Tracing the fate of mercury from vaccines in organisms: methods for the determination of ethylmercury, methylmercury, and total mercury in biological tissues

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Goal of the Study

Develop a procedure for the determination of MeHg, EtHg and Inorganic Hg in a tissue sample that yields acceptable recovery of SRMs and matrix spikes and yields reproducible results.

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Thimerosal

- Vaccine preservative
- 50% (*m/m*) EtHg
- Been in use since 1930s
- In 1999, FDA recommended discontinuing use
- 2013 AAP recommends continuing the use

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MeHg Analysis

- EPA 1630
- Used Brooks Rand Instruments MERX-M autoanalyzer

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Sodium Tetraethylborate

"Ethylation" with $NaBEt_4$

- Hg^0 → Hg^0 (no change-already volatile)
- CH_3-Hg^+ → CH_3-Hg -CH_2-CH_3 (methylethyl mercury)
- Hg^{2+} → CH_3 -CH_2- Hg -CH_2-CH_3 (diethyl mercury)

Why $NaBEt_4$ <u>can't</u> be used to determine EtHg

- CH_3 -CH_2- Hg^+ → CH_3 -CH_2- Hg -CH_2-CH_3 (diethyl mercury)

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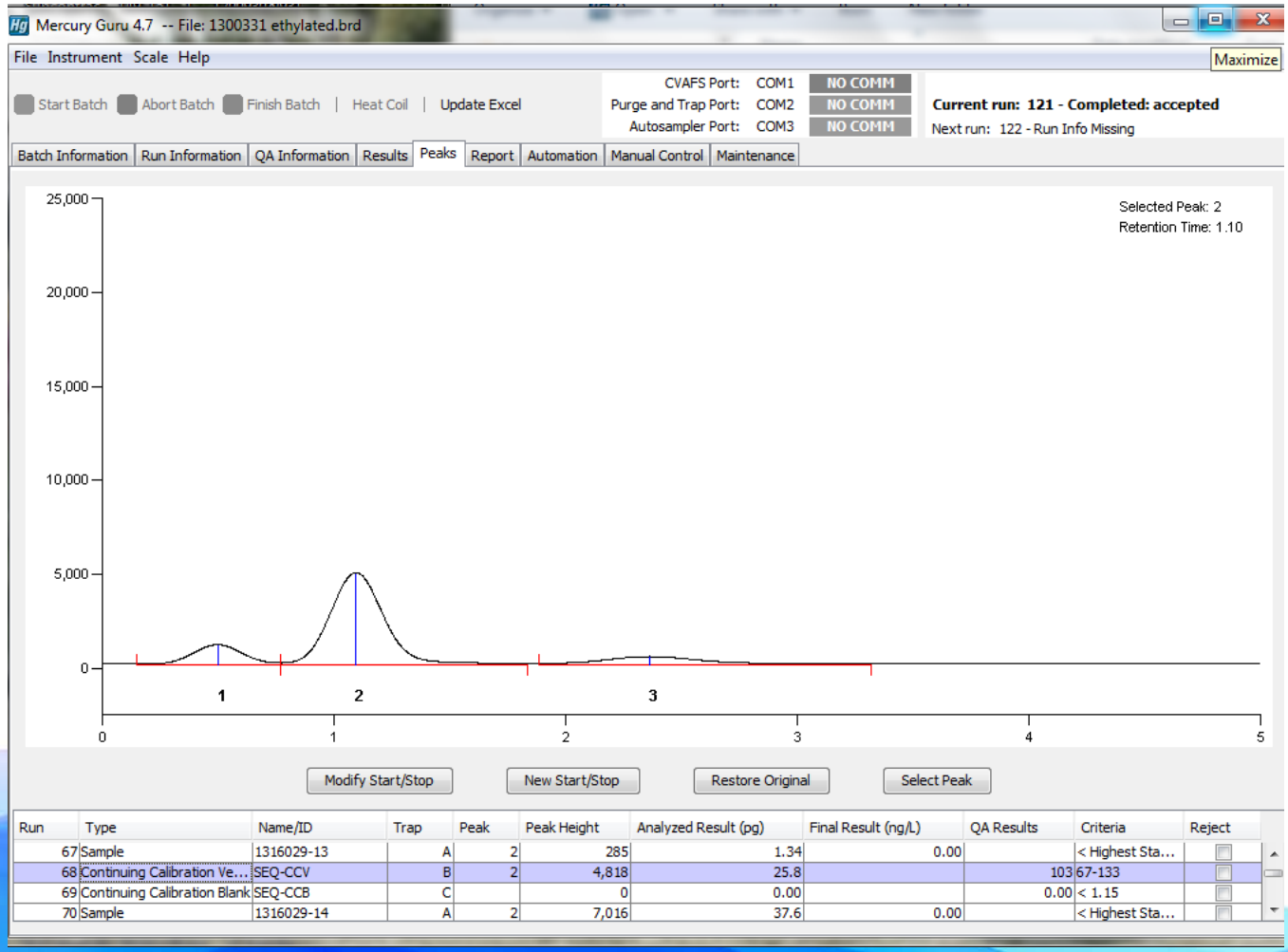
Chromatogram



Run	Type	Name/ID	Trap	Peak	Peak Height	Analyzed Result (pg)	Final Result (ng/L)	QA Results	Criteria	Reject
67	Sample	1316029-13	A	2	285	1.34	0.00		< Highest Sta...	
68	Continuing Calibration Ve...	SEQ-CCV	B	2	4,818	25.8		103	67-133	
69	Continuing Calibration Blank	SEQ-CCB	C		0	0.00		0.00	< 1.15	
70	Sample	1316029-14	A	2	7,016	37.6	0.00		< Highest Sta...	

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Sodium Tetra(n-propyl)borate

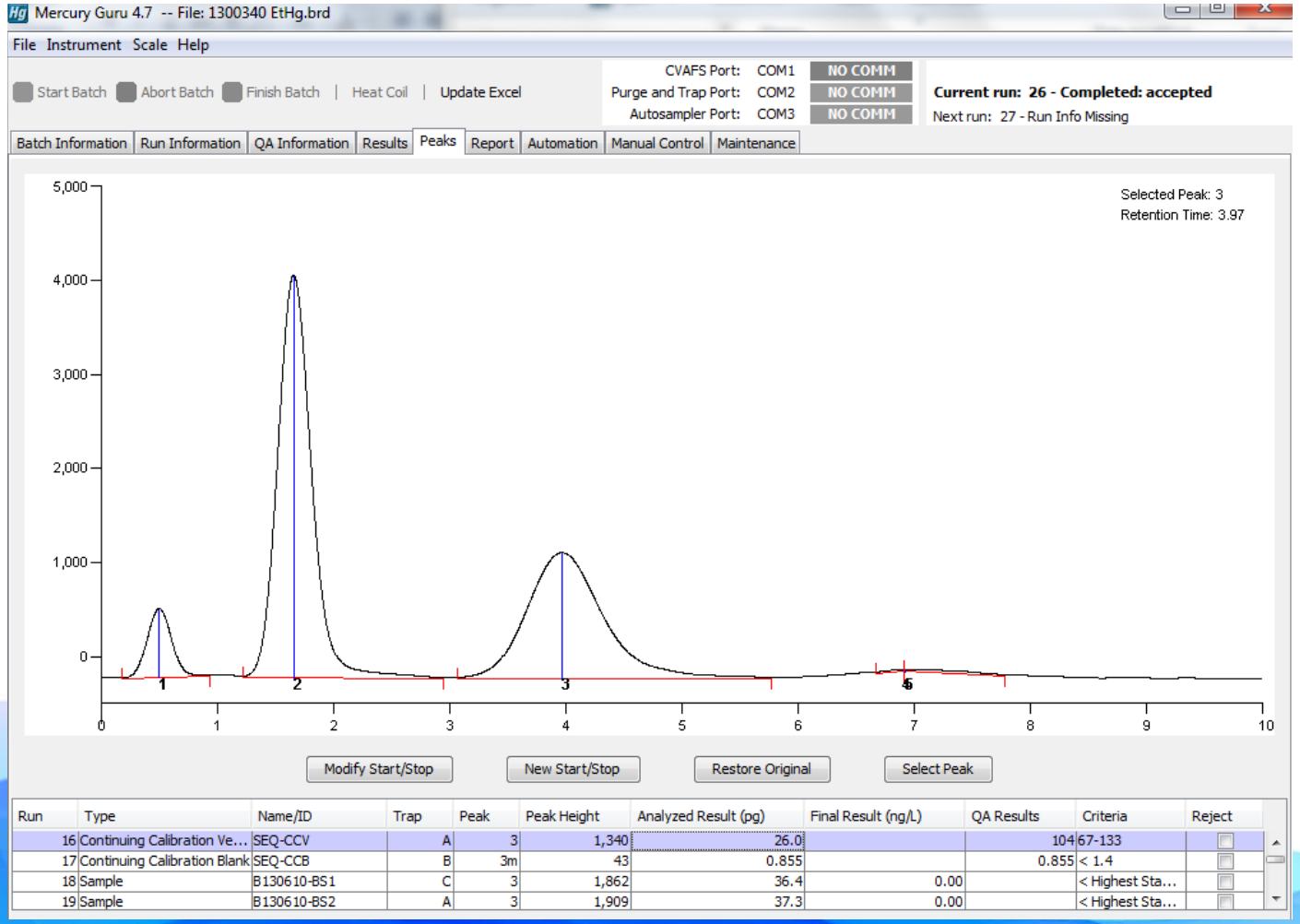
"Propylation" with $NaBPr_4$

- $Hg^0 \rightarrow Hg^0$ (no change-already volatile)
- $CH_3\text{-}Hg^+ \rightarrow CH_3\text{-}Hg\text{-}CH_2\text{-}CH_2\text{-}CH_3$ (methylpropyl mercury)
- $Hg^{2+} \rightarrow CH_3\text{-}CH_2\text{-}CH_2\text{-}Hg\text{-}CH_2\text{-}CH_2\text{-}CH_3$ (dipropyl mercury)

Why $NaBPr_4$ <u>can</u> be used to determine EtHg

- $CH_3\text{-}CH_2\text{-}Hg^+ \rightarrow CH_3\text{-}CH_2\text{-}Hg\text{-}CH_2\text{-}CH_2\text{-}CH_3$ (ethylpropyl mercury)

Chromatogram



Run	Type	Name/ID	Trap	Peak	Peak Height	Analyzed Result (pg)	Final Result (ng/L)	QA Results	Criteria	Reject
16	Continuing Calibration Ve...	SEQ-CCV	A	3	1,340	26.0		104	67-133	
17	Continuing Calibration Blank	SEQ-CCB	B	3m	43	0.855		0.855	< 1.4	
18	Sample	B130610-BS1	C	3	1,862	36.4	0.00		< Highest Sta...	
19	Sample	B130610-BS2	A	3	1,909	37.3	0.00		< Highest Sta...	

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Propylation Reagent

- Puchased from ABCR
- Solution of 1g $NaBPr_4$, 2 g KOH, 97 mL DIW
- Aliquoted into 40mL amber glass vials (~10 mL/vial)
- Stored frozen at -20°C

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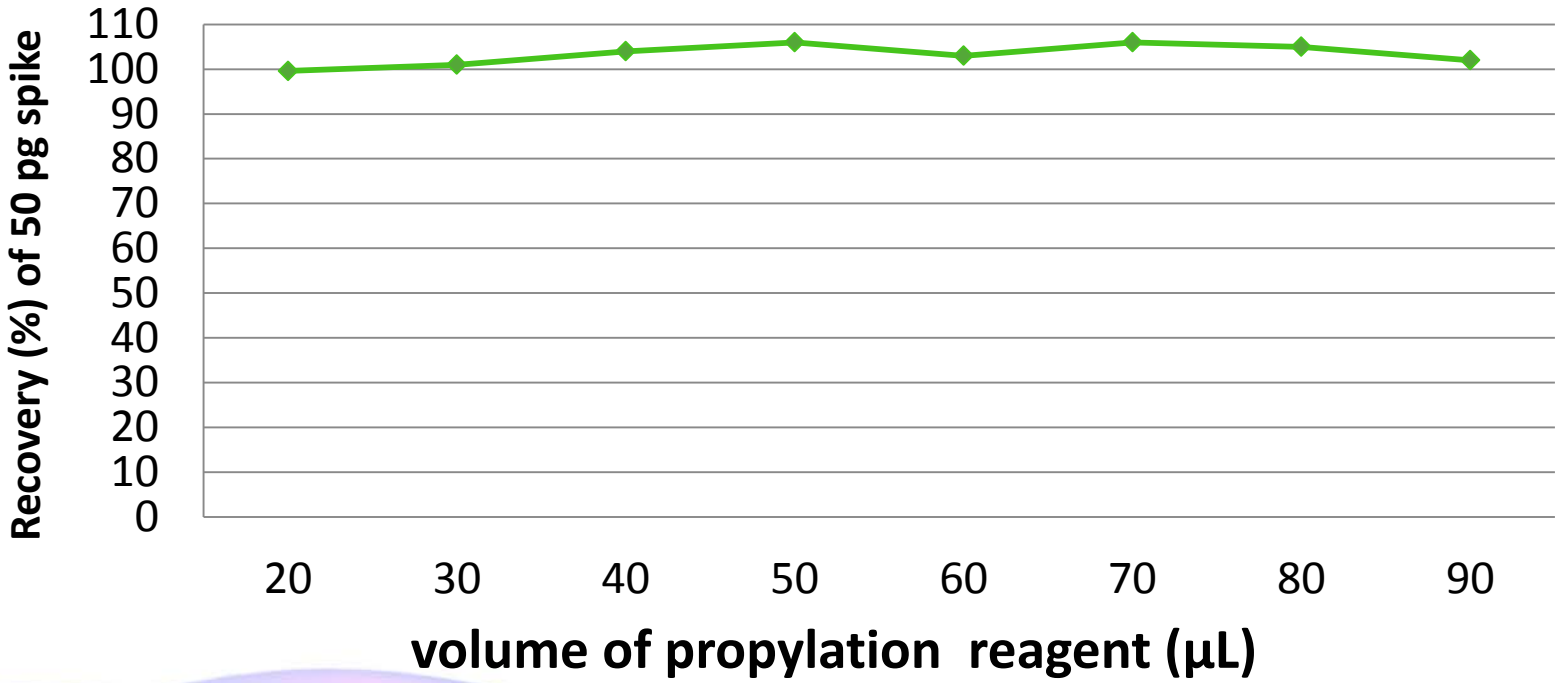
Volume to Use



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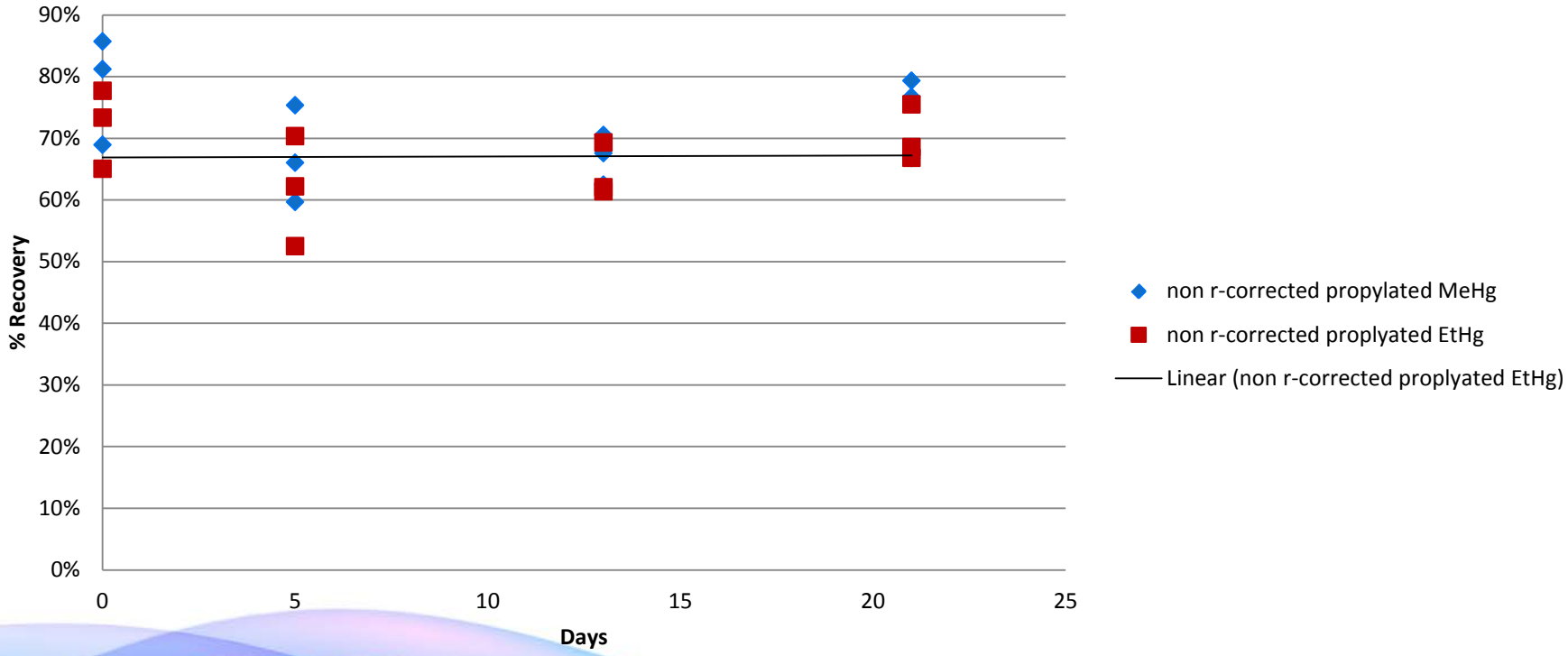
Propylation Reagent Holding Time



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Correction Factor

- EPA Method 1630 section 12.4.2

  F=100/R

  F=Empirically derived correction factor
  R=Recovery (the running mean of the last 30 IPR and OPR samples)

- Propylation is less efficient than ethylation
  - 1.15 (ethylated) and 1.39 (propylated) for MeHg
  - 1.49 for EtHg

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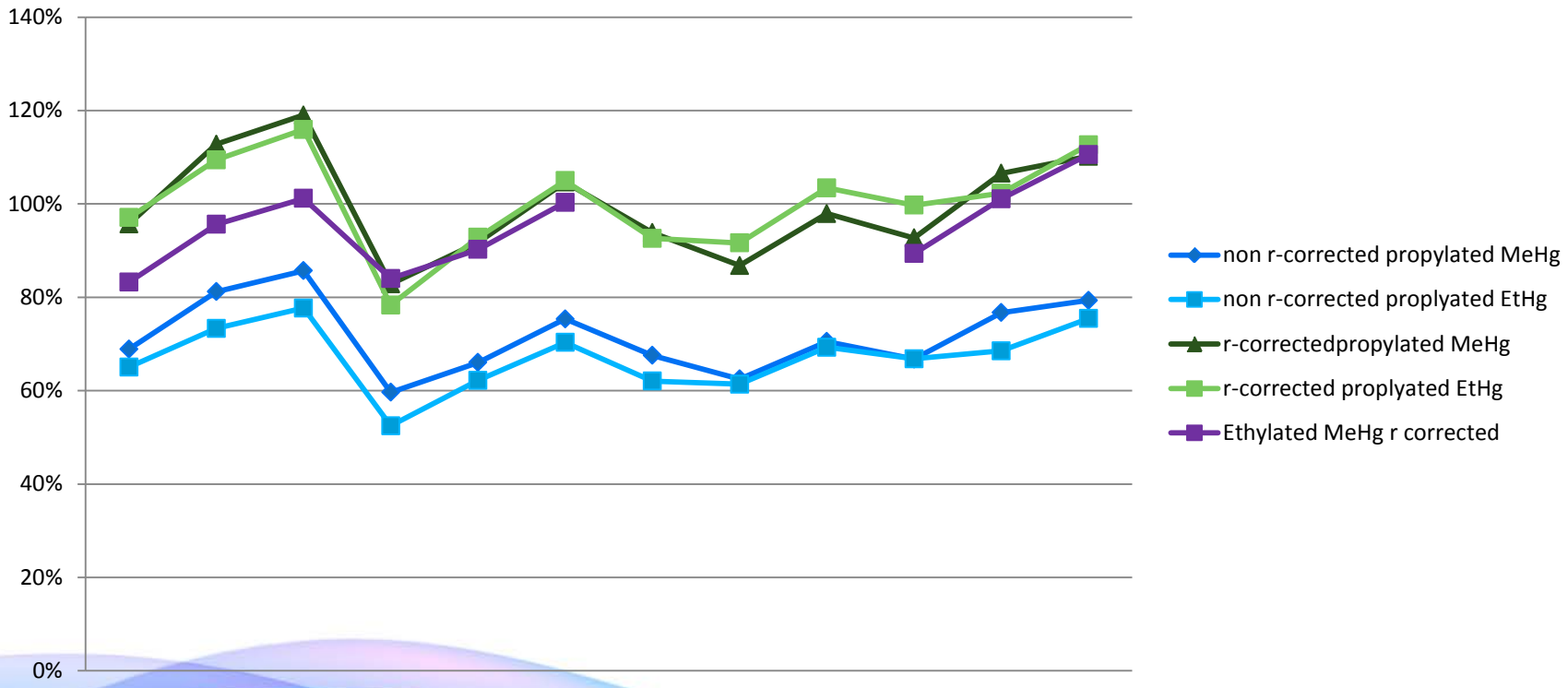
Propylation Reagent Holding Time Study



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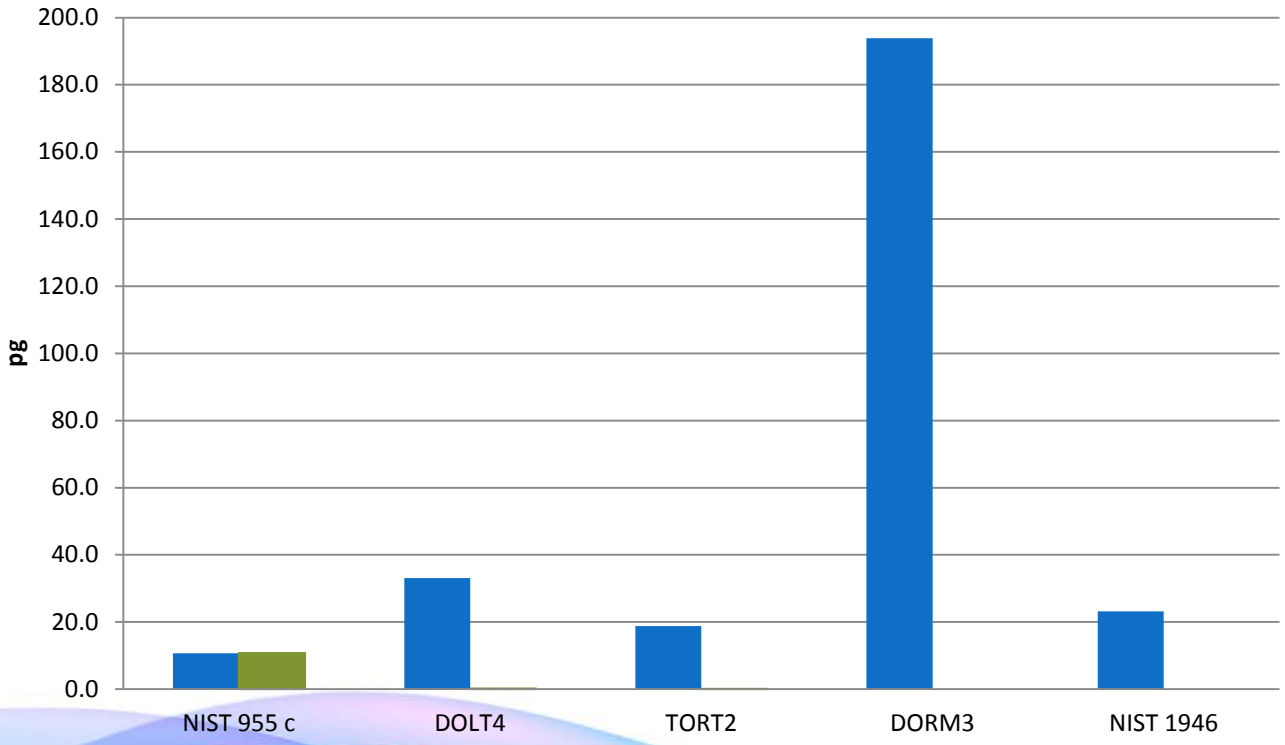
Species Conversion



	MeHg (pg)	EtHg (pg)
NIST 955 c	10.7	10.8
DOLT4	33.08	0.21
TORT2	18.8	0.0
DORM3	193.9	0.0
NIST 1946	23.1	0.0

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Sample Preparation – Experiment 1 TMAH

TMAH Digestion

- Add ~0.3 g sample and 2 mL 25% tetramethylammonium hydroxide (TMAH) solution to a glass vial. Bring volume to 10 mL with DI water.
- Seal the vial and heat to 65 °C in an oven for 4 hours. Cool to room temperature.
- Bring volume of samples up to constant level with DI water, to correct for volume loss.

Rodrigues, J. L.; Alvarez, C. R.; Fariñas, N. R.; Berzas Nevado, J. J.; Barbosa Jr, F.; Rodríguez Martín-Doimeadios, R. C. *Journal of Analytical Atomic Spectrometry* **2011**, *26*, 436

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Sample Preparation – Experiment 2 DCM

DCM Extraction

- Add sample and 5% H_2SO_4 + 18% KBr solution to a digestion vessel with a 1:4 (wt:v) ratio. Add 1 M $CuSO_4$ solution in a 1:5 (v:v) ratio with the H_2SO_4/KBr solution.
- Add dichloromethane in a 2:1 (wt:v) ratio with the H_2SO_4/KBr solution.
- Shake for 1 hour and let stand overnight.
- Centrifuge at 3,000 RPM for 15 mins. Separate the organic phase into a Teflon® bottle and add DI water in a ~5:2 (v:v) ratio. Heat to 70 °C until dichloromethane evaporates.

Gibičar, D.; Logar, M.; Horvat, N.; Marn-Pernat, A.; Ponikvar, R.; Horvat, M. *Analytical and Bioanalytical Chemistry* **2007**, *388*, 329–340.

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Sample Preparation – Experiment 3 KOH/MeOH plus DCM

- KOH/MeOH Digestion
- Add ~0.1 g sample and 1 mL 25% KOH/Methanol solution (wt/v) to a Teflon® digestion vial.
- Seal the vial and heat to 65 °C in an oven for 4 hours. Remove from the oven and bring the volume to 2.5 mL with methanol.
- Do DCM extraction procedure as previously described.

Bloom, N. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 1010–1017.

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Sample Preparation – Experiment 4 KOH/MeOH plus Distillation

- Follow the KOH/MeOH Digestion procedure
- Transfer the digestate to a distillation vial
- Distill following EPA Method 1630 for water samples.

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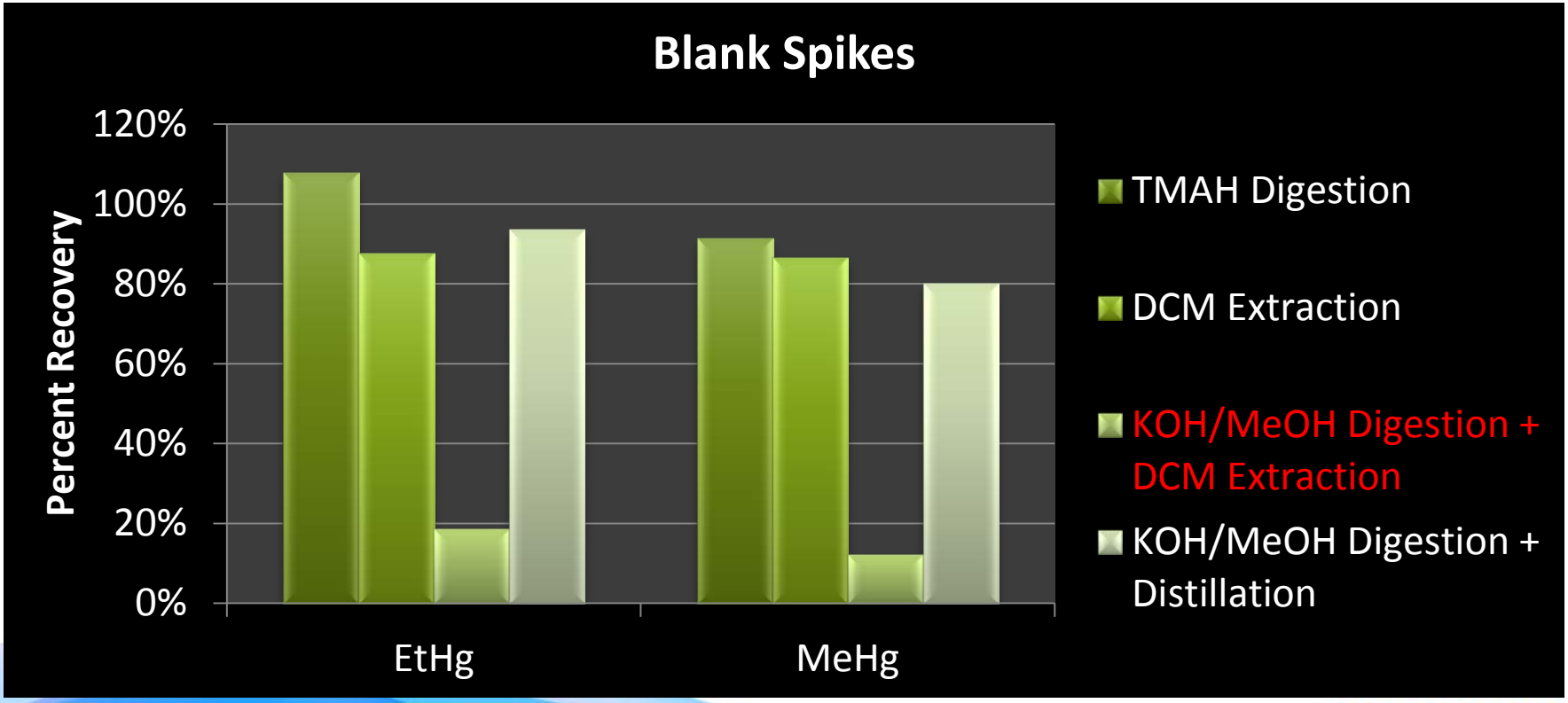
Blank Spikes



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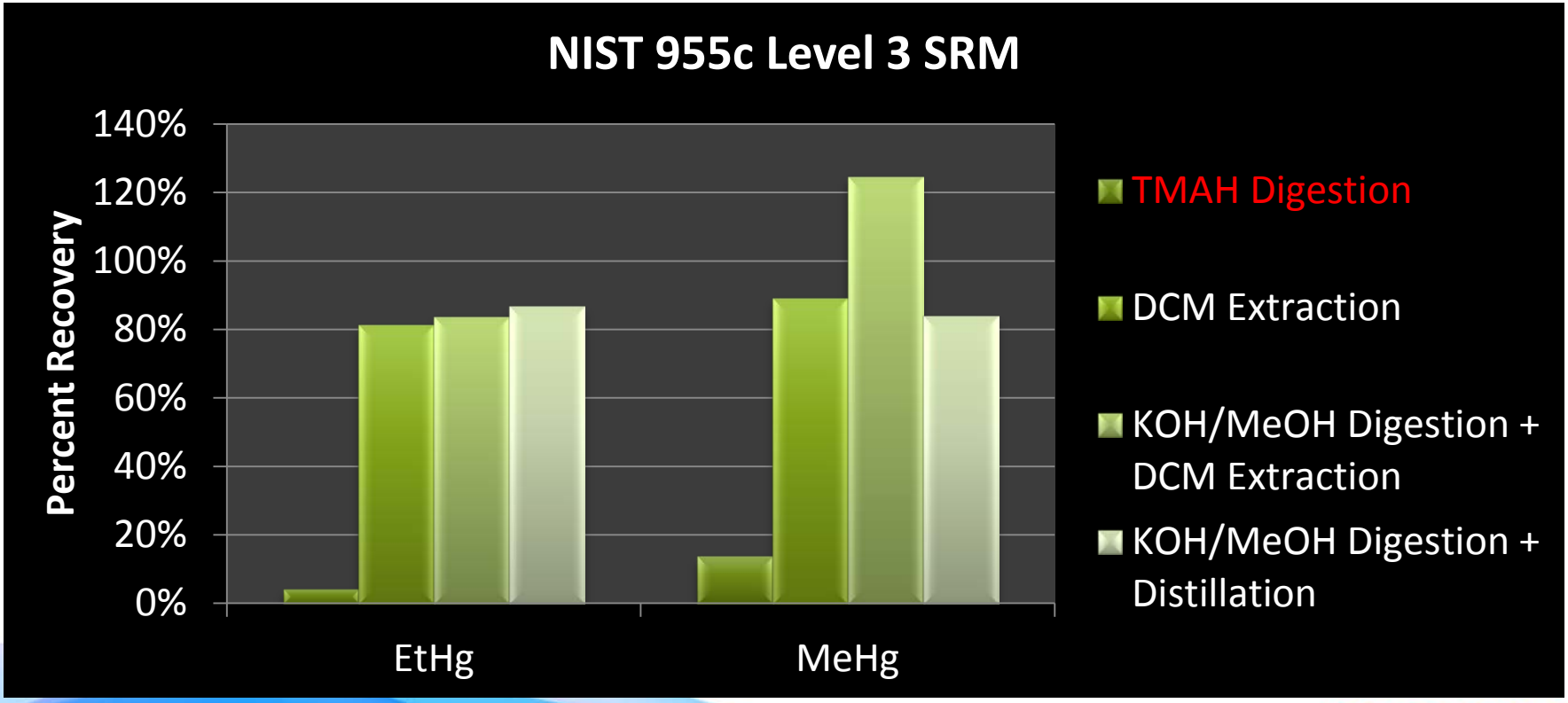
SRM Recovery



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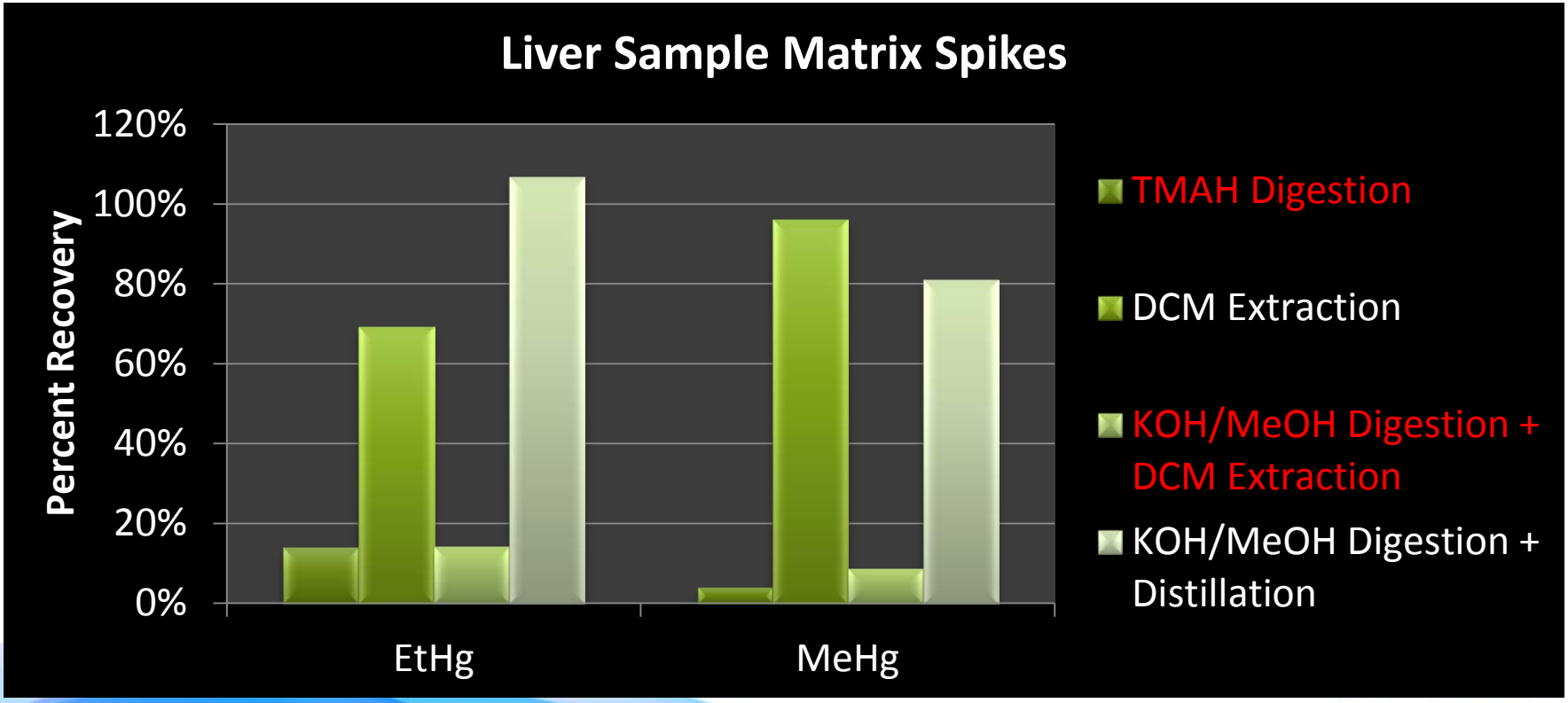
Matrix Spikes



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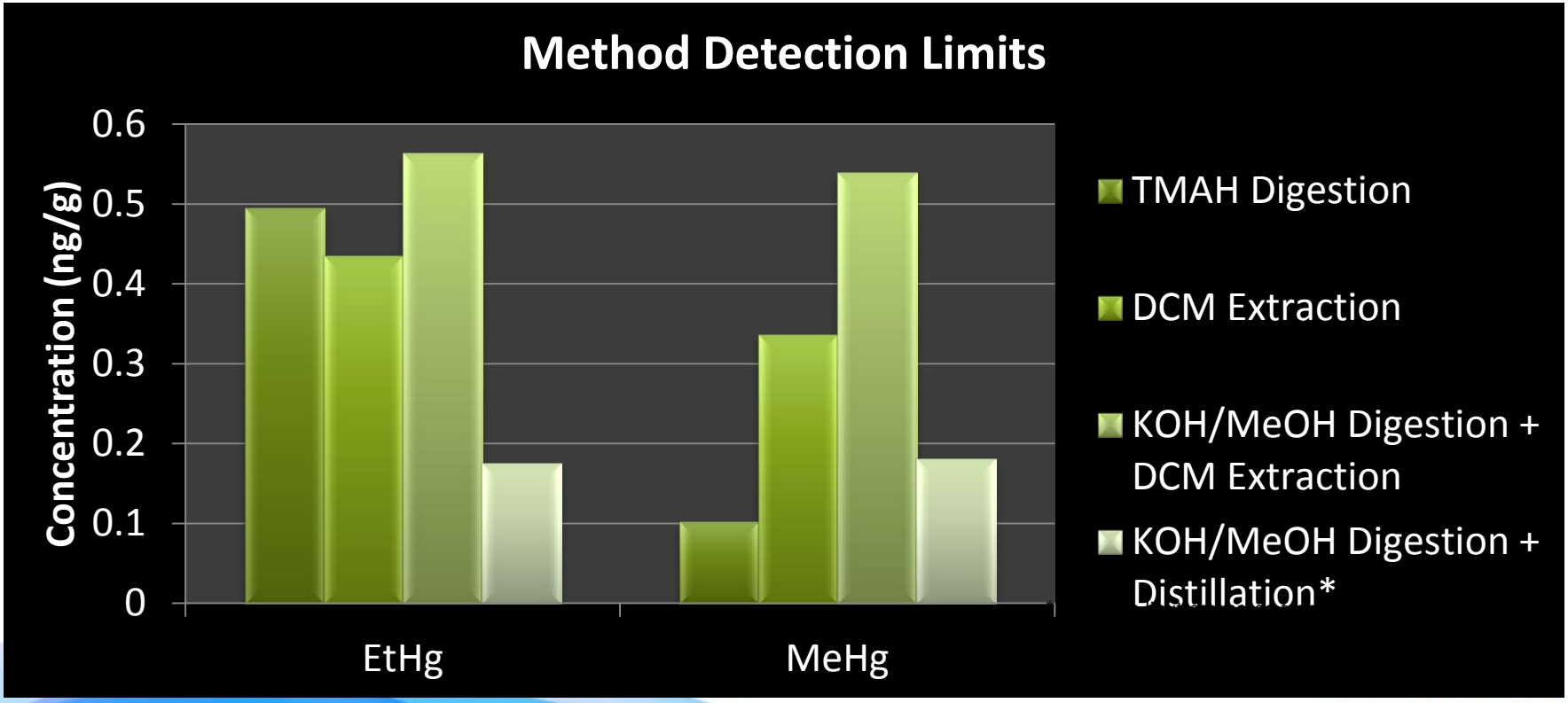
Detection Limit



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Additional Considerations

- incomplete digestion of liver and kidney matrix in the DCM prep
  - Because there is no certified reference material for ethylmercury in a solid tissue matrix, we could not be certain that this method was extracting all ethylmercury from the solid samples.

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Kidney Sample Analysis

- MeHg and EtHg (simultaneous)
  - KOH/MeOH digest and distill
  - Propylation
- MeHg only (for confirmation)
  - KOH/MeOH digest
  - ethylation
- Total Hg by 1631e Appendix
  - 70:30 Nitric:Sulfuric digest
  - CVAFS on BRI MERX-T
- Inorganic mercury
  - Determined by difference

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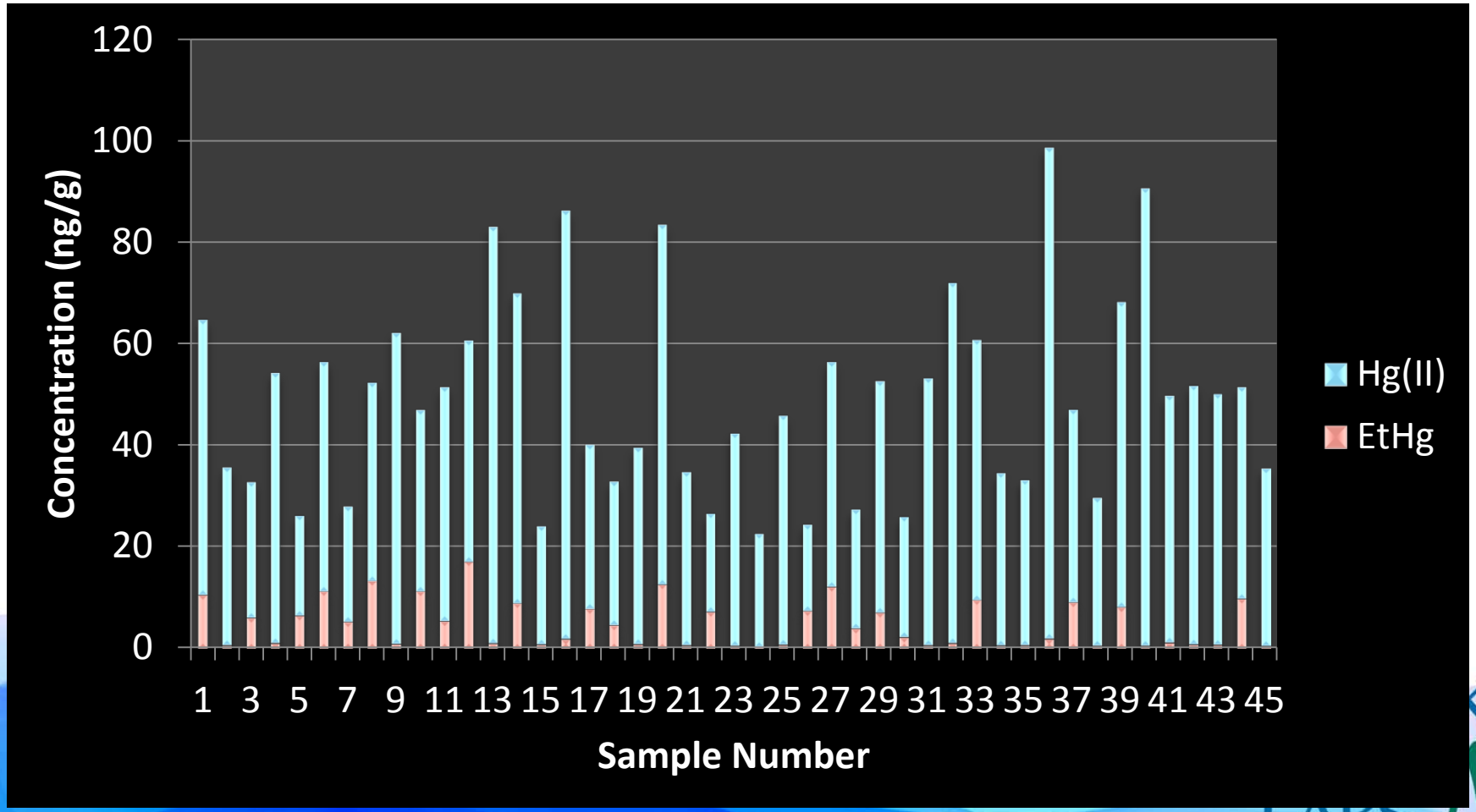
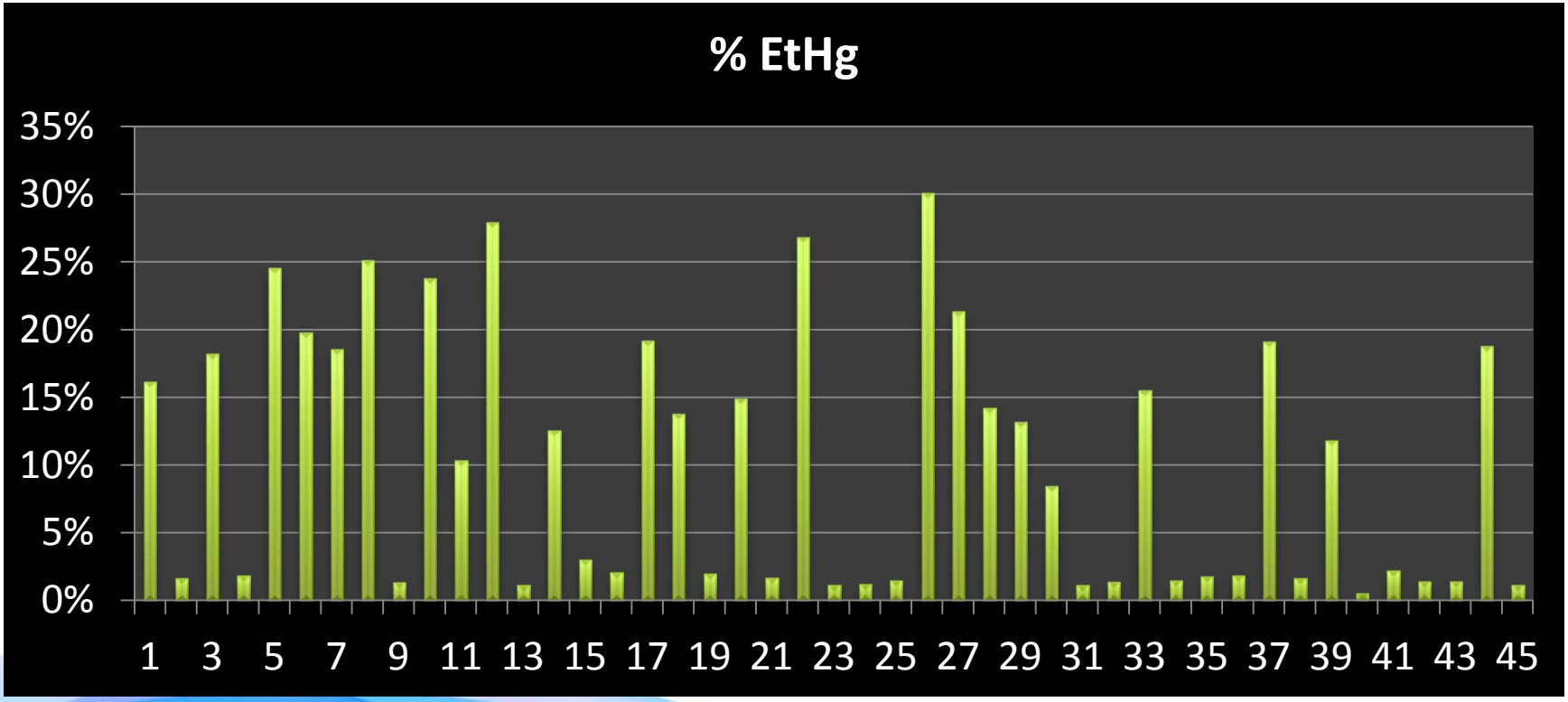
Sample results



% of THg that is EtHg



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Batch QC

	Ethylmercury	Methylmercury
Mean Matrix Spike Recovery	106.9%	81.2%
Mean Matrix Spike Duplicate Precision	6.0%	10.6%
Mean Sample Duplicate Precision	12.7%	Non-detect
Mean Recovery NIST 955c SRM	86.8%	84.0%

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Conclusions

- Substitution of $NaBPr_4$ for $NaBEt_4$ is an effective way to extend analytical equipment designed for MeHg analysis to the analysis of EtHg as well.
- The digest/distill method is effective for the simultaneous determination of EtHg and MeHg in liver and kidney samples
  - Methods developed for the analysis of blood and other bodily fluids are not necessarily applicable to tissue analysis due to their inability to disperse solid particles.
- There is a need for an animal tissue reference material certified for ethylmercury

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Thanks!

Becky Thorsness

Joel Creswell

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