SCAT DETECTION DOGS: A NONINVASIVE/NONDESTRUCTIVE APPROACH FOR THE ENVIRONMENTAL ASSESSMENT OF BFRs

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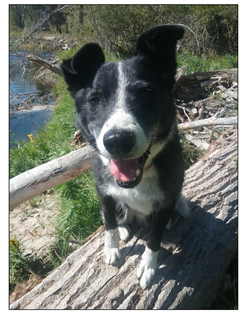

Abstract: **A prior exploratory study led by Working Dogs for Conservation (WD4C) in Montana confirmed that dispatching detection dog-handler teams to gather otter and mink scat for aquatic contaminants analysis considerably increased sampling efficiency and sample size. Building on those findings, additional target scats were collected by one of the dog-handler teams for development of a formalized analytical method for the analysis of brominated flame-retardants (BFRs) therein. Freeze-dried scat samples were extracted with dichloromethane. Extracts were purified by size exclusion and silica gel liquid chromatography and analyzed by ultra-performance liquid chromatography (UPLC)/atmospheric pressure photoionization (APPI) tandem mass spectrometry (MS/MS) for BFRs: polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl) tertabromophthalate (BEH-TEBP). Mink and otter scat samples contained several BFRs, ranging from 129 – 5130 ng g^{-1}, lipid weight. Method validation included analysis of surrogate and BFR spiked recoveries, duplicate analyses and the analysis of NIST's Standard Reference Material (SRM) #2781 (domestic sludge).**

Introduction: Scat (i.e., feces), particularly of sentinel species, is a useful noninvasive/nondestructive media for evaluating contaminant exposure in wildlife. Contaminant levels e.g. polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in the scat of mink and otter have been shown to correlate with their liver and adipose tissue levels.[1, 2] However, it is laborious and/or difficult to locate enough viable scat samples. Richards et al. (2018) recently confirmed that detection dogs could simultaneously and reliably find otter and mink scats (as confirmed by genetic testing) and preliminary analyses showed residues of brominated flame retardants (BFRs) could be detected therein.[3] However; the requisite lab capacity does not formally exist to analyze BFRs in wildlife fecal samples.

Building on this work, a WD4C dog-handler team opportunistically surveyed along several Montana rivers in autumn 2018 and spring 2019. The recovered samples were then used to develop an analytical method to determine BFR levels in scat based on procedures described for BFR analysis of sewage sludge by Hale et al. (2012) and sediments by La Guardia et al. (2013).[4, 5]

Figure 1., Dog handler-team locate fragments of an aged, crumbly otter latrine hidden in an offshore log-jam. (*photo credit: Marirose Kuhlman, MPG Ranch, MT.*)

Figure 2., Otter (top) and mink (bottom) scat.

References:

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2. Gutleb, A. C.; Kranz. A., (1998), Estimation of polychlorinated biphenyl (PCB) levels in livers of the otter (*Lutra lutra*) from concentrations in scats and fish. Water Air Soil Pollut., 106:481 – 491.
3. Richards, N. L. et al. (2018), Using scat detection dogs to monitor environmental contaminants in sentinel species and freshwater ecosystems. Pages: 193-262. *In* Using Detection Dogs to Monitor Aquatic Ecosystem Health and Protect Aquatic Resources (N.L. Richards, editor). Palgrave, Switzerland.
4. Hale, R. C. et al. (2012), Polybrominated diphenyl ethers in U.S. sewage sludge and biosolids: Temporal and geographical trends and uptake by corn following land application. Environ. Sci. Technol., 46, 2055 – 2063.
5. La Guardia, M. J. et al. (2013), Brominated flame-retardants in sub-Saharan Africa: Burdens in inland and coastal sediments in the eThekwini metropolitan municipality, South Africa, Environ. Sci. Technol., 47:9643 – 9650.
6. Davis, E. F. et al. (2012), Measurement of flame retardants and triclosan in municipal sewage sludge and biosolids. Environ. Int. 40:1 – 7.
7. National Institute of Standards and Technology, Standard Reference Material #2781 (Domestic Sludge) Gaithersburg, MD. USA

Scat collection: Searching by scent rather than sight, dog-handler teams help eliminate survey bias and offer comprehensive coverage of an area (see Figure 3). They often make finds in 'unexpected' or previously undocumented places. During the exploratory study, the dog-handler team's highest and lowest scat find rates for mink were 20.5/km and 0.3/km, for otter 5.9/km and 0.4/km, respectively. An informal performance comparison between a dog-handler team and an experienced surveyor along a 2 km shoreline yielded an 11:1 otter scat find rate (Richards et al. 2018).

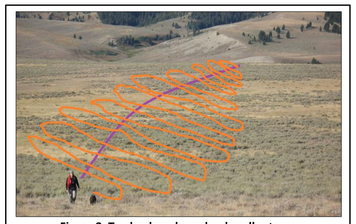


Figure 3, Tracks show how dog-handler teams thoroughly cover a survey area working together. Handler's path purple, Dog's path orange

Samples: Mink and otter scat samples (Figure 4) were collected by a dog-handler team from a small tributary of Madison R. and near a fishing access point on the lower Bitterroot R. Both sites are in relatively rural locations of western Montana (MT), USA (Figure 5). Additional samples were collected from the ground of the penning area at a now-defunct mink farm, those samples having been outside for >3 years when collected.

Figure 4, Mink and otter scat samples

Date	Amount	Species	Location
Aug. 2018	2	Otter	Madison River, MT
Aug. 2018	3	Mink	Mink Farm, MT
Sept. 2018	5	Mink	Bitterroot River, MT
Apr. 2019	1	Otter	Bitterroot River, MT

Figure 5, Montana, USA

Sample preparation and analysis [4, 5]***:***

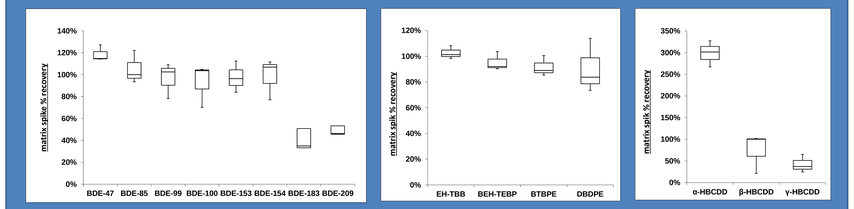
- **Samples, freeze-dried and homogenized**
- **~1 gm, Enhanced solvent extraction**
  - Accelerated Solvent Extractor (ASE 200), Thermo Scientific/Dionex
- **Size exclusion chromatography**
  - Envirosep-ABC, 350 x 21.1 mm, Phenomenex
- **Silica gel liquid chromatography**
  - 2gm silica gel (glass) columns, Biotage
    - Fraction #1, 3.5mL hexane
    - Fraction #2, 6.5mL hexane:DCM (60:40)
- **UPLC/APPI – MS/MS analysis for BFRs**
  - Acquity, Ultra Performance Liquid Chromatography, Waters
  - 3200 Q-trap LC/MS/MS System, SciEx

North American river otter (*Lontra Canadensis*)

American mink (Neovison vison)

Method validation results:

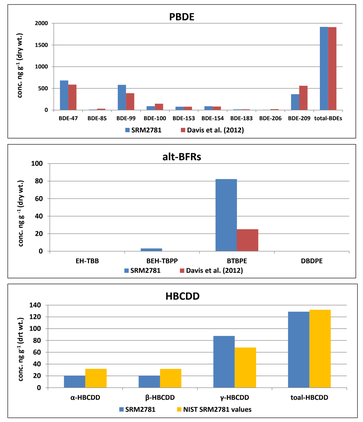
Figure 6, Matrix spike recovery (n =3):



- **Mean spiked recovery 86%, range 42 – 119%, except for $\alpha HBCDD$**
- **$\alpha HBCDD$ high recovery caused by elevated base-line. However, this result was only observed in the spiked samples**

Method validation (continued):

Figure 7, SRM-2781 analysis:



- PBDEs and alt-BFRs (except BTBPE) results are similar to published SRM data (Davis et al. 2012)[6], indicated by low Relative % Difference (RPD) values
  - << difference between total-PBDEs, 0.36% RPD
  - > discrepancy between BTBPE values, 107% RPD
- HBCDD values compare with NIST certified values[7], RPD 14.3% for total-HBCDD
- Duplicate analyses produced good analytical reproducibility, RPD range 2 - 44% for BDE-99, -100, -209 (Sample #19WDS05, Figure 8, below)

Results (native scat analysis):

Figure 8, BFR analysis mink and otter scat (ng g^{-1}, lipid wt.):

Sample ID	19WDS02	19WDS03	19WDS04	19WDS05	19WDS05 (dup)	19WDS07	19WDS08	19WDS09	19WDS10	19WDS11	19WDS12
location, date	Bitterroot 9/18	Bitterroot 9/18	Bitterroot 9/18	Madison 8/18	Madison 8/18	Bitterroot 4/19	Bitterroot 9/18	Bitterroot 9/18	Mink Farm 8/18	Mink Farm 8/18	Mink Farm 8/18
Species	Mink	Mink	Mink	Otter	Otter	Otter	Mink	Mink	Mink	Mink	Mink
BDE-47	97.3	207	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-99	32.1	9.28	111	20.6	32.0	20.7	27.8	315	57.6	47.8	59.8
BDE-100	33.6	10.5	142	20.5	31.9	40.0	53.2	373	7.09	nd	117
BDE-206	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	151
BDE-209	nd	31.5	444	87.7	68.1	92.8	45.8	4018	296	1933	1731
EH-TBB	nd	5.99	53.2	nd	nd	16.9	18.5	345	75.7	139	120
BEH-TBPH	nd	nd	9.75	nd	nd	3.24	7.23	82.3	35.2	123	42.9
DBDPE	nd	nd	nd	nd	nd	68.0	nd	nd	94.6	476	116
Total-BFRs	163	264	760	129	132	242	153	5130	566	2720	2340

- Multiple BFRs were detected in all samples, **100% detection rate**
  - Total-BFRs range 129 – 5130 ng g^{-1}
  - BDE-99 was detected in each sample, range 9.28 - 315 ng g^{-1}
  - BDE-209 was the most abundant BFR present, levels > 5000 ng g^{-1}
  - EH-TBB, BEH-TBPP and DBDPE were only detected in scat from the Bitterroot R. and mink farm, reaching 345, 82.3 and 476 ng g^{-1}, respectively
  - BDE-85, -153, -154, -183, BTBPE and HBCDD were not detected

Conclusions:

- **Scat analysis offers a noninvasive/nondestructive means of monitoring contaminants in mink and otter, among other sentinel species**
- **Analytical methodology has now been validated for several restricted and current use BFRs in otter and mink scats**
- **Analysis of mink and otter scat revealed several BFRs, totals exceeding part per million (ppm) levels**
- **Results indicate that pairing scat dog-handler team surveys for sample collection with BFR analysis of target species represents a valuable and efficient environmental monitoring tool.**