

# Development of Modified EPA Method 1668 for the Detection of 209 PCB Congeners in Fish Tissue



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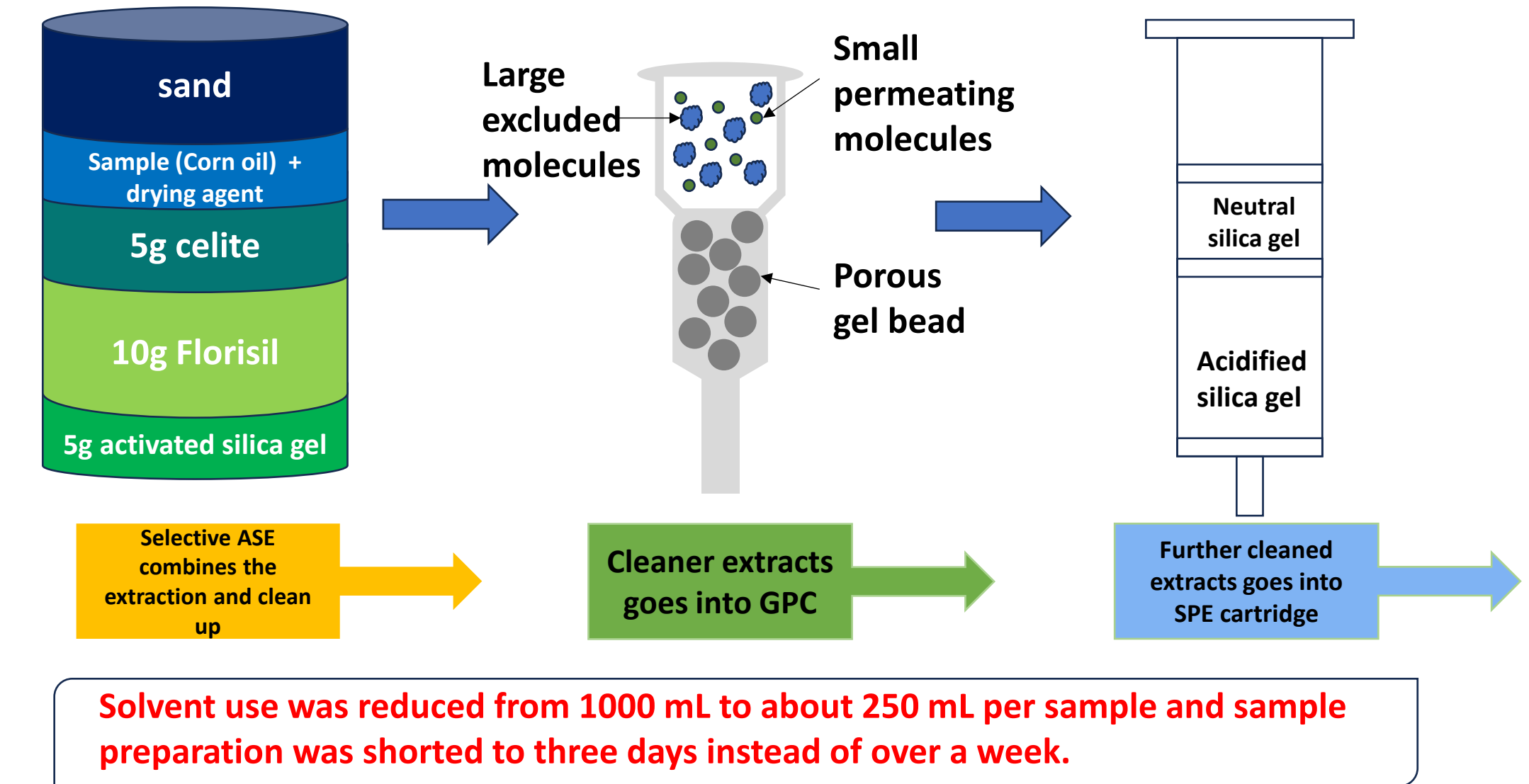
## Introduction

A growing body of scientific literature has associated Polychlorinated biphenyls (PCBs) with a multitude of health concerns, and the detection of these pollutants is a pertinent undertaking. The New Jersey Department of Health - Environmental and Chemical Laboratory Services (NJDOH-ECLS) is prompted to develop the inquired methods from the Bureau of Freshwater and Biological Monitoring of the NJ Department of Environmental Protection to perform whole tissue analyses of PCBs collected from their fish tissue monitoring program. The goal of current study is to develop and validate effective method by integration with modern automated sample preparation instruments to reduce solvent and labor cost in comparison to the original EPA method 1668B. This study presents the ongoing results of our investigation of feasibility of modified EPA method 1668 for the analysis in whole fish tissues by combining a serial of adsorbents in extraction cells, and developed the effective Gel Perpetration Chromatograph (GPC) for the subsequent clean-up of fish tissue extracts then followed by automated Solid Phase Extraction (SPE) modules for lipid removal.

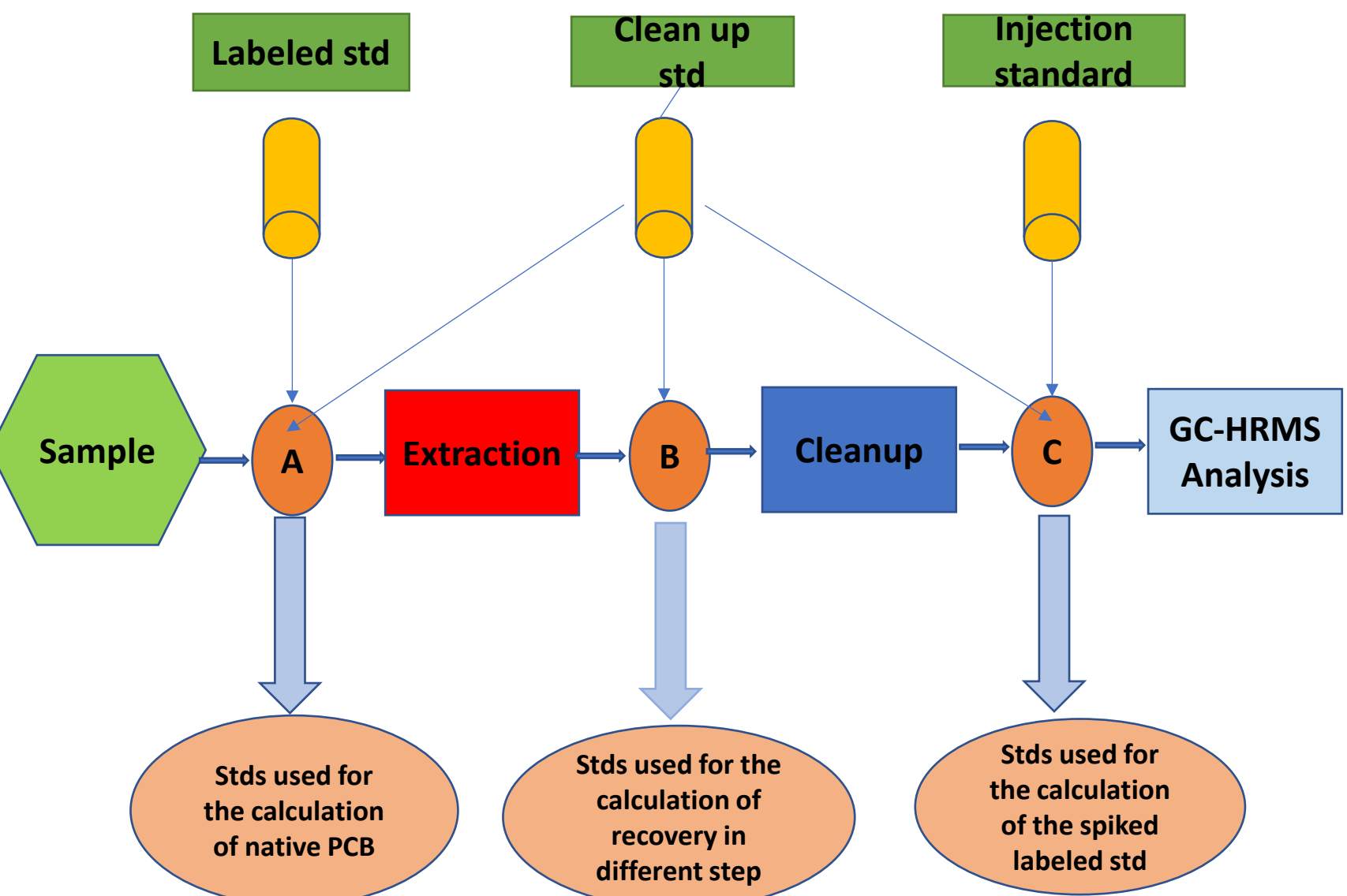
## Methodology

### Modified Sample Preparation from Method 1668C

- Original EPA Method 1668: Soxhlet extraction followed by GPC and multiple manually packed glass columns.
- Selective ASE followed by GPC automated SPE.



### Standard Addition of Isotope Dilution Quantification



## Results

Recovery of labeled PCBs in reference samples (n=7)

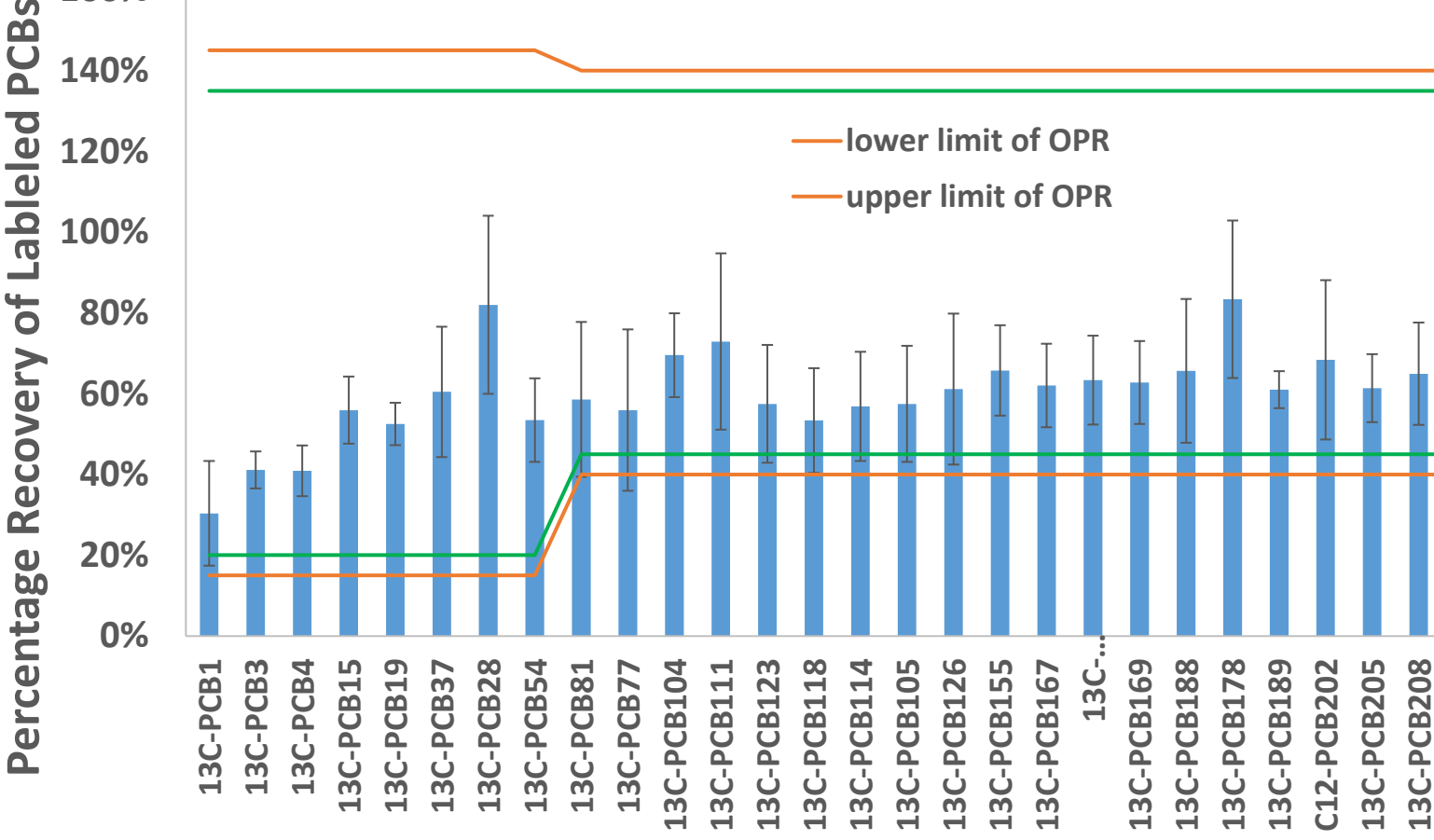


Figure 1. Comparison of the determined labeled compounds recovery in spiked corn oils (n=7) with the QC acceptance criteria for IPR, OPR.

Table 1. Comparison of the determined labeled compounds recovery in spiked corn oils (n=7) with the QC acceptance criteria for IPR, OPR.

	SA.01	SA.02	SA.03	SA.04	SA.05	SA.06	SA.07	Ave	Stdev	RSD	Acceptance Criteria
PCB1	103%	100%	80%	103%	105%	74%	99%	93%	13%	15%	70-130% 60-135% 25%
PCB3	98%	95%	72%	103%	105%	74%	99%	92%	13%	15%	70-130% 60-135% 25%
PCB4	112%	118%	98%	132%	131%	127%	107%	118%	13%	11%	70-130% 60-135% 25%
PCB15	182%	193%	151%	177%	192%	158%	114%	167%	28%	17%	70-130% 60-135% 25%
PCB19	87%	97%	71%	116%	116%	108%	107%	102%	8%	9%	70-130% 60-135% 25%
PCB37	269%	273%	222%	318%	317%	301%	133%	262%	66%	25%	70-130% 60-135% 25%
PCB54	94%	93%	75%	109%	110%	115%	97%	99%	14%	14%	70-130% 60-135% 25%
PCB81	102%	97%	79%	101%	100%	91%	93%	95%	8%	9%	70-130% 60-135% 25%
PCB77	123%	121%	98%	142%	115%	122%	123%	121%	13%	11%	70-130% 60-135% 25%
PCB104	94%	95%	72%	111%	104%	112%	94%	97%	14%	14%	70-130% 60-135% 25%
PCB123	117%	114%	87%	79%	140%	89%	105%	104%	21%	20%	70-130% 60-135% 25%
PCB118	1172%	1666%	954%	1302%	1111%	1348%	1448%	1152%	142%	15%	70-130% 60-135% 25%
PCB114	119%	116%	89%	120%	119%	119%	125%	115%	12%	10%	70-130% 60-135% 25%
PCB105	461%	426%	355%	515%	503%	518%	529%	473%	63%	13%	70-130% 60-135% 25%
PCB126	109%	100%	72%	100%	90%	88%	90%	96%	13%	13%	70-130% 60-135% 25%
PCB155	99%	99%	79%	114%	115%	117%	100%	103%	14%	13%	70-130% 60-135% 25%
PCB167	124%	125%	97%	145%	142%	138%	124%	128%	17%	13%	70-130% 60-135% 25%
PCB156/157	127%	128%	98%	146%	153%	142%	131%	132%	18%	14%	70-130% 60-135% 25%
PCB169	101%	100%	77%	108%	107%	101%	90%	99%	10%	11%	70-130% 60-135% 25%
PCB188	95%	95%	77%	110%	113%	108%	95%	99%	12%	12%	70-130% 60-135% 25%
PCB189	102%	102%	73%	104%	108%	100%	100%	98%	12%	12%	70-130% 60-135% 25%
PCB202	110%	105%	85%	123%	120%	120%	112%	114%	16%	14%	70-130% 60-135% 25%
PCB205	102%	101%	78%	114%	114%	104%	99%	102%	12%	12%	70-130% 60-135% 25%
PCB208	97%	98%	77%	111%	116%	105%	95%	100%	13%	13%	70-130% 60-135% 25%
PCB206	108%	106%	82%	118%	119%	116%	99%	107%	13%	13%	70-130% 60-135% 25%
PCB209	97%	96%	78%	108%	111%	109%	90%	99%	12%	12%	70-130% 60-135% 25%

Recovery of labeled PCBs in spiked Tilapia and Salmon (n=3)

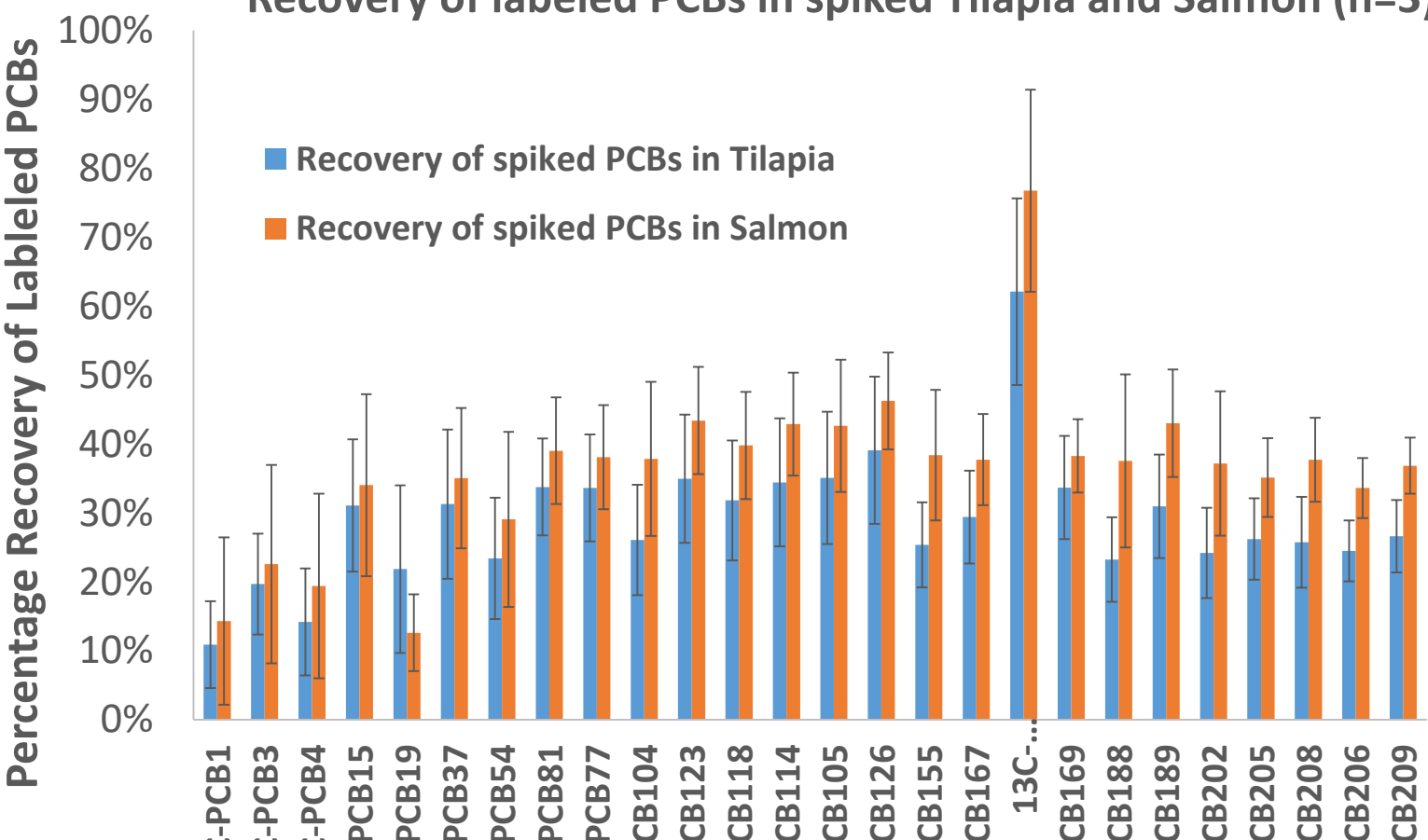


Figure 2. Labeled compounds recovery in high lipid content Salmon (n=3) and low lipid content Tilapia (n=3).

- Contamination of PCB 105, 118, 37 and 15 are the major issues for the current method.
- We have been tracking down the source of contamination along the sample preparation. The initial evidence pinpointed that the GPC is the significant source.

### Calibration Verification for Labeled and Native Compounds

Table 2. Response factor (RF) derived from single point in 209 congener is compared with the RF derived from the six level of the toxic congeners standard. The calibration verification results for labeled and native compounds are listed in left and right panel, respectively.

	RF from Verification	RF from cal curve	VER	Calculated ratio	Acceptable Range
13C-PCB1	1.13	1.22	93%	50-145%	
13C-PCB3	1.18	1.25	94%	50-145%	
13C-PCB4	0.56	0.58	97%	50-145%	
13C-PCB15	1.38	1.52	91%	50-145%	
13C-PCB19	0.42	0.43	96%	50-145%	
13C-PCB37	1.10	1.94	57%	50-145%	
13C-PCB28	1.08	1.13	96%	50-145%	
13C-PCB54	1.17	1.18	99%	50-145%	
13C-PCB81	1.88	2.25	83%	50-145%	
13C-PCB77	1.88	2.31	81%	50-145%	
13C-PCB104	0.74	0.62	119%	50-145%	
13C-PCB111	1.22	1.33	92%	50-145%	
13C-PCB123	1.30	1.46	90%	50-145%	
13C-PCB118	1.20	1.52	79%	50-145%	
13C-PCB114	1.24	1.50	83%	50-145%	
13C-PCB105	1.13	1.41	80%	50-145%	
13C-PCB126	1.13	1.13	101%	50-145%	
13C-PCB155	1.15	1.13	101%	50-145%	
13C-PCB167	1.23	1.34	92%	50-145%	
13C-PCB156/157	2.15	2.56	84%	50-145%	
13C-PCB169	0.97	1.28	76%	50-145%	
13C-PCB188	1.71	1.50	114%	50-145%	
13C-PCB178	1.13	0.96	118%	50-145%	
13C-PCB189	1.37	1.57	87%	50-145%	
13C12-PCB202	1.41	1.24	113%	50-145%	
13C-PCB205	1.18	1.45	81%	50-145%	
13C-PCB208	1.25	1.26	99%	50-145%	
13C-PCB206	0.77	0.95	82%	50-145%	
13C-PCB209	1.06	1.12	95%	50-145%	

	RF from Verification	RF from cal curve	VER	Calculated ratio	Acceptable Range
PCB1	1.01	1.07	94%	75-125%	
PCB3	1.00	1.08	93%	75-125%	
PCB4	0.71	0.77	92%	75-125%	
PCB15	0.67	0.70	96%	75-125%	
PCB19	1.07	1.16	92%	75-125%	
PCB37	1.07	1.11	96%	75-125%	
PCB54	1.24	1.03	120%	75-125%	
PCB81	0.93	1.33	70%	75-125%	
PCB77	1.16	1.21	96%	75-125%	
PCB104	1.37	1.15	119%	75-125%	
PCB123	0.98	1.14	86%	75-125%	
PCB118	1.10	1.28	86%	75-125%	
PCB114	1.10	1.30	84%	75-125%	
PCB105	0.99	1.55	64%	75-125%	
PCB126	1.16	1.35	86%	75-125%	
PCB155	1.14	1.10	104%	75-125%	
PCB167	1.18	1.88	63%	75-125%	
PCB156/157	1.10	0.90	122%	75-125%	
PCB169	1.29	1.47	88%	75-125%	
PCB188	1.15	1.28	90%	75-125%	
PCB189	1.10	1.38	80%	75-125%	
PCB202	1.10	1.44	76%	75-125%	
PCB205	1.06	1.37	77%	75-125%	
PCB208	1.04	1.46	71%	75-125%	
PCB206	1.10	1.43	77%	75-125%	
PCB209	1.02	1.51	67%	75-125%	

## Conclusions

- The recovery of all labeled compounds are within the acceptance range of EPA Method 1668.
- The recovery of majority of native compounds are within the acceptance range of EPA method 1668 with the exception of PCB 105, 118, 15 and 37.
- The recovery of spiked labeled compounds in Salmon and Tilapia tissues representative of high and low lipid content are comparable to the range of recovery from variety of fish species in EPA method 1668C.
- New sample cleanup method lowers preparation time from one week to three days. Additionally, sample preparation also reduces solvent consumption by half.
- Method development results demonstrated that this method was precise, accurate and robust with high-throughput potential that suitable for monitoring studies.
- Future work will address the background contamination, particularly PCB 105 and 118. In addition, we also plan to test the current method with certified fish tissue samples.

## References

- U.S. Environmental Protection Agency (EPA). Method 1668C: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS. EPA-820-R-10-005, April 2010.
- Subedi, B., Usenko, S. (2012). Enhanced pressurized liquid extraction technique capable of analyzing polychlorodibenzo-p-dioxins, polychlorodibenzofurans, and polychlorobiphenyls in fish tissue. *Journal of Chromatography A*, Volume 1238, 30-37.

## Acknowledgements

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