Product Deformulation to Identify Exposure Pathways for ToxCast Chemicals

John Wambaugh[1], Alice Yau[2], Katherine Phillips[3,4], Kristin A. Favela[2], Chantel Nicolas[1,4], Derya Biryol[3,4], Christopher Grulke[1,5], Ann M. Richard[1], Paul Price[3], Antony Williams[1], Kristin Isaacs[3], Russell Thomas[1]

National Center for Computational Toxicology[1] and National Exposure Research Laboratory[3], U.S. Environmental Protection Agency, Office of Research and Development
Southwest Research Institute[2], Oak Ridge Institute for Science and Education[4], Lockheed Martin[5]

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ORCID: 0000-0002-4024-534X
John Wambaugh | wambaugh.john@epa.gov | 919-451-5400

Abstract

High throughput screening (HTS) data that characterize chemically induced biological activity have been generated for thousands of chemicals by the US interagency Tox21 and the US EPA ToxCast programs. In many cases there are no data available for comparing bioactivity from HTS with relevant human exposures. The EPA's ExpoCast program is developing high-throughput approaches to generate the needed exposure estimates using existing databases and new, high-throughput measurements. The exposure pathway (i.e., the route of chemical from manufacture to human intake) significantly impacts the level of exposure. The presence, concentration, and formulation of chemicals in consumer products and articles of commerce (e.g., clothing) can therefore provide critical information for estimating risk. We have found that there are only limited data available on the chemical constituents (e.g., flame retardants, plasticizers) within most articles of commerce. Furthermore, the presence of some chemicals in otherwise well characterized products may be due to product packaging. We are analyzing sample consumer products using 2D gas chromatograph (GC) x GC Time of Flight Mass Spectrometry (GCxGCTOF/MS), which is suited for forensic investigation of chemicals in complex matrices (including toys, cleaners, and food). In parallel, we are working to create a reference library of retention times and spectral information for the entire Tox21 chemical library. In an examination of five plastic children's toys, as many as 114 and as few as 56 chemicals were identified in each toy, with between 0 and 40 unidentified chemicals in each. Putative endocrine disrupter, Bisphenol A (BPA), was identified as present in a product marked "BPA free". Information on chemical constituents of products, while only a prerequisite to actual exposure, provides heuristics for estimating likely human exposure pathways for thousands of chemicals.

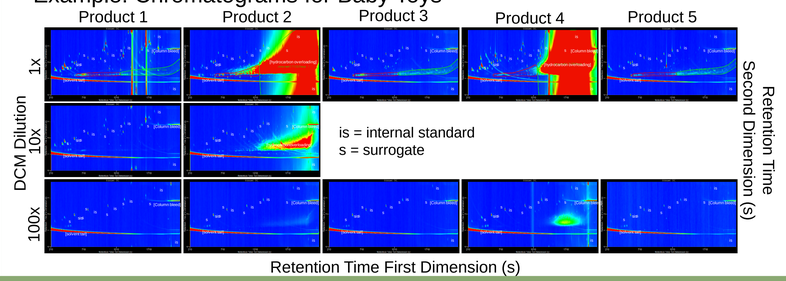
Introduction

- In most cases there are limited human exposure data available to compare with bioactivity from high-throughput screening (HTS) (Egeghy et al., 2012)
- The EPA's Exposure Forecasting (ExpoCast) program is developing high-throughput approaches to generate the needed exposure estimates
- The presence of chemicals in consumer products and elsewhere in the home ("near field" sources) is a key driver of high exposure levels in Centers for Disease Control (CDC) National Health and Nutrition Survey (NHANES) biomonitoring data (Wambaugh et al., 2014)

Methods

- Southwest Research Institute conducted analytical chemistry screening for large numbers of chemicals in consumer products and articles of commerce
  - Five sample products were arbitrarily selected from -each of twenty different categories
- Products were analyzed using two dimensional gas chromatograph (GC) x GC Time of Flight Mass Spectrometry
  - Chemical presence and approximate quantitation relative to reference chemicals (internal standards) was determined
  - All dilutions and extractions used Dichloromethane (DCM) *(Hexane:Ether was also examined initially, but had a higher background)*
  - Dilution level and processing were tailored to Mass spectra for some each sample; 1x, 10x and/or 100x
- Data processing
- GC features were matched to NIST 07 spectral database for tentative chemical identification
  - Compounds within some chemical classes are very similar, making definitive identifications difficult
  - Some peaks have a large, unresolved region of hydrocarbons in the C17-C32 range
    - Classifications used to manage hydrocarbon regions were ambiguous

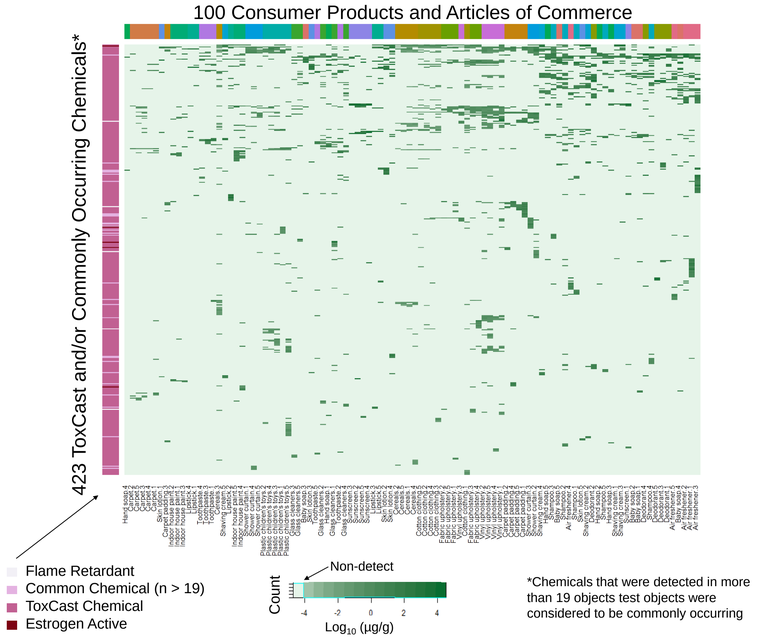
Example: Chromatograms for Baby Toys



Results

A total of 3803 unique chemical signatures were observed in test objects. Of these, 1597 were associated with a tentative chemical identification and 41 had confirmed chemical identities. Test objects consisted of five arbitrary products in each of the following twenty categories:

- Air freshener
- Baby soap
- Carpet
- Carpet padding
- Cereals
- Cotton clothing
- Deodorant
- Fabric upholstery
- Glass cleaners
- Hand soap
- Indoor house paint
- Lipstick
- Plastic children's toy
- Shampoo
- Shaving cream
- Shower curtain
- Skin lotion
- Sunscreen
- Toothpaste
- Vinyl upholstery



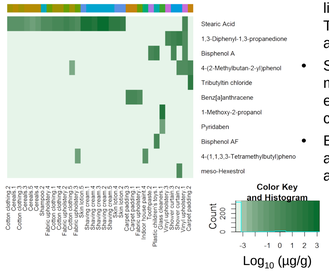
*Chemicals that were detected in more than 19 objects test objects were considered to be commonly occurring

1606 tentative and confirmed chemical identifications

- 184 of 1797 chemicals with previously known consumer product use (CPCPdb[4])
- 520 of 8948 Tox21 chemicals
- 393 of 3805 ToxCast chemicals
- 11 of 96 ToxCast ER active chemicals
- 17 of 178 EDSP List 1 and 2 chemicals
- 94 of 1172 ToxRefDB chemicals
- 32 of 452 NHANES chemicals
- 1 of 670 pharmaceuticals (Obach, 2008)
- 9 of 67 flame retardants[1,7,9]

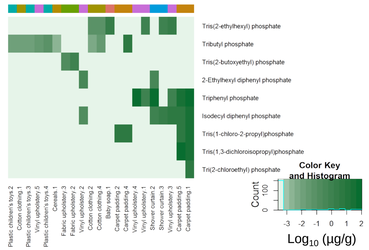
Estrogen Actives and Flame Retardants

Estrogen active chemicals



- Judson et al. (2015) analyzed HTS bioactivity data to identify likely estrogen receptor (ER)-active chemicals among 1800 ToxCast chemicals – here we examine those chemicals that were among the top 25% most potent ER active chemicals
- Stearic acid is a naturally occurring fatty acid known to be used in many detergents, soaps, shampoos, and shaving creams[10] Other estrogen active chemicals were found in articles like shower curtains, upholstery, and carpet padding
- Bisphenol A was found in toothpaste and one children's toy, while a replacement for Bisphenol A, Bisphenol AF, was found in another toy

Flame retardant chemicals



- We used ToxCast chemical annotation and public information[1,7,9] to identify chemicals that are sometimes used as flame retardants
- Chemicals with flame retardant application were indicated in most fabric and vinyl upholsteries, carpet paddings, cotton clothing, shower curtains, and children's toys, as well as in one hand soap, baby soap, and breakfast cereal" (*likely used as an anti-foaming agent)

Summary

- We characterized the ability of GCxGCTOF/MS to screen 100 consumer products and articles of commerce for chemical content
- We found 3803 unique chemical signatures
  - 1606 were either confirmed (41) or tentatively identified (1597) in some products – additional confirmation using ToxCast library standards is ongoing
  - 1422 of 1606 were not in a databases of chemicals with known consumer product use[4]
  - The numbers of chemicals are likely an underestimate since only a single extraction and analytical method was employed
- An average of 109 chemical signatures were detected (52 were tentatively identified) per test object
  - Low of 11 (Hand soap #4), high of 574 (Carpet padding #1)
- All of the detected chemicals have potential for "near field" exposure; however, chemical presence in an object does not necessarily mean that it is bioavailable
- **These data will allow new exposure predictions for these chemicals, as well as refined models for chemical formulation and weight fraction for other chemicals**

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