Memorandum

To: Ralph Markarian, ENTRIX Inc.

Cc: Robert Taylor, Ericka Hailstocke-Johnson, and Lisa DiPinto, National Oceanic and Atmospheric Administration

From: Michel Gielazyn and Rob Ricker, National Oceanic and Atmospheric Administration

Date: April 20, 2011

Subject: Preparation of water-accommodated fractions for toxicity testing

The natural resource trustees for the *Deepwater Horizon* oil spill will conduct toxicity testing using several fish and invertebrate species. Trustees anticipate exposing test organisms to water-accommodated fractions (WAFs) of field-collected oil samples prepared with seawater. In addition to WAF tests, other exposure matrices also may be tested, including oiled sediment, tar balls, droplets, and surface-floated oil.

The composition and concentration of petroleum hydrocarbons in WAFs can differ depending on the protocol used to mix oil and water. These protocols address the ratio of oil to water added to the mixture, oil composition, ratio of dispersant to oil added to the mixture (if any), energy and duration of mixing, post-mixing settling time, and other variables. The WAF, which is typically drawn off near the bottom of the mixing container, may contain dissolved and particulate oil, depending on the protocol used to prepare the mixture. This memorandum describes the protocols that we anticipate using to prepare WAFs.

Trustees currently anticipate preparing WAFs using three field-collected oils. These oils include (1) source oil collected on July 26, 2010 from the hold of the barge *Massachusetts*, which received oil from the hold of the *Discoverer Enterprise*, which was receiving oil directly from the Macondo Well riser (sample ID 072610-01); (2) slick oil collected on July 19, 2010 by the *USCGC Juniper* (sample ID GU2888-A0719-OE701); and (3) oil collected on July 19, 2010 from the hold of barge number CTC02404, which was receiving slick oil from various skimmer vessels (sample ID CTC02404-02). Additionally, the Trustees may artificially weather the source oil (see Appendix A for weathering protocol) in order to remove benzene, toluene, ethylbenzene, and xylene (BTEX) components from the oil and use this slightly weathered source oil to prepare WAFs for testing.

As a first step, we evaluated several alternative WAF preparation methods. The WAF mixing protocols we tested included (1) low-energy and (2) chemically enhanced preparation methods described by Aurand and Coelho (2005), and (3) a high-energy preparation developed by the National Oceanic and Atmospheric Administration (NOAA; see Appendix B for all three protocols). WAFs were analyzed for BTEX and polycyclic aromatic hydrocarbons (PAHs) in unfiltered and (or) filtered (0.7-µm GF/F filter) samples (see Appendix C for oil and WAF

chemistry results). We compared the BTEX and PAH compositions of the various WAFs to water samples collected during the release.

The trustee toxicity testing will include exposing test organisms to multiple WAF preparations using source oil, a weathered batch of source oil (BTEX removed), and the two slick oils. Additionally, depending on the test objectives, WAFs may be filtered (0.7-μm GF/F filter) prior to use. The study design for each toxicity test will include multiple WAF preparations that represent different chemical compositions that may have existed in the field.

Reference

Aurand, D. and G. Coelho (eds.). 2005. *Cooperative Aquatic Toxicity Testing of Dispersed Oil and the "Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF)."* Technical Report 07-03. Ecosystem Management & Associates, Inc. Prepared for the American Petroleum Institute.

A. Protocols for Preparing Water-Accommodated Fractions

Prepared by
Environmental Conservation Division
NOAA Northwest Fisheries Science Center
Seattle, WA
and
Mote Marine Laboratory
Sarasota, FL

Standard Operating Procedures (SOPs) are intended to provide detailed and explicit instructions for the research staff in the collection of study data and should be fully reviewed by staff so that:

- They are versed on study objectives, methods, procedures, and details before proceeding
- Data are collected systematically and consistently throughout the study
- Each staff member understands and adheres to the requirements.

Events may arise during this study that require derivations from the procedures documented.

Derivations from the procedures should be documented in writing, with a detailed explanation of why the derivation was necessary. Revisions to the procedures will be conducted only after the approval of the Principal Investigator and/or Co-Principal Investigators. This SOP should be considered a draft document that describes procedures which are still under development.

Controls should be prepared for all WAF types. Controls should be prepared using the same WAF technique, with the exception of the addition of oil. All transfers and filtration steps should be replicated. Chemically-enhanced WAFs (CEWAFs) may require additional control preparations with dispersant only.

SOP for High-Energy Water-Accommodated Fractions (HEWAF)

Materials:

- Waring™ CB15 commercial food blender
- Seawater or embryo culture solution
- 1-L or larger graduated cylinder
- 1-L or larger separatory funnels and ring stand
- Whatman GFD and GF/F (0.7μ pore size) 4.7-cm filters
- Filter-holder apparatus
- 1-L sidearm Erlenmeyer flasks
- Vacuum source and tubing to fit sidearms
- Heavy-duty aluminum foil, cut into 12-in. squares, rinsed with acetone/dichloromethane (DCM)
- Laboratory-grade soap such as Sparkleen or Liqui-Nox
- Reagent-grade acetone and Teflon wash bottle
- Reagent-grade DCM and Teflon wash bottle
- Reagent-grade hexane and Teflon wash bottle
- KimWipes
- Nitrile gloves
- Aluminum weigh boats
- Glass gastight syringes with Teflon plunger (appropriate volumes)
- Stainless steel spatulas.

General considerations:

Wash all equipment that will come in contact with the sample (glassware, spatulas, stir bars, etc.) with Sparkleen soap and hot water. Soap and hot water can be blended on low for 1 minute. Rinse 3 times with reverse osmosis (RO) water to remove any soap residue. Rinse all equipment with acetone followed by hexane followed by DCM, three rinses for each solvent. Allow sufficient time for full evaporation of solvent. Use gloved hands throughout all prep steps, and use common-sense lab safety when handling solvents.

Hexane is helpful in removing visible residue from heavily oiled equipment. Underside of blender prongs may need to be carefully scrubbed by hand with solvent-soaked KimWipes, particularly after use with weathered surface oil samples. Use gloved hands throughout all prep steps. Note: it is quite easy to puncture a glove on the blender blades.

Procedure:

A. Preparation of blender lid (this is repeated for each WAF prep; see photos in the attachment)

1. Invert blender lid on bench top.

2. Center foil square over inside of lid and carefully push down into the lid. Push and fold inward to avoid tearing and keep foil centered.

3. Fold excess out over the lip so it can be trimmed with scissors.

4. Trim around the edge, leaving ~ 1 cm to fold over the first sealing ridge.

5. Press around the edges to make the foil as flat as possible over the sealing ridge.

6. Discard and replace foil with each prep.

B. Prepare oil HEWAF

1. Measure appropriate volume of water into pre-cleaned blender pitcher

2. Add desired amount of oil to the blender pitcher

    a. **Source oil** should be added using a pre-cleaned gastight syringe. It is best to fill the syringe with oil and dispense it prior to taring it on a balance. This will fill the needle and any voids, allowing for a more accurate dispensing weight. Fill the syringe with the desired weight and record the initial weight. After dispensing, record the final weight and determine the actual amount added by mass difference. Note: one gram of oil is equivalent to about 1.2 mL of oil. It is best to have a syringe dedicated to source oil to avoid contamination.

    b. **Slick oil** should be weighed in a pre-cleaned aluminum weigh boat. Tare a weigh boat and 2–3 KimWipes on the top loading balance. Using a stainless steel spatula, add slightly more than the desired mass of oil onto the weigh boat. With the weigh boat over the blender pitcher, slightly bend the weigh boat to create a narrower spout. Carefully transfer the oil using a spatula to scrape the oil into the pitcher. Wipe any oil remaining on the spatula using the tared KimWipes. Reweigh the weigh boat and KimWipes to calculate and record the actual mass transferred. See the Photograph Attachment.

3. Close blender lid

4. Blend 30 seconds on low

5. Transfer contents to a pre-cleaned separatory funnel

6. Note time of transfer.

C. Separation and filtration

1. Transfer separatory funnel to a ring stand, preferably in a hood.

2. Allow 1 hour separation.

3. Unfiltered HEWAF may be used directly; be sure not to use the top layer ~ 100 mL.

4. If filtered HEWAF is required follow steps 5–9.

5. Set up filter apparatus.

6. Drain slightly more than the desired amount from the separatory funnel. For example, if you need 400 mL, drain 450 mL into an intermediate vessel. Be sure not to use the top layer ~ 100 mL.

7. While running at low vacuum, transfer sample to the first filter apparatus (GFD).

8. Remove the filter apparatus, and pour first filtrate into second filter apparatus with GF/F filter (or disassemble filter holder and replace with GF/F).

9. Final filtrate can be measured into exact volumes and sampled for analytical chemistry or exposure assay.

Refer to the Photographic Attachment for:

- Filter apparatus
- Steps for lining the blender lid with solvent-rinsed foil.

SOP for Low-Energy (LEWAF) and Chemically-Enhanced (CEWAF)

Materials:

- Seawater or embryo culture solution
- 1-L or larger graduated cylinder
- Filter-holder apparatus
- 1-L sidearm Erlenmeyer flasks
- Vacuum pump
- Laboratory-grade soap such as Sparkleen or Liqui-Nox
- Reagent-grade acetone and Teflon wash bottle
- Reagent-grade DCM and Teflon wash bottle
- Reagent-grade hexane and Teflon wash bottle
- Aluminum foil
- Stir plate
- Stir bars, Teflon coated
- Aspirator bottles
- Tubing
- Syringes, glass/Teflon gastight
- Container for seawater, 20-L Nalgene carboy with spigot
- Top-loading bench scale (should have ≥ 300 g limit)
- Aluminum weigh boats
- KimWipes
- Nitrile gloves.

General considerations:

Wash all equipment that will come in contact with the sample (glassware, spatulas, and stir bars) with Sparkleen soap and hot water. Rinse three times with RO water. Rinse all equipment with acetone followed by hexane followed by DCM before each prep, three rinses for each solvent. Allow sufficient time for full evaporation of solvent. Use gloved hands throughout all prep steps, and use common-sense lab safety when handling solvents.

Procedure:

A. Preparing LEWAF

1. Obtain seawater from a clean source (e.g., sand filtered, ozonated) and record temperature and salinity.

2. Place clean aspirator bottles on stir plates.

3. Secure Tygon tubing and clamps onto bottom outlet.

4. Place stir bar in bottom of aspirator bottle (do not forget this step as it cannot be done after oil has been added), 1 in. for 1-L, 2 in. for 2-L.

5. Add desired amount of seawater to each aspirator bottle.

6. Begin to stir with no vortex (180–240 rpm for 2-L with 2-in. stir bar).

7. Add desired amount of oil to aspirator bottle.

    a. **Source oil** should be added using a pre-cleaned gastight syringe. It is best to fill the syringe with oil and dispense it prior to taring it on a balance. This will fill the needle and any voids, allowing for a more accurate dispensing weight. Fill the syringe with the desired weight and record the initial weight. After dispensing, record the final weight and determine the actual amount added by mass difference. Note: one gram of oil is equivalent to about 1.2 mL of oil. It is best to have a syringe dedicated to source oil to avoid contamination.

    b. **Slick oil** should be weighed in a pre-cleaned aluminum weigh boat. Tare a weigh boat and 2–3 KimWipes on the top loading balance. Using a stainless steel spatula, add slightly more than the desired mass of oil onto the weigh boat. With the weigh boat over the aspirator bottle, slightly bend the weigh boat to create a narrower spout (see the Photograph Attachment). Carefully transfer the oil using a spatula. Wipe off any oil remaining on the spatula with the tared KimWipes. Reweigh the weigh boat and KimWipes to calculate and record the actual mass transferred (see the Photograph Attachment).

8. Cover with aluminum foil and stir for 18–24 hours. No settling time is required for LEWAF. It is best if it is used immediately to avoid VOC loss, but may sit for up to 24 hours if necessary.

9. Prior to use, allow 20–40 mL to drain to waste to clear any water that has been sitting in the Tygon tubing.

10. Unfiltered LEWAF may be used directly, be sure not to use the top layer ~ 100 mL.

11. For filtered samples, assemble filtration apparatus and filter using a 47-mm GF/F filter.

12. While running at low vacuum, pour the sample through the filter.

13. Final filtrate can be measured into exact volumes and sampled for analytical chemistry or exposure assay.

B. Preparing CEWAF

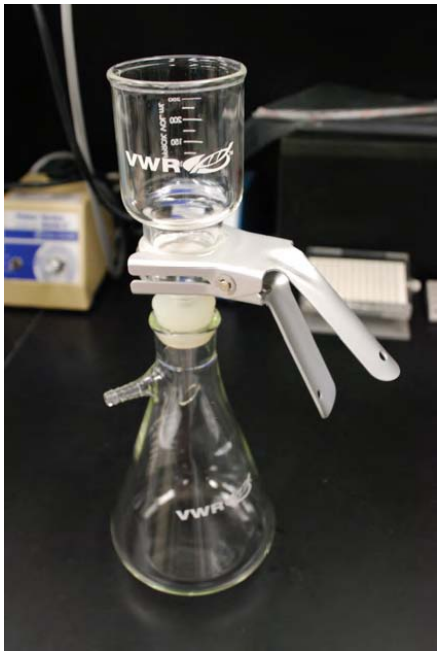
1. Follow steps 1–5 as for LEWAF.
2. Begin to stir with minimal vortex.
3. Add oil to the center of the vortex as described in Step 7 for LEWAF (source or slick oil).
4. Increase the mixing speed if the vortex decreases to less than 25% of the solution height.
5. Add dispersant to center of vortex using a gastight syringe, and again calculate the delivery mass by difference. Prior to taring the syringe, prefill and dispense the syringe in order to achieve a more accurate weight, as was done with the source oil. It is best to have a syringe dedicated to Corexit to avoid contamination.
6. Adjust vortex to 25%.
7. Stir for 18–24 hours. Turn stirrer off. Let settle for 3–6 hours.
8. Prior to use, allow 20–40 mL to drain to waste in order to clear any water that has been sitting in the Tygon tubing.
9. Unfiltered CEWAF may be used directly, be sure not to use the top layer ~ 100 mL.
10. For filtered samples, assemble filtration apparatus and filter using a 47-mm GF/F filter.
11. While running at low vacuum, pour the sample through the filter.
12. Final filtrate can be measured into exact volumes and sampled for analytical chemistry or exposure assay.

Refer to the Photograph Attachment for:

- Transferring slick oil to aspirator bottle
- Transferring source oil to aspirator bottle
- Taring KimWipes and aluminum weigh boat
- Reweighing scraped weigh boat and KimWipe used to clean spatula.

Photograph Attachment

Steps for lining the blender lid with solvent-rinsed foil.



Filter apparatus.

Transferring slick oil to aspirator bottle.

Transferring source oil to aspirator bottle.

Taring KimWipes and aluminum weigh boat.

Reweighing scraped weigh boat and KimWipe used to clean spatula.

B. Artificial Weathering of Source Oil

Based on:

Marty, G.D., J.W. Short, D.M. Dambach, N.H. Willits, R.A. Heintz, S.D. Rice, J.J. Stegeman, and D.E. Hinton. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology* 75:989–1007.

The purpose of this weathering process is to remove BTEX components from neat oil.

A volume (e.g., 2 L) of source oil is slowly stirred and heated at 70°C in an open-top container (e.g., beaker) and weighed periodically until a pre-determined mass is lost from the oil. This pre-determined mass corresponds to the mass of BTEX in the oil (e.g., 20%). Once the appropriate mass loss is achieved, the oil is mixed, subsampled, and analyzed to confirm loss of BTEX from the oil.

C. Chemical Composition of Oil and Water-Accommodated Fractions

This appendix contains analytical data for WAFs prepared using the LEWAF and CEWAF protocols described in Appendix A. The HEWAF method used to mix oil and water for which data are reported in this appendix differed from the protocol described in Appendix A. The protocol used to produce the HEWAF data presented here substituted the blender mixing procedure described in Appendix A with 5 minutes of vigorous shaking by hand in a separatory funnel, based on Carls et al. (2008).

The oil loading rate for all WAFs was 1:100 oil:water. The dispersant (Corexit 9500) loading rate was 1:10 dispersant:oil for all CEWAFs.

Reference

Carls, M.G., L. Holland, M. Larsen, T.K. Collier, N.L. Scholz, and J.P. Incardona. 2008. Fish embryos are damaged by dissolved PAHs, not oil particles. *Aquatic Toxicology* 88:121–127.

Table C.1. Composition of three field-collected oils used to prepare WAFs (mg/kg)

Oil	Source	Slick A	Slick B
Client ID	**072610-01**	**CTC02404-02**	**GU2888-A0719-OE701**
Collection date	**7/26/2010**	**7/29/2010**	**7/19/2010**
Analyte			
Benzene (B)	2,880.0	0.0	NA
Toluene (T)	7,060.0	0.0	NA
Ethylbenzene (EB)	1,310.0	0.0	NA
p/m-Xylene (MPX)	6,880.0	0.0	NA
o-Xylene (OX)	2,490.0	0.0	NA
Sum PAH (no BTEX)			
Naphthalene (N0)	1,140.0	0.2	0.3
C1-Naphthalenes (N1)	2,480.0	9.6	1.0
C2-Naphthalenes (N2)	3,060.0	319.0	10.9
C3-Naphthalenes (N3)	1,970.0	717.0	55.8
C4-Naphthalenes (N4)	898.0	504.0	79.6
Biphenyl (B)	284.0	14.0	0.8
Dibenzofuran (DF)	41.2	9.6	0.6
Acenaphthylene (AY)	11.1	1.7	0.1
Acenaphthene (AE)	33.2	7.7	0.3
Fluorene (F0)	204.0	68.0	5.9
C1-Fluorenes (F1)	424.0	261.0	56.1
C2-Fluorenes (F2)	537.0	411.0	160.0
C3-Fluorenes (F3)	377.0	319.0	191.0
Anthracene (A0)	16.1	10.6	3.1
Phenanthrene (P0)	439.0	299.0	97.3
C1-Phenanthrenes/Anthracenes (PA1)	934.0	818.0	454.0
C2-Phenanthrenes/Anthracenes (PA2)	879.0	824.0	612.0
C3-Phenanthrenes/Anthracenes (PA3)	454.0	431.0	333.0
C4-Phenanthrenes/Anthracenes (PA4)	216.0	195.0	136.0
Retene (RET)	0.0	0.0	0.0
Dibenzothiophene (DBT0)	78.8	48.8	12.0
C1-Dibenzothiophenes (DBT1)	217.0	182.0	72.9
C2-Dibenzothiophenes (DBT2)	270.0	261.0	184.0
C3-Dibenzothiophenes (DBT3)	204.0	216.0	176.0
C4-Dibenzothiophenes (DBT4)	97.9	99.6	83.9

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.1. Composition of three field-collected oils used to prepare WAFs (mg/kg) (cont.)

Oil	Source	Slick A	Slick B
Client ID	**072610-01**	**CTC02404-02**	**GU2888-A0719-OE701**
Collection date	**7/26/2010**	**7/29/2010**	**7/19/2010**
Analyte			
Benzo(b)fluorine (BF)	14.7	10.4	0.0
Fluoranthene (FL0)	3.3	2.8	2.6
Pyrene (PY0)	18.8	17.4	12.3
C1-Fluoranthenes/Pyrenes (FP1)	108.0	89.9	54.9
C2-Fluoranthenes/Pyrenes (FP2)	161.0	142.0	75.1
C3-Fluoranthenes/Pyrenes (FP3)	216.0	184.0	114.0
C4-Fluoranthenes/Pyrenes (FP4)	172.0	162.0	121.0
Naphthobenzothiophenes (NBT0)	26.0	29.3	26.9
C1-Naphthobenzothiophenes (NBT1)	83.2	83.8	84.1
C2-Naphthobenzothiophenes (NBT2)	111.0	118.0	103.0
C3-Naphthobenzothiophenes (NBT3)	79.9	74.9	64.6
C4-Naphthobenzothiophenes (NBT4)	59.1	58.0	45.8
Benz[a]anthracene (BA0)	9.5	6.4	1.5
Chrysene/Triphenylene (C0)	78.6	92.9	86.8
C1-Chrysenes (BC1)	175.0	184.0	155.0
C2-Chrysenes (BC2)	218.0	203.0	145.0
C3-Chrysenes (BC3)	228.0	174.0	97.4
C4-Chrysenes (BC4)	135.0	94.8	54.2
Benzo[b]fluoranthene (BBF)	7.0	7.8	7.9
Benzo[k]fluoranthene (BJKF)	0.9	0.6	0.7
Benzo[a]fluoranthene (BAF)	0.0	0.0	0.0
Benzo[e]pyrene (BEP)	13.8	14.2	13.1
Benzo[a]pyrene (BAP)	3.5	2.2	1.1
Perylene (PER)	0.4	0.2	0.0
Indeno[1,2,3-cd]pyrene (IND)	1.3	0.8	0.5
Dibenz[a,h]anthracene (DA)	2.6	2.0	0.9
Benzo[g,h,i]perylene (GHI)	2.0	2.2	1.9
Sum PAH	17,193.8	7,784.3	3,996.7

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.2. WAFs prepared using source oil (072610-01) (μg/L)

Preparation method	HEWAF		LEWAF	CEWAF	
Filtration (0.7 μ GF/F)	**Unfiltered**	**Filtered**	**Unfiltered**	**Unfiltered**	**Filtered**
Analyte					
Benzene (B)	5,300.0	NA	5,800.0	6200	NA
Toluene (T)	5,000.0	NA	5,900.0	6300	NA
Ethylbenzene (EB)	280.0	NA	360.0	370	NA
p/m-Xylene (MPX)	1,500.0	NA	1,700.0	1800	NA
o-Xylene (OX)	620.0	NA	790.0	820	NA
Sum PAH (no BTEX)					
Naphthalene (N0)	19.4	0.9	36.9	117.4	106.2
C1-Naphthalenes (N1)	67.1	6.2	38.3	101.0	67.1
C2-Naphthalenes (N2)	207.4	20.3	52.9	189.1	79.2
C3-Naphthalenes (N3)	150.8	8.7	15.3	105.2	20.3
C4-Naphthalenes (N4)	97.3	2.5	4.2	49.7	4.8
Fluorene (F0)	11.4	1.7	2.6	6.5	3.0
C1-Fluorenes (F1)	21.0	1.7	2.7	11.9	2.9
C2-Fluorenes (F2)	27.0	0.9	0.7	13.3	1.9
C3-Fluorenes (F3)	25.9	0.6	0.4	11.5	1.5
Dibenzothiophene (DBT0)	2.5	0.5	0.5	1.6	0.6
C1-Dibenzothiophenes (DBT1)	4.3	0.7	0.9	3.6	0.9
C2-Dibenzothiophenes (DBT2)	7.7	0.3	0.5	3.7	0.7
C3-Dibenzothiophenes (DBT3)	3.9	0.0	0.3	2.6	0.0
C4-Dibenzothiophenes (DBT4)	2.7	0.0	0.0	1.8	0.0
Phenanthrene (P0)	14.7	2.5	3.1	9.0	3.0
Anthracene (A0)	0.0	0.0	0.3	0.8	0.3
C1-Phenanthrenes/Anthracenes (PA1)	39.3	2.8	3.3	21.7	3.5
C2-Phenanthrenes/Anthracenes (PA2)	71.8	1.7	2.6	37.8	3.2
C3-Phenanthrenes/Anthracenes (PA3)	42.9	0.5	0.6	23.1	1.4
C4-Phenanthrenes/Anthracenes (PA4)	17.7	0.0	0.0	10.7	0.0
Fluoranthene (FL0)	0.0	0.0	0.0	0.0	0.0
Pyrene (PY0)	0.0	0.0	0.0	0.0	0.0
C1-Fluoranthenes/Pyrenes (FP1)	2.7	0.0	0.1	1.6	0.0
C2-Fluoranthenes/Pyrenes (FP2)	4.1	0.0	0.1	1.9	0.0
C3-Fluoranthenes/Pyrenes (FP3)	4.0	0.0	0.0	1.6	0.0
C4-Fluoranthenes/Pyrenes (FP4)	2.3	0.0	0.0	1.5	0.0

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.2. WAFs prepared using source oil (072610-01) (μg/L) (cont.)

Preparation method	HEWAF		LEWAF	CEWAF	
Filtration (0.7 μ GF/F)	**Unfiltered**	**Filtered**	**Unfiltered**	**Unfiltered**	**Filtered**
Analyte					
Benz[a]anthracene (BA0)	2.4	0.0	0.0	0.3	0.0
Chrysene/Triphenylene (C0)	0.9	0.0	0.0	1.2	0.0
C1-Chrysenes (BC1)	5.5	0.0	0.0	2.4	0.0
C2-Chrysenes (BC2)	5.5	0.0	0.0	2.8	0.0
C3-Chrysenes (BC3)	3.8	0.0	0.0	1.6	0.0
C4-Chrysenes (BC4)	0.0	0.0	0.0	0.0	0.0
Benzo[e]pyrene (BEP)	0.2	0.0	0.0	0.0	0.0
Benzo[a]pyrene (BAP)	0.5	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene (BBF)	0.0	0.0	0.0	0.0	0.0
Benzo[k]fluoranthene (BJKF)	0.0	0.0	0.0	0.0	0.0
Sum PAH	866.9	52.6	166.1	736.8	300.5

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.3. WAFs prepared using Slick A oil (CTC02404-02) (µg/L)

Preparation method	HEWAF		LEWAF	CEWAF	
Filtration (0.7 µ GF/F)	**Unfiltered**	**Filtered**	**Unfiltered**	**Unfiltered**	**Filtered**
Analyte					
Benzene (B)	0.0	NA	0.0	1.3	NA
Toluene (T)	0.0	NA	0.20	2.4	NA
Ethylbenzene (EB)	0.0	NA	0.0	0.16	NA
p/m-Xylene (MPX)	0.0	NA	0.0	0.87	NA
o-Xylene (OX)	0.0	NA	0.0	0.27	NA
Sum PAH (no BTEX)					
Naphthalene (N0)	0.0	0.0	0.0	0.0	0.0
C1-Naphthalenes (N1)	0.3	0.0	0.0	17.4	4.8
C2-Naphthalenes (N2)	34.0	1.4	2.0	51.2	24.9
C3-Naphthalenes (N3)	89.3	4.6	3.5	69.8	31.5
C4-Naphthalenes (N4)	67.9	5.2	0.8	36.3	16.3
Fluorene (F0)	5.0	0.7	0.8	4.5	2.7
C1-Fluorenes (F1)	20.2	7.1	6.8	19.3	11.4
C2-Fluorenes (F2)	41.0	9.8	9.5	25.9	16.2
C3-Fluorenes (F3)	28.7	6.4	4.7	20.2	12.8
Dibenzothiophene (DBT0)	1.8	0.4	0.4	1.8	1.1
C1-Dibenzothiophenes (DBT1)	7.7	3.2	0.8	4.8	2.7
C2-Dibenzothiophenes (DBT2)	10.8	0.6	0.7	5.0	2.8
C3-Dibenzothiophenes (DBT3)	7.2	0.0	0.0	2.8	1.5
C4-Dibenzothiophenes (DBT4)	5.4	0.0	0.0	0.0	0.0
Phenanthrene (P0)	14.4	2.6	2.6	11.9	6.7
Anthracene (A0)	0.0	0.0	0.0	0.0	0.4
C1-Phenanthrenes/Anthracenes (PA1)	46.4	4.7	3.5	30.5	16.4
C2-Phenanthrenes/Anthracenes (PA2)	91.2	7.0	4.7	49.2	27.8
C3-Phenanthrenes/Anthracenes (PA3)	58.2	3.0	2.3	30.3	14.2
C4-Phenanthrenes/Anthracenes (PA4)	26.6	0.0	0.0	10.9	5.3
Fluoranthene (FL0)	0.0	0.0	0.0	0.0	0.0
Pyrene (PY0)	0.0	0.0	0.1	0.0	0.0
C1-Fluoranthenes/Pyrenes (FP1)	1.0	0.4	0.4	1.5	0.6
C2-Fluoranthenes/Pyrenes (FP2)	1.8	0.4	0.4	2.0	0.9
C3-Fluoranthenes/Pyrenes (FP3)	4.4	0.1	0.2	1.3	0.5
C4-Fluoranthenes/Pyrenes (FP4)	1.9	0.0	0.0	0.0	0.0

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.3. WAFs prepared using Slick A oil (CTC02404-02) (µg/L) (cont.)

Preparation method	HEWAF		LEWAF	CEWAF	
Filtration (0.7 µ GF/F)	**Unfiltered**	**Filtered**	**Unfiltered**	**Unfiltered**	**Filtered**
Analyte					
Benz[a]anthracene (BA0)	0.6	0.1	0.1	0.3	0.1
Chrysene/Triphenylene (C0)	3.8	0.1	0.0	1.6	0.8
C1-Chrysenes (BC1)	7.4	0.0	0.0	2.4	1.4
C2-Chrysenes (BC2)	6.7	0.0	0.0	1.7	0.8
C3-Chrysenes (BC3)	1.5	0.0	0.0	0.0	0.0
C4-Chrysenes (BC4)	0.0	0.0	0.0	0.0	0.0
Benzo[e]pyrene (BEP)	0.0	0.0	0.0	0.0	0.0
Benzo[a]pyrene (BAP)	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene (BBF)	0.0	0.0	0.0	0.0	0.0
Benzo[k]fluoranthene (BJKF)	0.0	0.0	0.0	0.0	0.0
Sum PAH	585.1	57.5	44.5	402.6	204.9

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.4. WAFs prepared using Slick B oil (GU2888-A0719-OE701) (μg/L)

Preparation method	HEWAF		LEWAF	CEWAF	
Filtration (0.7 μ GF/F)	**Unfiltered**	**Filtered**	**Unfiltered**	**Unfiltered**	**Filtered**
Analyte					
Benzene (B)	0.0	NA	0.0	0.0	NA
Toluene (T)	0.0	NA	0.21	0.34	NA
Ethylbenzene (EB)	0.0	NA	0.0	0.0	NA
p/m-Xylene (MPX)	0.0	NA	0.0	0.0	NA
o-Xylene (OX)	0.0	NA	0.0	0.0	NA
Sum PAH (no BTEX)					
Naphthalene (N0)	0.0	0.0	0.0	0.0	0.4
C1-Naphthalenes (N1)	0.1	0.0	0.0	0.0	4.0
C2-Naphthalenes (N2)	0.7	0.0	0.0	0.0	3.4
C3-Naphthalenes (N3)	4.5	0.0	0.5	0.0	4.1
C4-Naphthalenes (N4)	6.2	0.0	0.0	0.0	3.5
Fluorene (F0)	0.4	0.0	0.2	1.4	0.4
C1-Fluorenes (F1)	3.6	0.1	1.3	15.3	3.9
C2-Fluorenes (F2)	9.0	0.3	2.3	34.2	8.9
C3-Fluorenes (F3)	11.1	0.4	2.2	42.2	11.7
Dibenzothiophene (DBT0)	0.4	0.0	0.2	1.8	0.5
C1-Dibenzothiophenes (DBT1)	2.9	0.0	0.6	10.8	2.9
C2-Dibenzothiophenes (DBT2)	5.0	0.1	0.8	20.8	5.5
C3-Dibenzothiophenes (DBT3)	4.2	0.1	0.6	11.3	3.6
C4-Dibenzothiophenes (DBT4)	2.5	0.0	0.2	3.5	1.6
Phenanthrene (P0)	3.3	0.1	0.9	12.9	3.3
Anthracene (A0)	0.0	0.0	0.0	0.0	0.2
C1-Phenanthrenes/Anthracenes (PA1)	18.1	0.4	2.0	77.2	17.5
C2-Phenanthrenes/Anthracenes (PA2)	48.6	0.6	2.3	202.0	44.5
C3-Phenanthrenes/Anthracenes (PA3)	27.8	0.5	0.6	128.4	24.3
C4-Phenanthrenes/Anthracenes (PA4)	16.3	0.2	0.0	20.2	6.5
Fluoranthene (FL0)	0.0	0.0	0.0	1.2	0.3
Pyrene (PY0)	1.7	0.0	0.0	2.4	0.7
C1-Fluoranthenes/Pyrenes (FP1)	1.9	0.0	0.1	7.3	1.4
C2-Fluoranthenes/Pyrenes (FP2)	1.1	0.0	0.2	7.0	1.4
C3-Fluoranthenes/Pyrenes (FP3)	2.0	0.0	0.0	5.0	1.3
C4-Fluoranthenes/Pyrenes (FP4)	1.6	0.0	0.0	4.1	0.9

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

Table C.4. WAFs prepared using Slick B oil (GU2888-A0719-OE701) (µg/L)

Preparation method	HEWAF		LEWAF	CEWAF	
Filtration (0.7 µ GF/F)	**Unfiltered**	**Filtered**	**Unfiltered**	**Unfiltered**	**Filtered**
Analyte					
Benz[a]anthracene (BA0)	2.5	0.0	0.1	8.2	1.6
Chrysene/Triphenylene (C0)	0.0	0.0	0.0	0.0	0.0
C1-Chrysenes (BC1)	4.1	0.1	0.0	11.2	2.5
C2-Chrysenes (BC2)	3.7	0.1	0.0	5.5	1.4
C3-Chrysenes (BC3)	1.6	0.0	0.0	1.2	0.0
C4-Chrysenes (BC4)	0.0	0.0	0.0	0.0	0.0
Benzo[e]pyrene (BEP)	0.0	0.0	0.0	0.0	0.0
Benzo[a]pyrene (BAP)	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene (BBF)	0.0	0.0	0.0	0.0	0.0
Benzo[k]fluoranthene (BJKF)	0.0	0.0	0.0	0.0	0.0
Sum PAH	185.3	3.1	14.8	635.0	162.3

Note that table totals may not sum due to rounding.
0 = below detection limit.
NA = not analyzed.

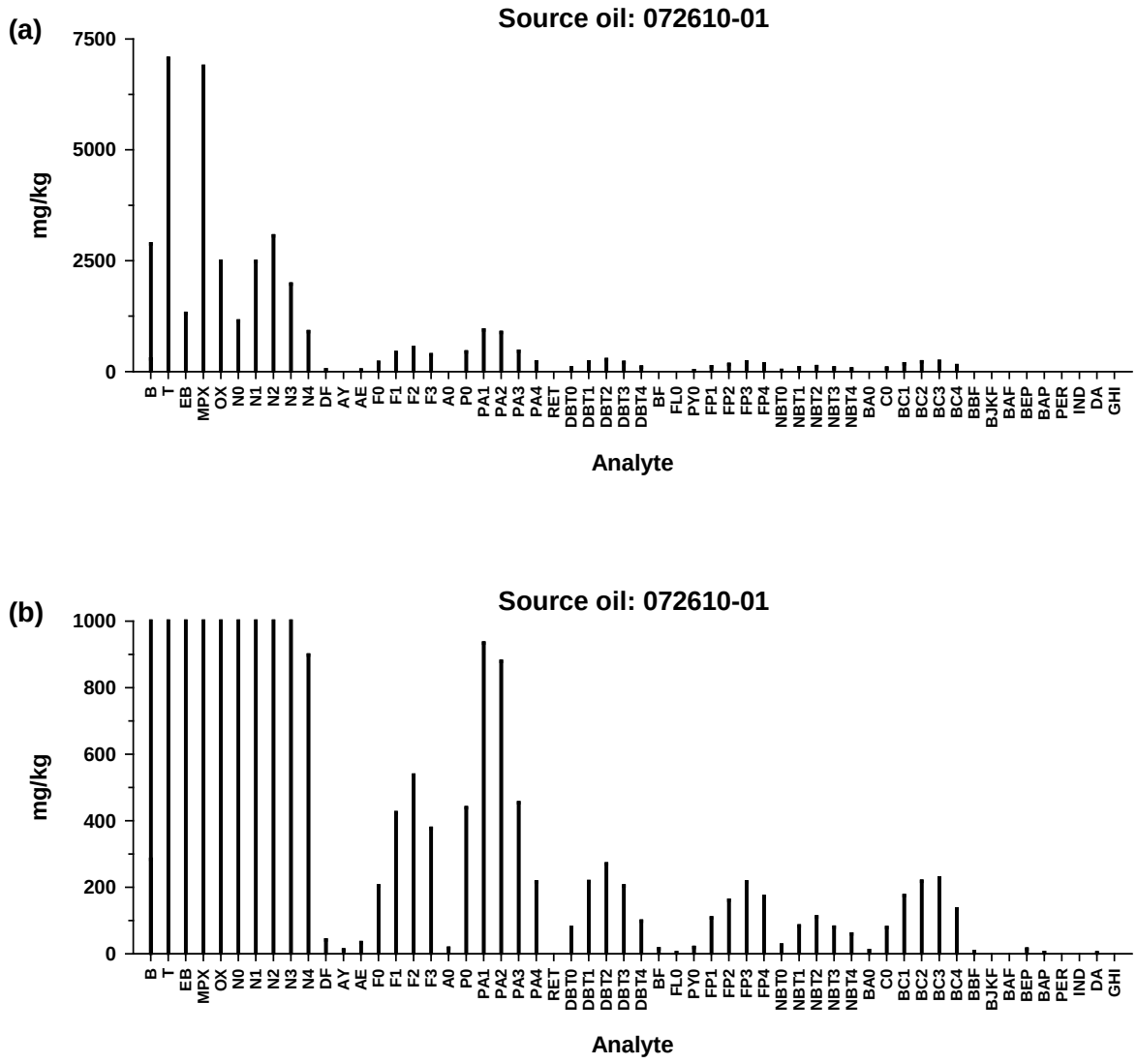


Figure C.1. Composition of source oil. Figures (a) and (b) contain the same data but are presented with different y-axis scales. See Table C.1 for analyte abbreviations and concentrations.

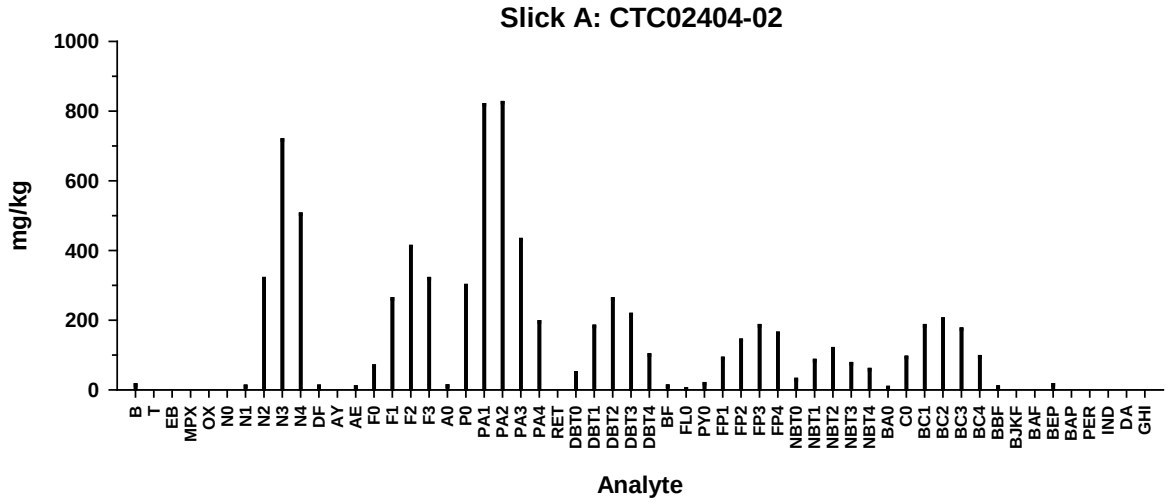


Figure C.2. Composition of Slick A oil. See Table C.1 for analyte abbreviations and concentrations.

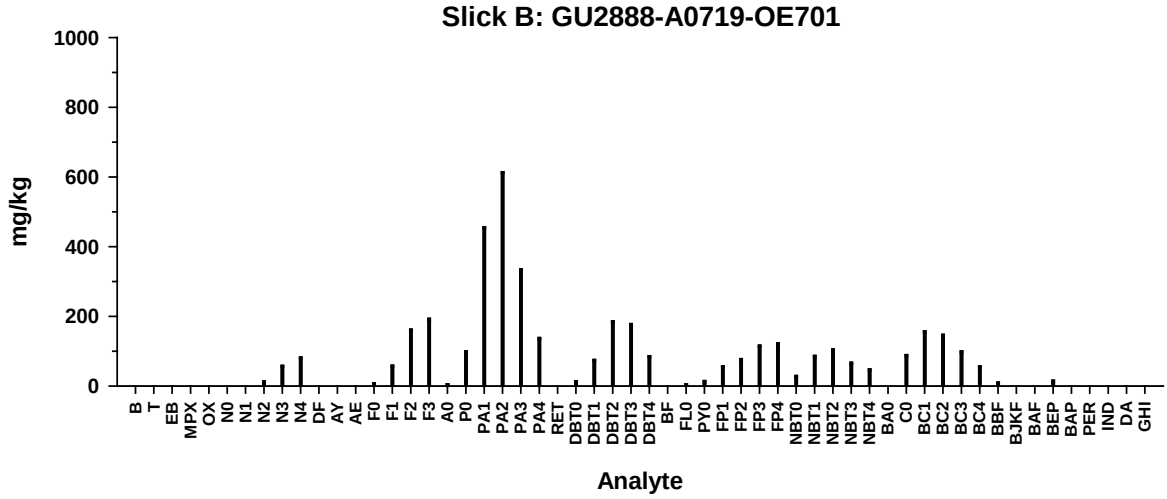


Figure C.3. Composition of Slick B oil. See Table C.1 for analyte abbreviations and concentrations.

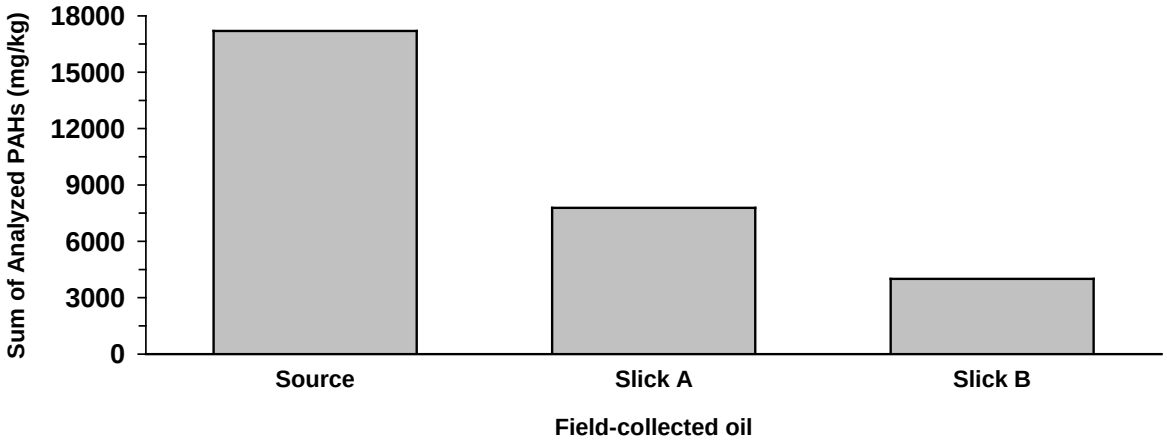


Figure C.4. Sum of analyzed PAHs in the source, Slick A, and Slick B oils. See Table C.1 for PAH concentrations.

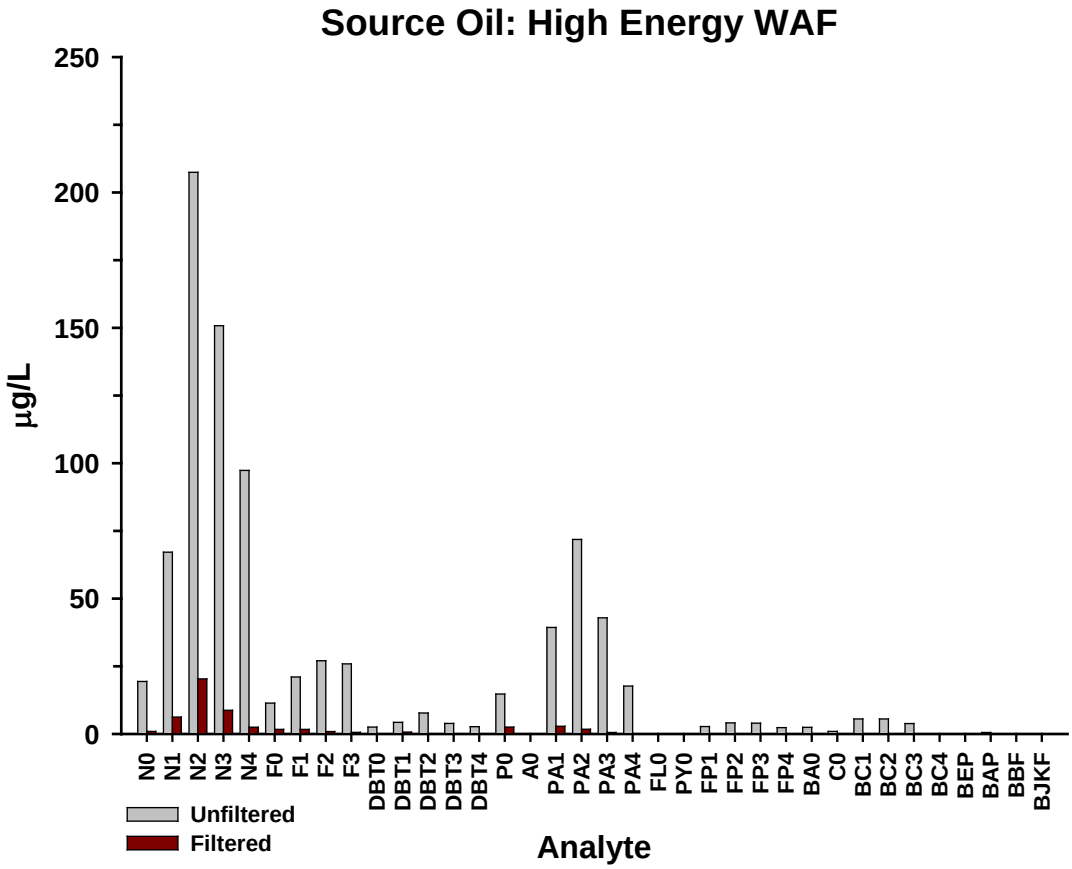


Figure C.5. Composition of a HEWAF (hand-shaking preparation) produced using source oil. See Table C.2 for analyte abbreviations and concentrations.

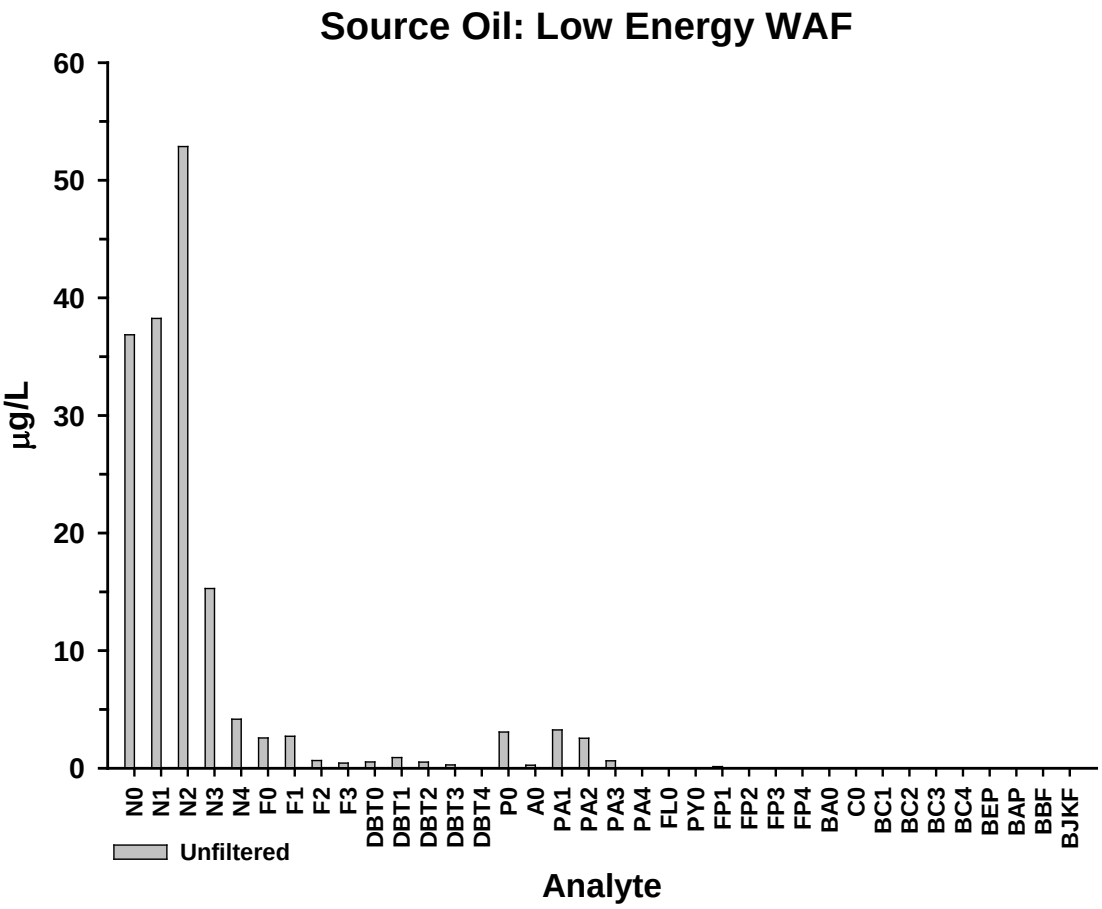


Figure C.6. Composition of a LEWAF produced using source oil. See Table C.2 for analyte abbreviations and concentrations.

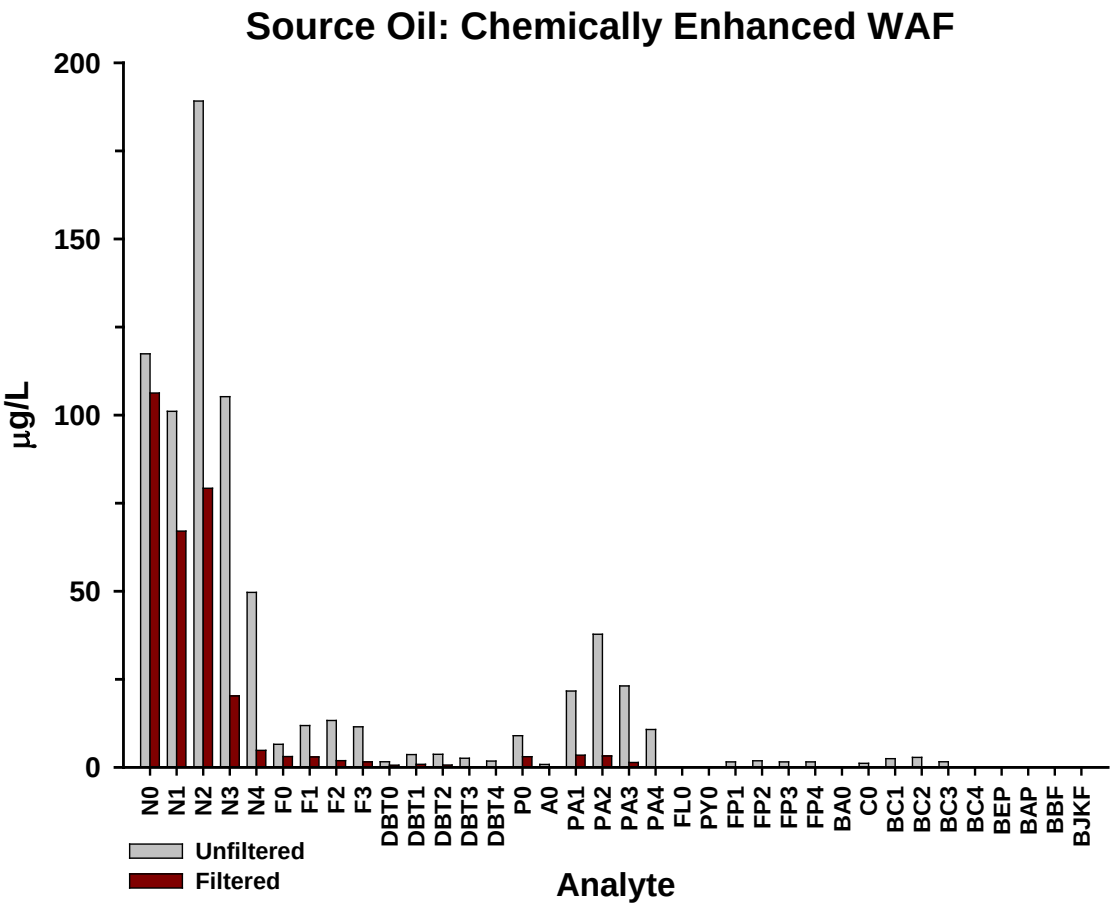


Figure C.7. Composition of a CEWAF produced using source oil. See Table C.2 for analyte abbreviations and concentrations.

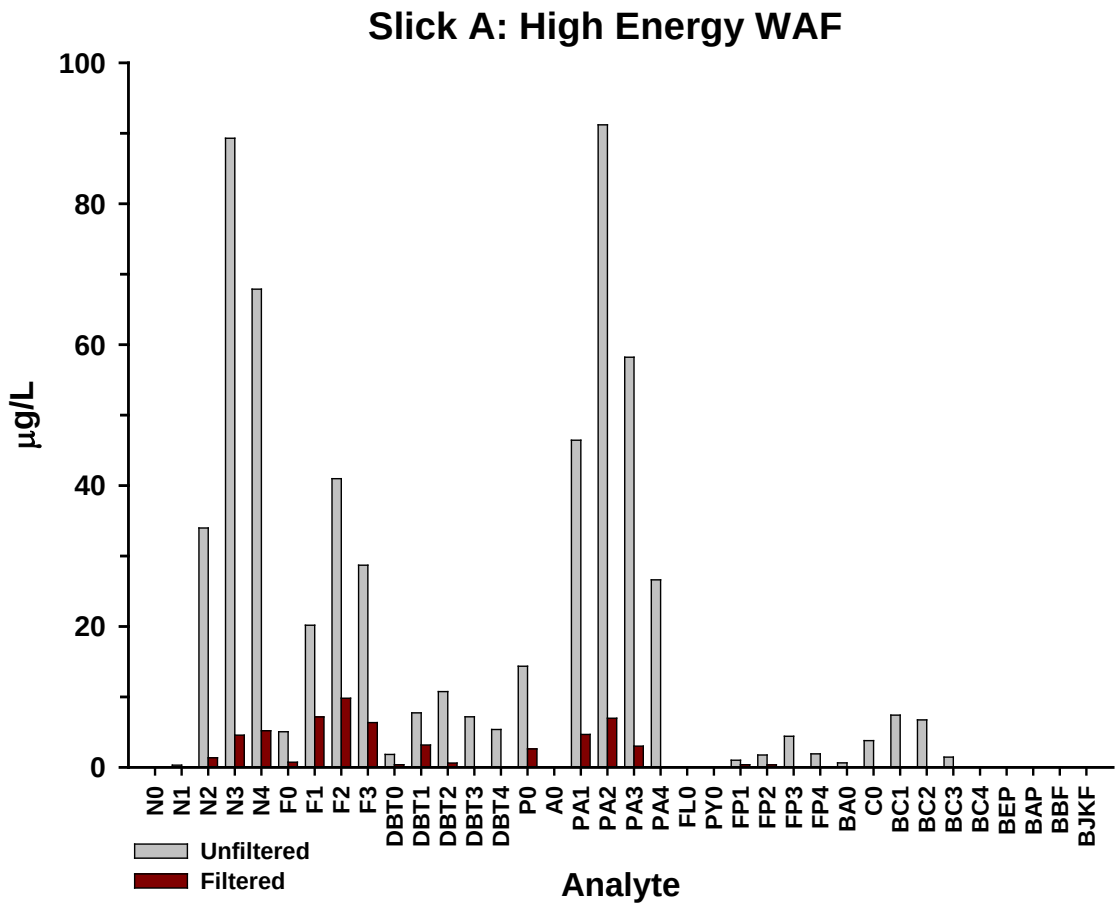


Figure C.8. Composition of a HEWAF (hand-shaking preparation) produced using Slick A oil. See Table C.3 for analyte abbreviations and concentrations.

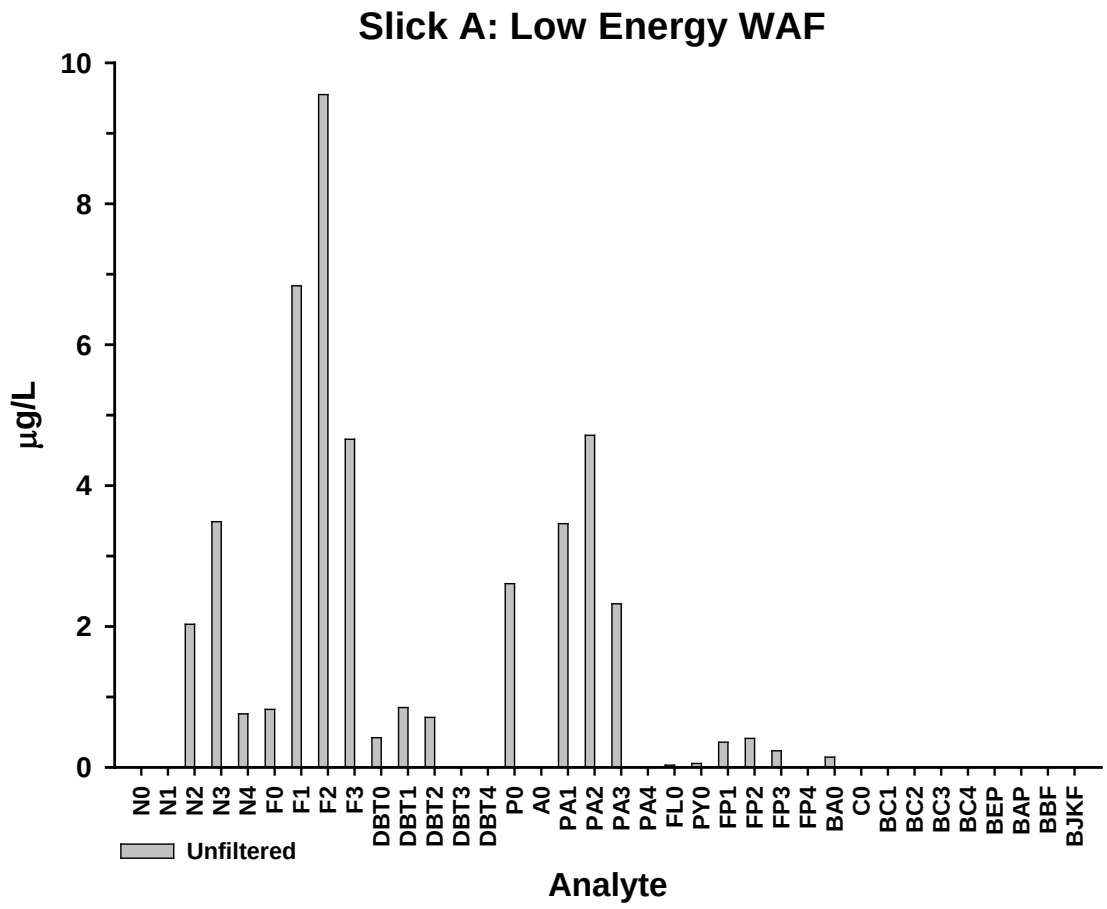


Figure C.9. Composition of a LEWAF produced using Slick A oil. See Table C.3 for analyte abbreviations and concentrations.

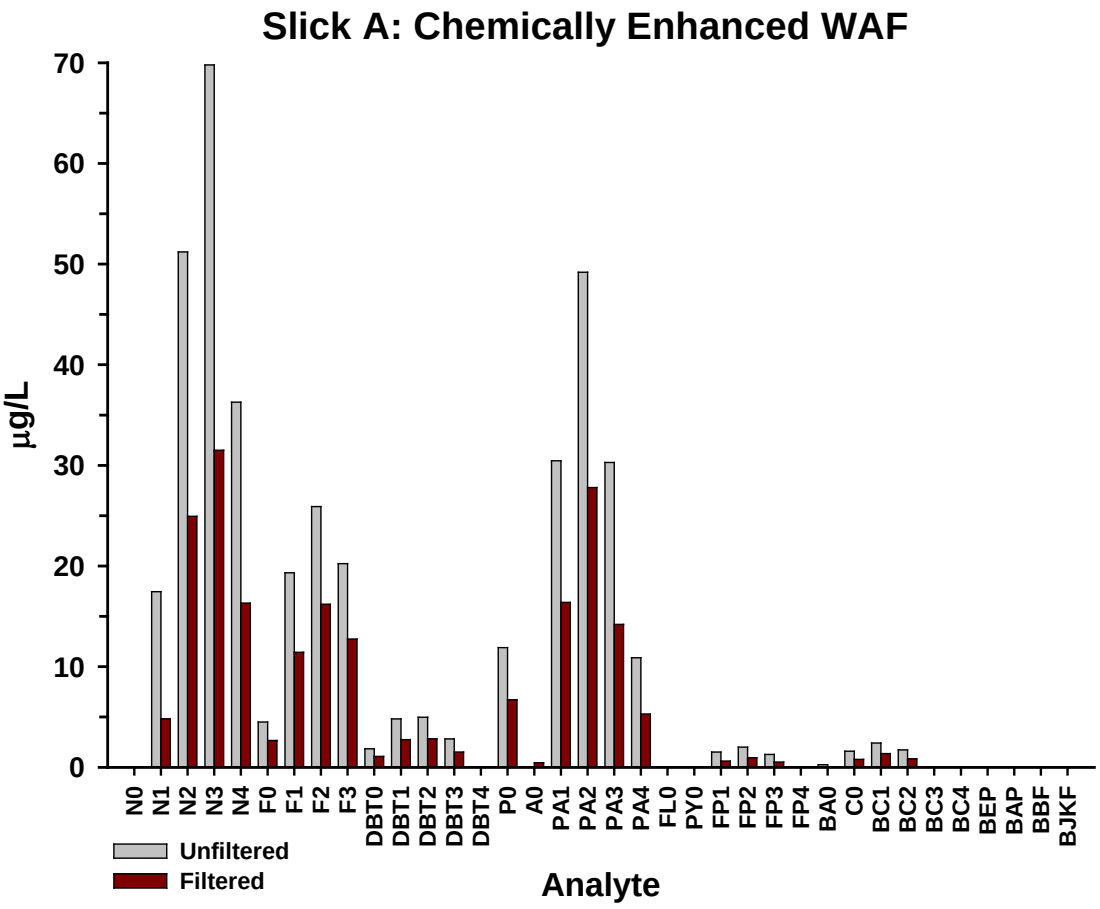


Figure C.10. Composition of a CEWAF produced using Slick A oil. See Table C.3 for analyte abbreviations and concentrations.

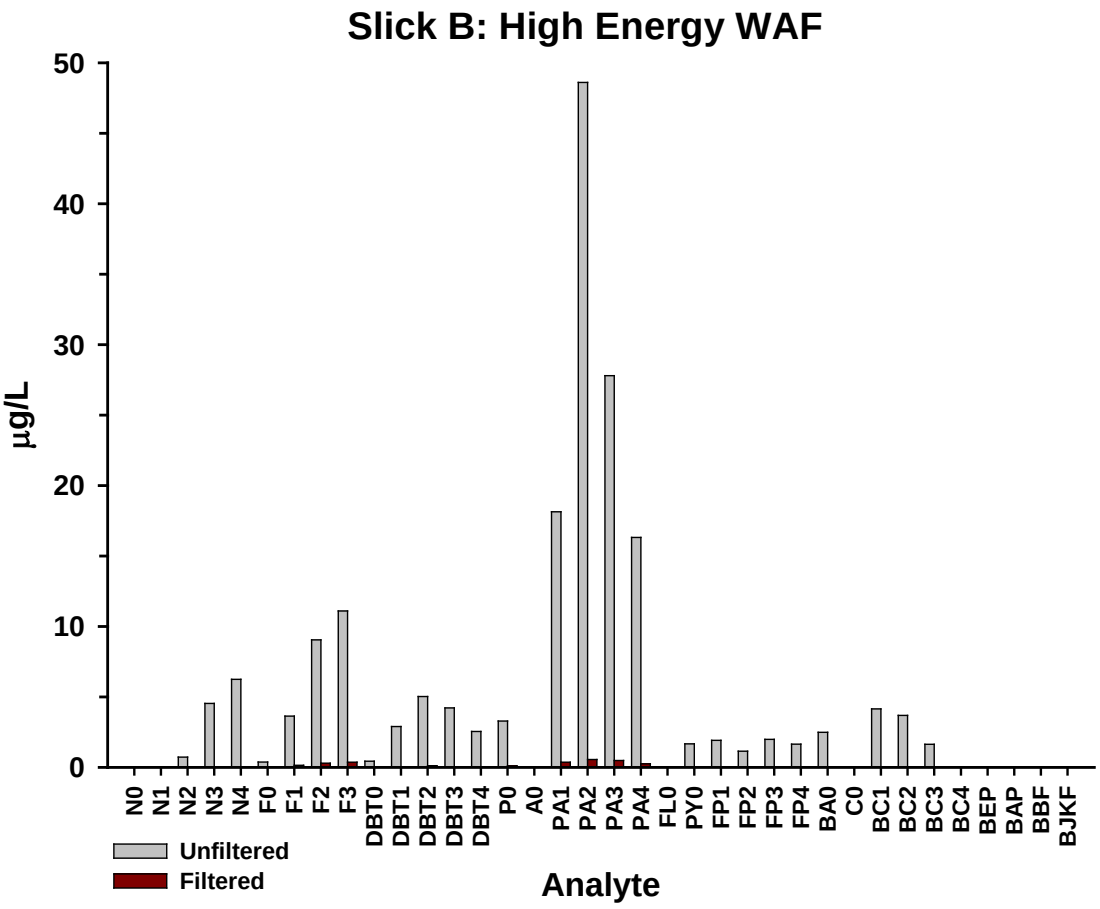


Figure C.11. Composition of a HEWAF (hand-shaking preparation) produced using Slick B oil. See Table C.4 for analyte abbreviations and concentrations.

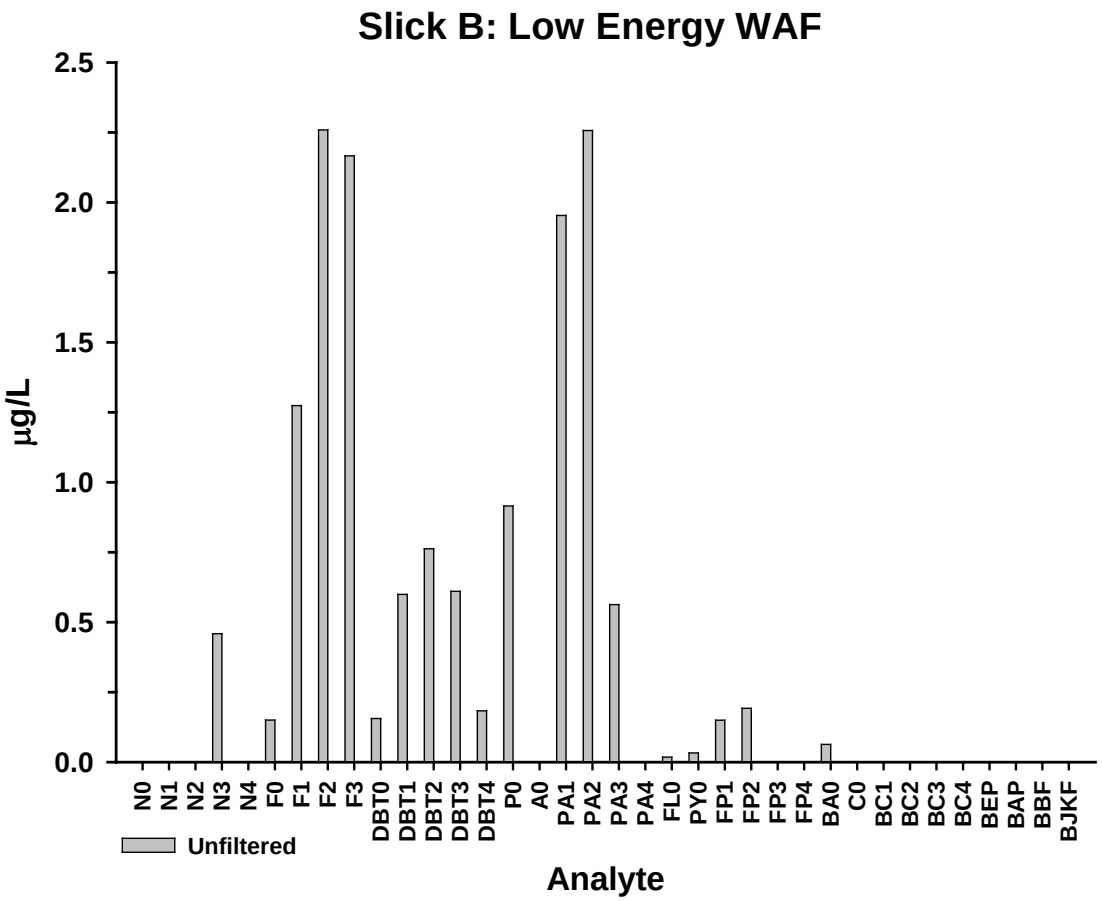


Figure C.12. Composition of a LEWAF produced using Slick B oil. See Table C.4 for analyte abbreviations and concentrations.

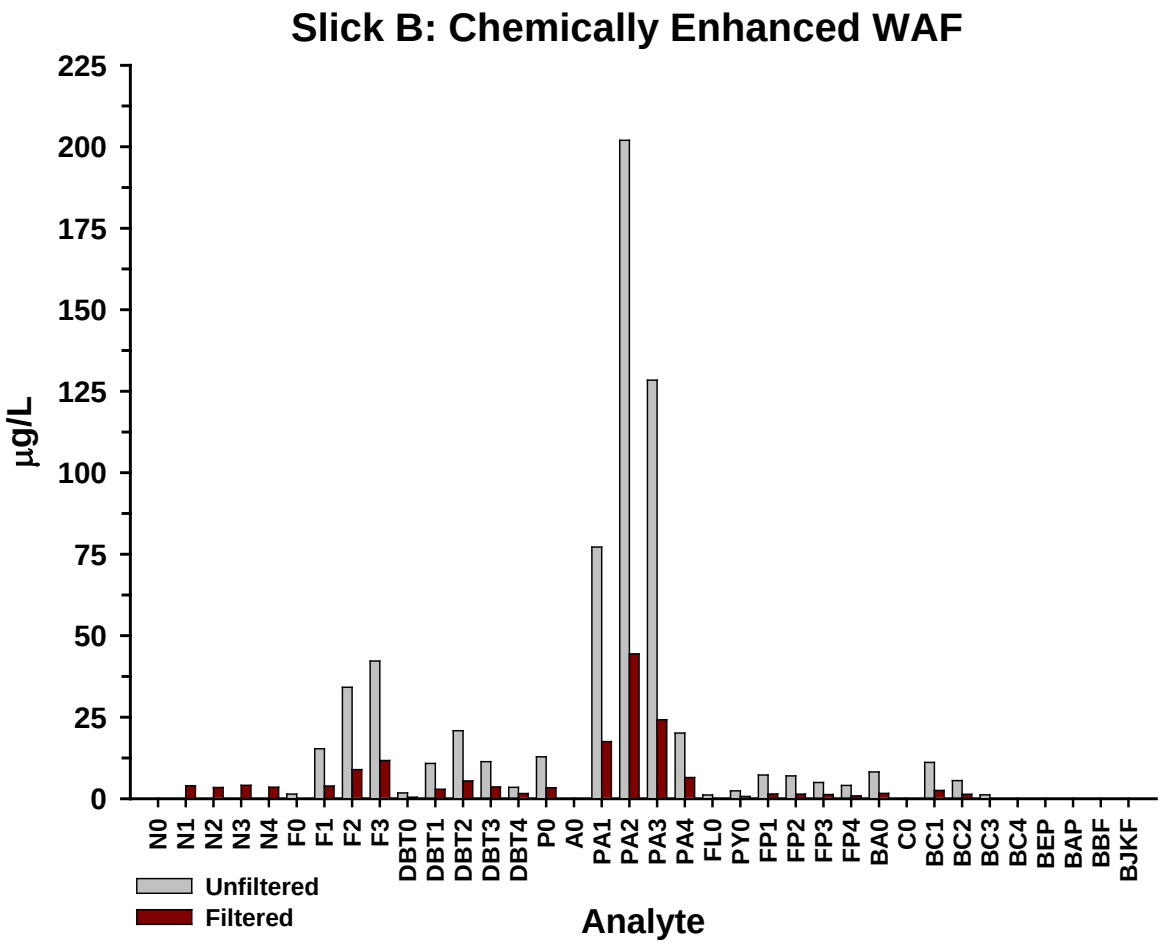


Figure C.13. Composition of a CEWAF produced using Slick B oil. See Table C.4 for analyte abbreviations and concentrations.

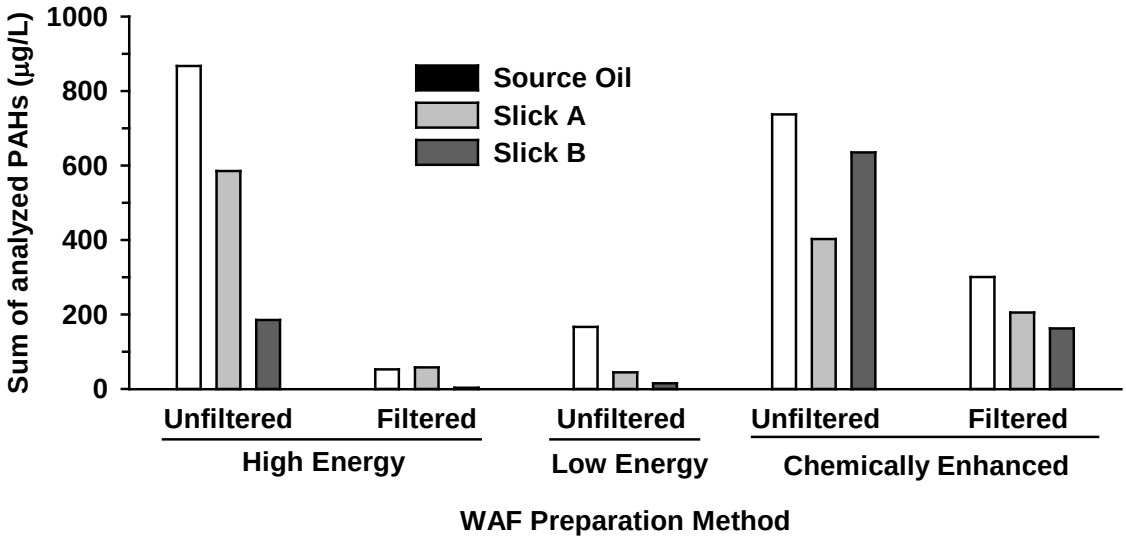


Figure C.14. Sum of analyzed PAHs in the source, Slick A, and Slick B oils for each preparation method. See Tables C.2–C.4 for PAH concentrations.