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 with 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-tetrabromobenzene (EH-TBB), and tetrabromomethylphosphate (BEH-TMB). 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. 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. However, the requisite labor 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 in sewage sludge by Hale et al. (2012) and sediments by La Guardia at al. (2013).

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 undetected 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 5.0/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).

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 the Bitterroot river basin 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.

Results:
BFR concentrations were detected in all mink and otter scat samples, BDE-209 levels were particularly high, reaching 345, 82.3 and 476 ng g⁻¹ in the mink scat samples in Madison, Bitterroot and Bitterroot R., respectively. BDE-154 was also detected in each sample, range 9.28 – 45.8 ng g⁻¹. BDE-206 was the most abundant BFR present, levels > 5000 ng g⁻¹. BEH-TBB, BEH-TMB and DBDE 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 in any of the samples.

Methodology:
Method validation (continued):

Results (native scat analysis):

Conclusions:
Scat detection 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.