J. Albertini (University of Vermont)

Funding:
American Chemistry Council, NIH P01 CA-138338, R01-CA-100670