Targeted and Non-Targeted Analysis of Serum Pools to Provide Chemical Exposure Data for Exposure Modeling and Chemical Prioritization[1]

ABSTRACT

Biomonitoring data can help inform the development and calibration of high-throughput exposure modeling for use in chemical prioritization and risk evaluation. A pilot project was conducted to evaluate the feasibility of using pooled banked blood samples to generate initial data on population blood concentrations for compounds not to date routinely assessed in biomonitoring efforts such as the National Health and Nutrition Examination Survey (NHANES). Serum samples were obtained from the NIEHS Clinical Research Unit. Serum pools were constructed from 25 individual 1 ml aliquots. Four pools each were constructed based on samples stratified by age (<45 vs. 45 and greater) and male vs. female, for a total of 16 pools. Samples were analyzed in triplicate using GCxGC-TOFMS and LC-qTOFMS analysis (positive and negative mode). An exposure- and risk-based prioritization scheme was used to identify approximately 130 chemicals of interest subjected to additional processing and evaluation. Targeted GCxGC was used to assess seven chemicals: triclosan, bisphenol A, dibutyl phthalate (DBP), dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-octylphthalate (DNOP). Although these chemicals are rapidly metabolized, levels exceeding those in method blanks were detected in most samples for all but DNOP and DMP. Among the gender and age stratifications, only DEP showed a significant ($p < 0.05$) difference between groups, with age >45 having a mean of 0.24 (sd 0.06) and age ≤45 having mean 0.17 (0.04). Concentrations of DEP and triclosan in serum were similar to those previously reported in other studies. Non-targeted GC analysis found an average of 355 detections per sample. Females had a significantly higher (p-value 0.00018) number of detections (mean 440, sd 89) than males (mean 270, sd 26). There was evidence of contamination with ubiquitous analytical contaminant compounds, both in solvent blanks and, indirectly, in field collection steps. For compounds with NHANES serum data, results here were generally consistent. This research provides only limited support for a broader effort to measure infrequently biomonitored chemicals in serum and highlights issues associated with sample collection, storage, QA/QC when using stored samples collected for biomonitoring.

1. BACKGROUND

To date, human biomonitoring (HBM) in blood or serum has been applied across a limited set of chemicals with known or likely human health impacts. For example, CDC's National Biomonitoring Program measures about 300 environmental chemicals in people, but many of these are cogeners of PCBs and PCDDs.

As high throughput and high content in vitro screening for evaluating bioactivity of chemicals has accelerated, there's a pressing need for HBM data across the larger commercial chemical space to:

1. To evaluate high-throughput exposure models designed to predict external exposure rates;
2. To evaluate in vitro-in vivo extrapolation (IVIVE) models that predict internal concentrations from external exposures; and
3. To compare to in vitro bioactivities in an exposure/risk context.

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2. OBJECTIVE

This project assesses the feasibility of using pooled banked serum to provide a valid representation of population central tendency concentrations of selected chemicals in blood to inform those goals.

3. SERUM SAMPLES

Serum samples were obtained from the NIEHS Clinical Research Unit specimen bank of 3500 adults recruited from the Durham, NC area. Sixteen pools were constructed, 4 each from 4 demographic groups:

- Women < 45 years;
- Women ≥ 45 years;
- Men < 45 years;
- Men ≥ 45 years.

Each pool was constructed with twenty-five 1mL aliquots, with samples selected randomly from the available specimens in the respective category.

4. CHEMICAL SELECTION FOR TARGETED ANALYSIS

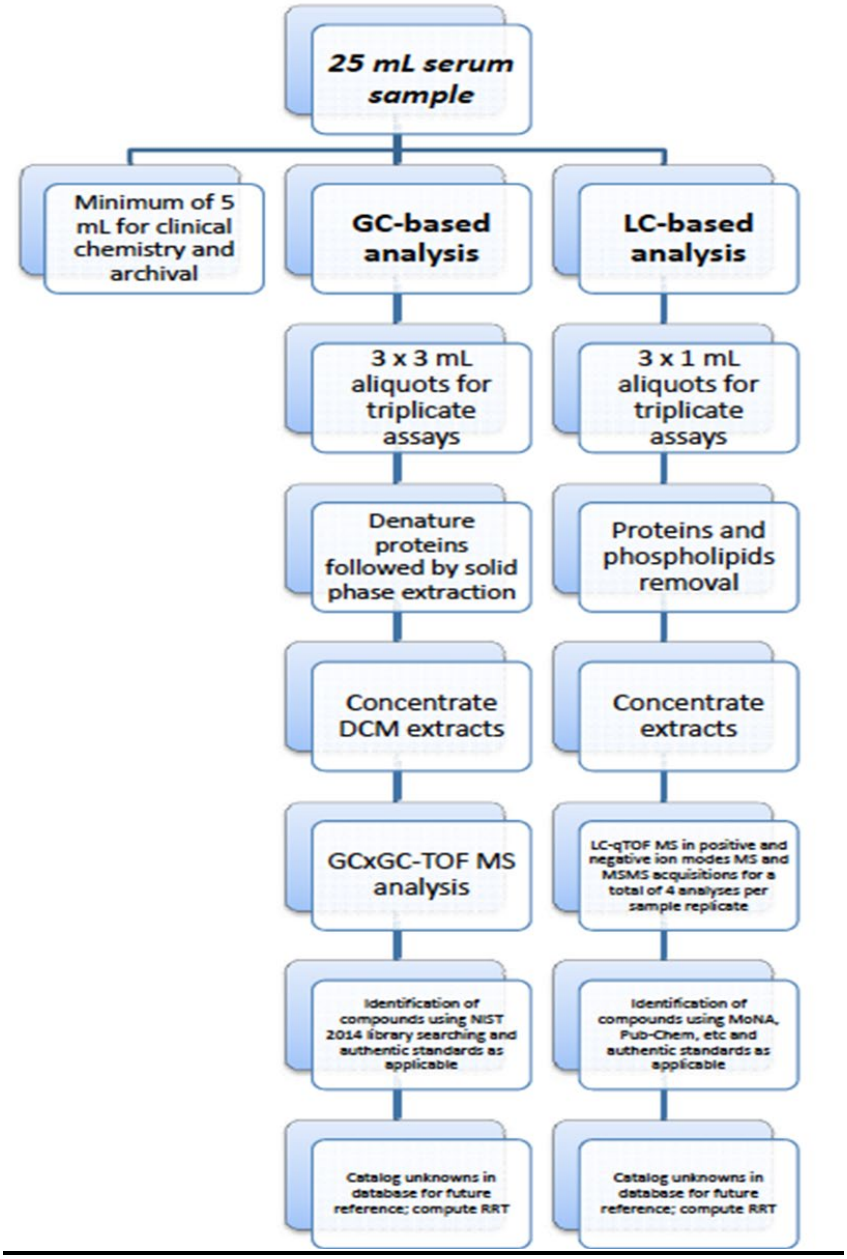
- 35 chemicals were selected from a starting list of 7986 Tox21 Library chemicals for which high-throughput chemical descriptors could be obtained, weighted for presence of:
  - NHANES serum data;
  - human physiologically-based pharmacokinetic (PBPK) models;
  - NHANES urine, IVIVE toxicokinetic (TK), and urinary excretion data;
  - presence of IVIVE TK data;
  - Top 100 predicted external exposures per Shin et al., 2014 and Wambaugh et al., 2015 (+ the bottom 100 from Wambaugh).
  - chemicals with low activity:exposure ratios (AERs) per Wetmore et al., 2015, and Browne et al., 2015.

5. ANALYTICAL WORKFLOW AND EVALUATION

The analytical workflow is presented in Figure 1. Samples were analyzed in triplicate using two-dimensional gas chromatography-time of flight mass spectrometry (GCxGC-TOFMS) and liquid chromatography quadrupole-TOFMS analysis (positive and negative mode). Chemical reference standards were provided by EPA. Detection limits or measured concentrations were assessed in a variety of ways to evaluate the sensitivity of the approach and value of data.
Comparisons were made to:

- NHANES serum biomonitoring data (where available).
- AC50 data for active ToxCast assays
- High-throughput TK predictions (Wetmore et al., 2015) were used to estimate oral equivalent doses (OEDs) corresponding to detection limits or measured concentrations (where applicable). OEDs were compared to:
- Reference doses (RfDs), where available
- ExpoCast exposure predictions (median estimate of population average) (Wambaugh et al., 2014).

Figure 1. Analytical Workflow



6. RESULTS

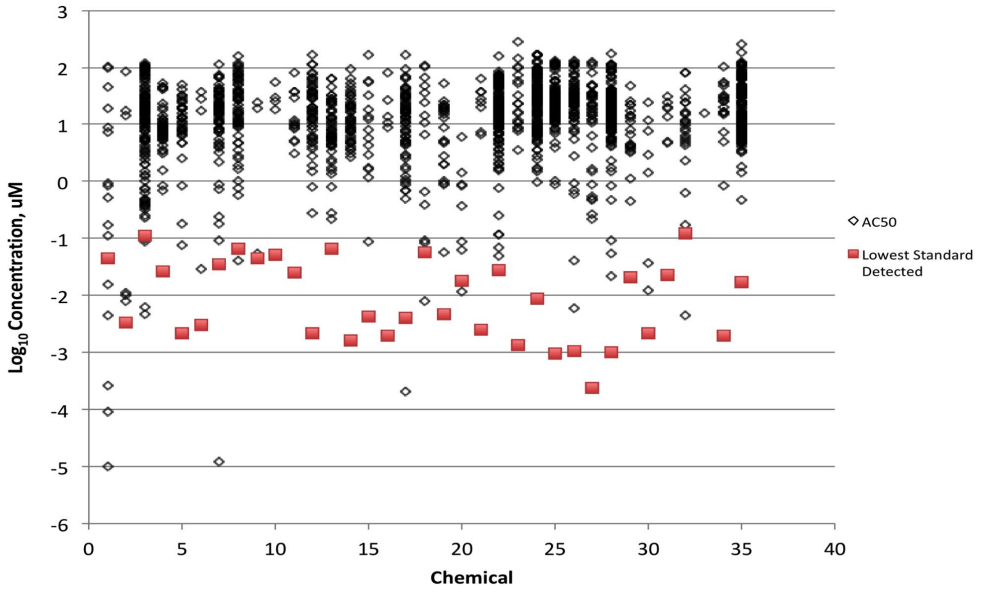
6.1 Chemicals Analyzed: The list of top 35 prioritized chemicals is presented in Table 1 along with lowest detected standard, estimated corresponding OED, RfDs (where available), and ExpoCast population average exposure estimates for adults. OEDs associated with lowest detected standard concentrations were often sensitive enough relative to RfDs, but not compared to ExpoCast exposure estimates. The methods used were designed to provide a broad scan for compounds, not optimized for individual chemicals, thus they are not as quantitatively precise as potentially achievable. All samples were run in triplicate and internal standards added to samples. For QA/QC, solvent blanks, but there were no field collection blanks available.

Table 1. Comparisons of analytical parameters and exposure metrics of interest for target chemicals. Experimental, detection limit (DL), dose-response (OEDs, RfDs), and exposure predictions (e.g., ExpoCast) are listed. Green cells indicate analytical sensitivity sufficient to detect concentrations associated with OEDs below existing RfDs and/or ExpoCast predictions.

#	Chemical Name	CASRN	LC+/LC-/GC	Lowest standard detection (ng/g)	OED for lowest detected standard, mg/kg-d	RfD (mg/kg-d)	Ref.	Sensitivity for RfD?	ExpoCast prediction (mg/kg-d)	Sensitivity for ExpoCast?
1	2,4-Dichlorophenoxyacetic acid	94-75-7	LC-	10	1.0E-03	0.21	1	Y	7E-07	N
2	Dioctyl phthalate	117-84-0	GC	10	6.1E-04				0.0002	N
3	Perfluoroundecanoic acid	2058-94-8	LC-	5	3.2E-05				4E-07	N
4	Dibutyl phthalate	84-74-2	GC	10	2.2E-02	0.1	4	Y	0.0001	N
5	Carbendazim	10605-21-7	LC+	5	1.1E-01	0.025	2	N	9E-09	N
6	Methyleugenol	93-15-2	GC	10	3.9E-02				7E-09	N
7	Acetaminophen	103-90-2	LC+	0.5	9.3E-03				1E-08	N
8	Mirex	2385-85-5	GC	10	1.8E-03	0.0002	4	N	3E-06	N
9	PFDA	335-76-2	LC-	0.5	3.2E-06				6E-07	N
10	Endrin	72-20-8	GC	25	2.1E-04	0.0003	4	Y	2E-07	N
11	Dimethyl phthalate	131-11-3	GC	10	1.7E-02				8E-05	N
12	PFNA	375-95-1	LC-	0.5	3.2E-06				9E-06	Y
13	Thiacloprid	111988-49-9	LC+	0.5	1.8E-03	0.004	3	Y	1E-08	N
14	Icaridin	119515-38-7	LC+	1	1.4E-02				9E-09	N
15	Simazine	122-34-9	GC	25	1.9E-01	0.005	4	N	9E-09	N
16	Pymetrozine	123312-89-0	LC+	5	1.9E-01	0.008		N	1E-08	N
17	Imidacloprid	138261-41-3	LC+	0.5	4.5E-03	0.057	3	Y	1E-08	N
18	Monuron	150-68-5	LC+	0.5	2.9E-04				1E-08	N
19	Metribuzin	21087-64-9	LC+	1	1.2E-03	0.013	3	Y	1E-08	N
20	Fenamiphos	22224-92-6	LC+	0.5	4.8E-03	0.0003	3	N	1E-08	N
21	Pirimicarb	23103-98-2	LC+	5	7.1E-02				1E-08	N
22	Propamocarb hydrochloride	25606-41-1	LC+	0.5	9.1E-03	0.12	2	Y	1E-08	N
23	Diuron	330-54-1	LC+	0.5	4.0E-03	0.002	4	N	1E-08	N
24	Linuron	330-55-2	LC+	1	1.3E-02	0.0077	2	N	1E-08	N
25	Carboxin	5234-68-4	LC+	0.5	8.3E-03	0.1	4	Y	1E-08	N
26	Strychnine	57-24-9	LC+	10					1E-08	Y
27	Cyromazine	66215-27-8	LC+	0.5	1.2E-03	0.015	2	Y	7E-09	N
28	Dieldrin	60-57-1	GC	25	2.1E-03	0.00005	4	N	1E-07	N
29	o,p'-DDT	789-02-6	GC	10	1.4E-03				1E-07	N
30	PFOS	1763-23-1	LC-	0.5	6.5E-06				6E-07	N
31	PFOA	335-67-1	LC-	0.1	4.6E-06				1E-05	Y
32	Triclosan	3380-34-5	LC-	25	1.1E-02	0.3	3	Y	2E-06	N
33	Perfluoroheptanoic acid	375-85-9	LC-	0.5	3.2E-06				5E-07	N
34	Bisphenol A	80-05-7	GC	25	1.3E-01	0.05	4	N	6E-05	N
35	Diethyl phthalate	84-66-2	GC	10	NA	0.8	4		0.0001	
Notes: A: priority ranking; B: Analytical method (LC in negative or positive ion mode; GC);										
C: Lowest detected standard concentration on calibration curve; D: OED, Wetmore *et al.,* 2015; E: OED for DL<RfD; F: Median prediction of average population exposure from Expocast (Wambaugh et al. 2015); G: OED for DL<Expocast prediction										
Refs: 1)	USEPA Office of Pesticide Programs 2013. Memorandum: 2,4-D. Human Health Risk Assessment. DP No. D389455; 2) US EPA Office of Water HHBP; 3) US EPA OCSPP Pesticide Database; 4) US EPA IRIS									

In general, methods were sensitive enough to detect concentrations below ToxCast bioactive concentrations. The detection limits are compared to active concentrations (AC50s) from ToxCast bioassays in Figure 2.

Figure 2: AC50s and lowest detected standard concentrations by chemical. Chemical number refers to Table 1.



6.2 Results of GCxGC-TOFMS Targeted Analysis Was Used to Screen for 15 Compounds:

Six of the 15 compounds screened were detected: Phthalates esters (DEP, DMP, DBP and DiNOP), Triclosan, and BPA. The results are summarized in Table 2. Phthalate diesters and BPA were detected in method blanks. Interpretation of these detected pool concentrations is difficult because 1) no field blanks are available, and contamination issues are widely documented for these compounds; 2) phthalate diesters are likely sampling/storage artifacts because the diesters would be expected to be metabolized in vivo to monoester; 3) BPA also unlikely to be present as free parent compound, but is known to be a ubiquitous contaminant in sample collection/analysis. Triclosan concentrations were similar across pools, at approximately 20 ng/g, within the range of free triclosan concentrations previously reported in controlled dosing studies.

Table 2. Discussion of the 6 Chemicals Detected

Compound	Comments
DEP, DBP, DiNOP, BPA	Frequently detected in solvent blanks No field blanks available Known ubiquitous contaminants in sample collection materials, lab ware, etc. CONCLUSION: Results are unreliable
DMP	1 detection in 1 replicate of 1 pool Not detected in solvent blanks No field blanks available Unlikely to be present in metabolized form CONCLUSION: Results are unreliable
Triclosan	Not detected in solvent blanks No field blanks available Reported concentrations below limit of quantitation CONCLUSION: Results are unreliable

6.3 Results of LC-qTOFMS Targeted Analysis Was Used to Screen for 23 Compounds: Nine of the 23 compounds screened were detected

- Acetaminophen, all pools (except 1 male); concentrations consistent with those expected related to therapeutic use.
- PFOA, PFOS, PFNA, PFDeA, all female pools and most male pools; average concentrations generally consistent with most recent NHANES serum data for these compounds (Table 3).
- Linuron, propamocarb, fenamiphos, metribuzin, in a few male pools (but no female pools); levels reported are close to LOD.

Table 3: Average serum pool concentrations for detected PFASs, ng/g. NHANES data from 2013-2014 (GM= geometric mean; P95=95th percentile).

	PFOS	PFOA	PFNA	PFDeA
Women<=45	4.1 (2.1)	0.4 (0.3)	1.0 (0.09)	0.1 (0.07)
Women>45	7.3 (3.8)	0.8 (0.5)	1.1 (0.2)	0.1 (0.09)
Men<=45	3.9 (1.1)	0.6 (0.7)	0.9 (0.09)	0.05 (0.03)
Men>45	3.9 (1.1)	ND	0.9 (0.05)	0.03 (0.02)
NHANES GM	5.2	2.0	0.7	0.19
NHANES P95	19.5	5.6	2.0	0.8

6.4 Summary of Results

A strategy was proposed to conduct serum biomonitoring for a wide range of compounds to provide information for evaluation of high-throughput exposure models, evaluate *in vitro-in vivo* extrapolation (IVIVE) models that predict internal concentrations given an external exposure, and comparison of *in vitro* bioactivities in an exposure/risk context.

A target list of 35 chemicals was identified from previously assessed ToxCast chemicals, emphasizing chemicals with data to allow interpretation of measured concentrations. Pooled serum samples from the NIEHS Clinical Research Unit were used.

- The analytical methods were sufficiently sensitive in the context of bioactive concentrations in ToxCast, but not sufficiently sensitive to provide information to inform ExpoCast modeling.
- Of 35 prioritized chemicals, 15 were detected in 1 or more pools. However, at least 5 of these (phthalate diesters and BPA) were likely to be present as contaminants, highlighting the difficulty of using samples collected for purposes other than biomonitoring.
- Selected PFASs and acetaminophen were frequently detected at plausible concentrations.
- Subsequent evaluation of laboratory blank data indicated that the detected PFAS compounds were also present in laboratory blanks, rendering the results suspect for these compounds.

7. CONCLUSIONS

There was evidence of contamination with ubiquitous analytical contaminant compounds, both in solvent blanks and, indirectly, in field collection steps. For compounds with NHANES serum data, results here were generally consistent. The detection limits for the non-detected compounds were above NHANES concentrations. The use of IVIVE and comparison with RfDs was not possible for the majority of substances evaluated because of issues with detection limits and contamination. In nearly all cases detected and non-detected concentrations were below the bioactive concentrations

This research provides only limited support for a broader effort to measure infrequently biomonitored chemicals in serum and highlights issues associated with sample collection, storage, QA/QC when using stored samples collected for biomonitoring.

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