Non-targeted and Suspect Screening of DSS-Tox Chemicals in Human Serum Samples

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Outline:

- Description of the Samples
- Suspect Screening
  - Examples of formulas/compounds found
- Non-targeted Approach
  - Examples of formulas/compounds found

The Samples

- 99 de-identified serum samples

Photo source: http://acceleratingscience.com/proteomics/proteomic-workflows-for-human-plasma/

- From women ages 33-44 in the Research Triangle Park (RTP), NC area

- Extracted and analyzed on an Agilent 6200 series time-of-flight mass spectrometer (TOFMS) operated in positive and negative mode

- Molecular features present in blanks were excluded from the main findings

Suspect Screening: Matching to DSS-Tox

- Molecular features were matched against the DSS-Tox (Distributed Structure-Searchable Toxicity) database.

  DSS-Tox includes information on environmental contaminants, pharmaceuticals, & industrial chemicals.

- A molecular feature is a single accurate mass with a specific retention time and an integrated area count.

  It is identified by the Agilent software according to ions that likely represent present compounds, after background noise has been excluded.

Strynar, M., et al. (2015). "Identification of Novel Perfluoroalkyl Ether Carboxylic Acids (PFECAs) and Sulfonic Acids (PFESAs) in Natural Waters Using Accurate Mass Time-of-Flight Mass Spectrometry (TOFMS)." Environ Sci Technol **49**(19): 11622-11630.

Rager, J. E., et al. (2016). "Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring." Environ Int **88**: 269-280.

Suspect Screening: Matching to DSS-Tox (continued)

- 2,031 molecular features matched to formulas in the DSS-Tox database with a match score greater than or equal to 90.

  Matching criteria: exact mass,
  isotope distribution,
  and isotope spacing

- Molecular features with mass defects greater than -0.2 and less than 0.1 were selected as most likely to include xenobiotic halogenated compounds.

- Out of the 2,031 molecular features that matched to DSS-Tox, 452 were within the designated mass defect range.

Determining the Best Candidate Chemical for a Given Formula

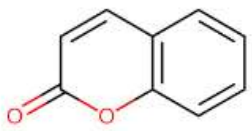
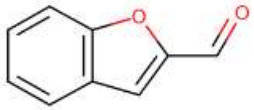
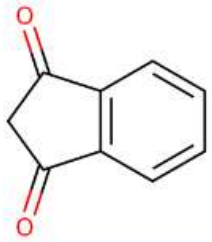
- EPA's Chemistry Dashboard provides access to data associated with over 700,000 chemicals.

  https://comptox.epa.gov/dashboard
  - includes information from US EPA's ExpoCast™ program and the US interagency Tox21 consortium

- A previous study showed that among chemicals that all correspond to the same formula, the chemical with the highest number of references was most likely to be the compound of interest.

  Little, J. L., et al. (2012). "Identification of "known unknowns" utilizing accurate mass data and ChemSpider." J Am Soc Mass Spectrom 23(1): 179-185.

Chemistry Dashboard Results for $C_9H_6O_2$

Structure	Preferred Name	CAS-RN	QC Level	Number of Sources
	Coumarin	91-64-5	DSSTox_Low	34
	2-Benzofurancarboxaldehyde	4265-16-1	DSSTox_Low	6
	1,3-Indandione	606-23-5	Public_High	2

ToxPi Score Criteria

- **Bioactivity:** percent of assays that had positive results, or “hits”

  Many compounds have been screened for bioactivity across hundreds of assays. We consulted the results of 4 different assays: AhR, AR, ERα, and PPARγ

- **Frequency:** number of samples (out of 99) in which the formula was detected

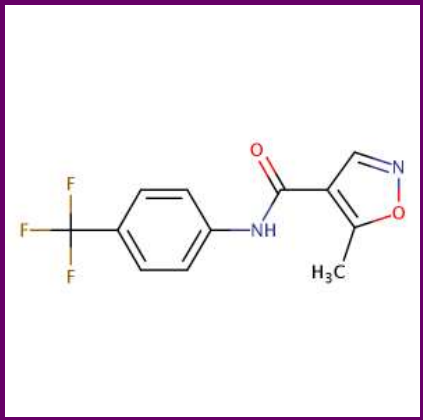
- **Abundance:** average volume (or area) of a peak corresponding to a given formula

Calculating the ToxPi Score

$$2\left(\frac{\log(Bioactivity_{sample})}{\log(Bioactivity_{max})}\right) + 1\left(\frac{\log(Frequency_{sample})}{\log(Frequency_{max})}\right) + 1\left(\frac{\log(Abundance_{sample})}{\log(Abundance_{max})}\right)$$

*Exposure data from the Chemistry Dashboard is listed separately from the ToxPi score.

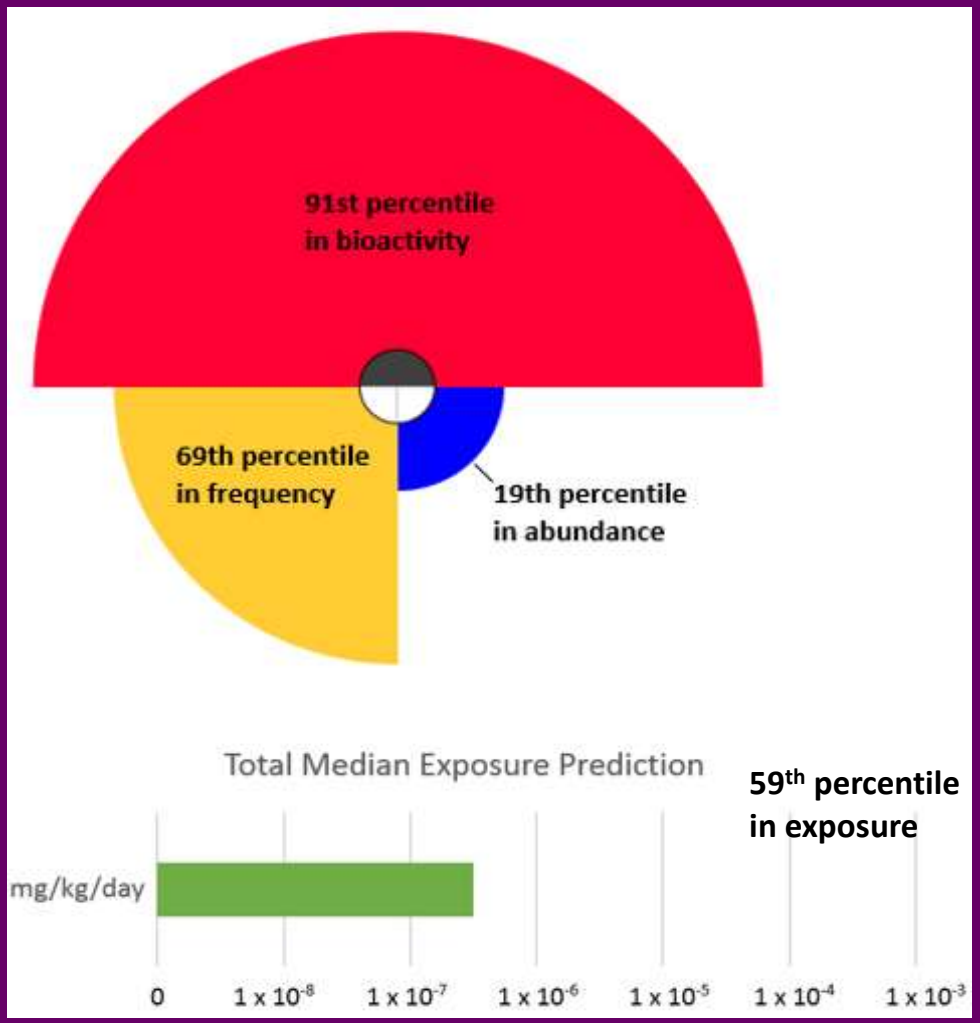
Leflunomide



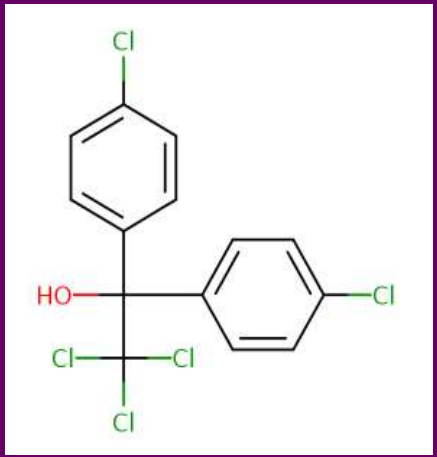
$C_{12}H_9F_3N_2O_2$ detected in 5 samples

ToxPi score = 2.38 out of 4

Sources: drug (antiviral, anticancer, anti-parasitic, anti-rheumatic, immunosuppressive)



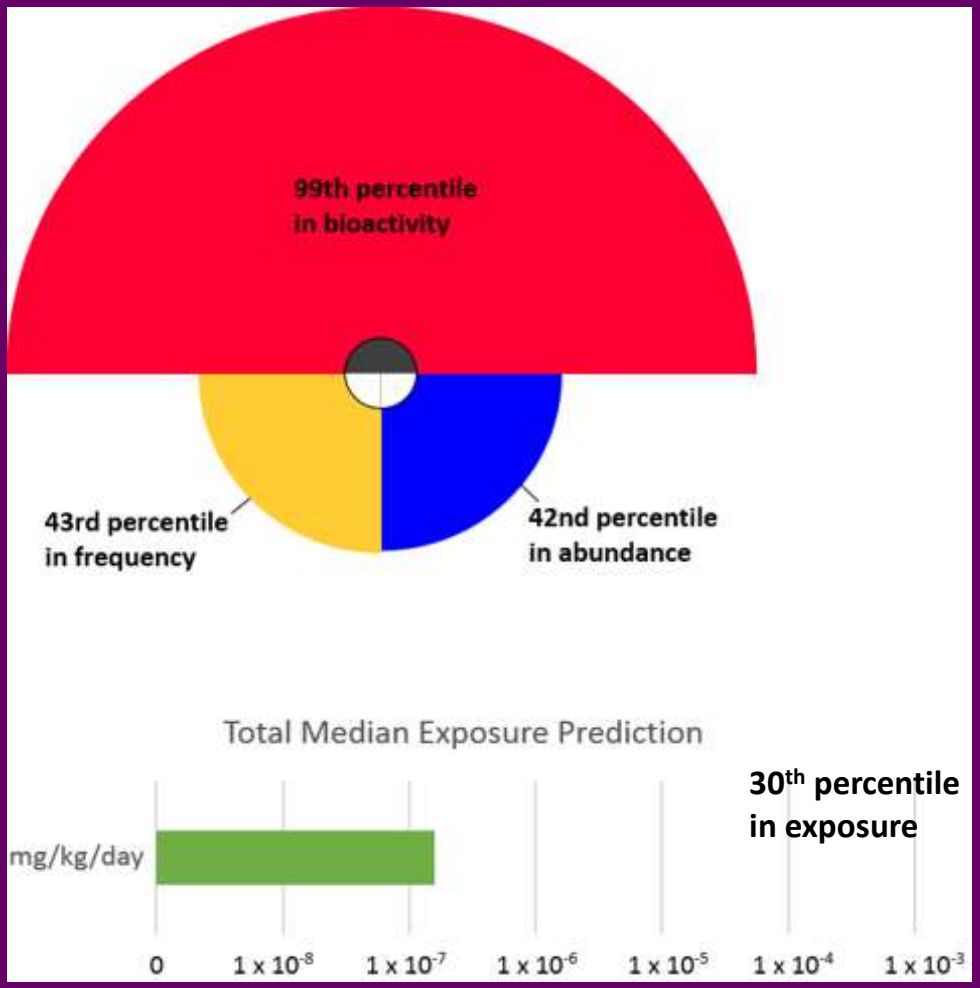
Dicofol



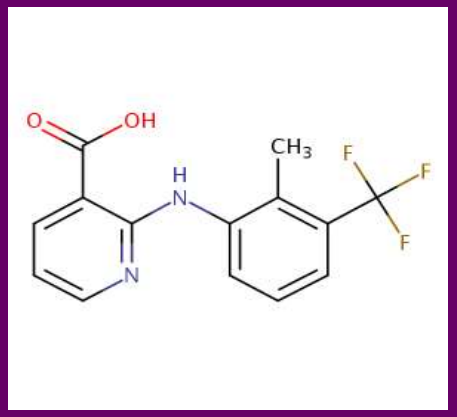
$C_{14}H_9Cl_5O$ detected in 2 samples

ToxPi score = 2.67 out of 4

Sources: organochlorine pesticides related to DDT



Flunixin

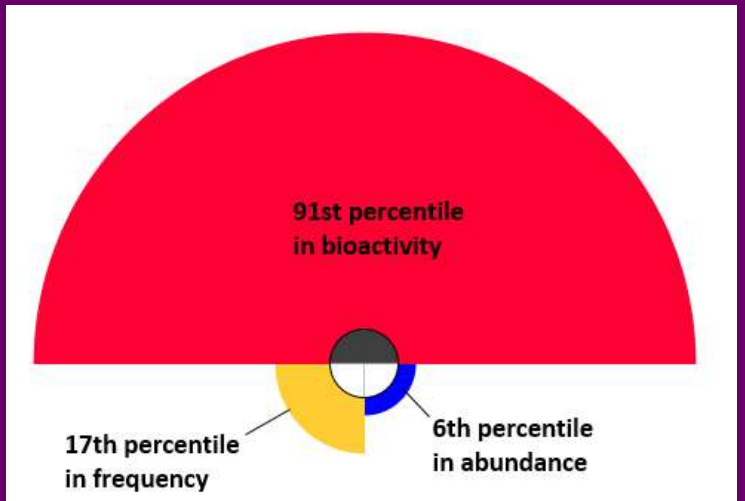


$C_{14}H_{11}F_3N_2O_2$ detected in 1 sample

ToxPi score = 1.91 out of 4

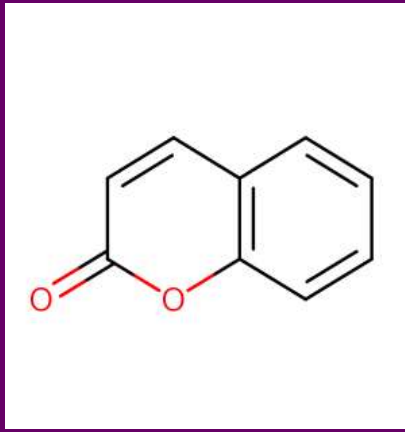
Sources: drug

(Administered to horses and cattle as Banamine®, can cause kidney problems in humans.)



No Total Median Exposure Prediction available.

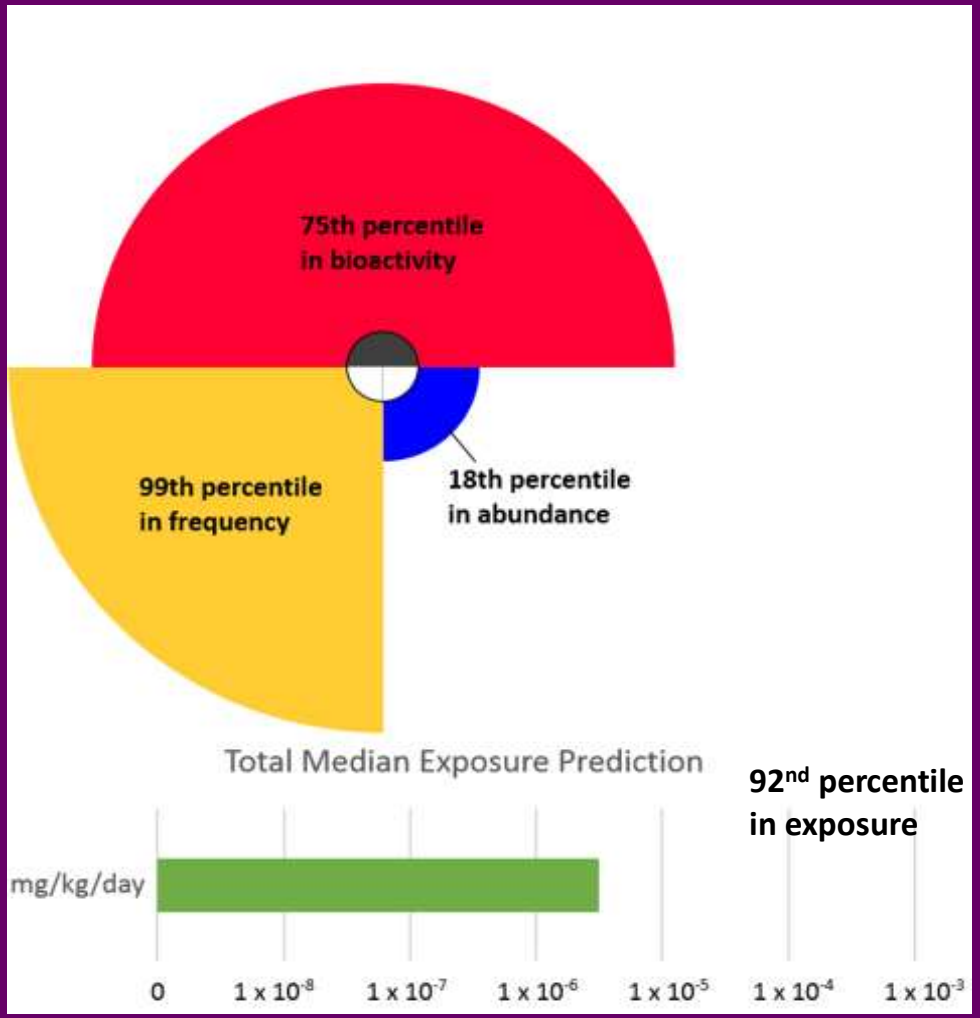
Coumarin



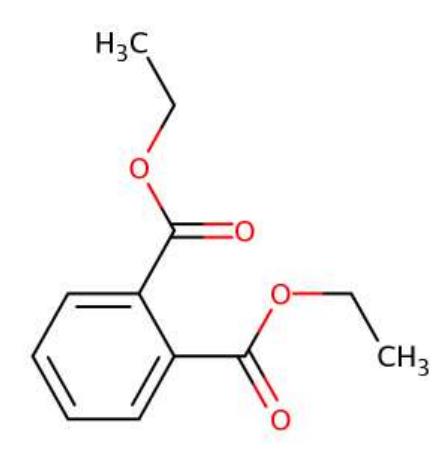
$C_9H_6O_2$
detected in 32 samples

ToxPi score = 2.41 out of 4

Sources: drug (anticoagulant), lubricant, machine manufacturing, fragrance, personal care/cosmetics, detergent, industrial cleaning, building construction



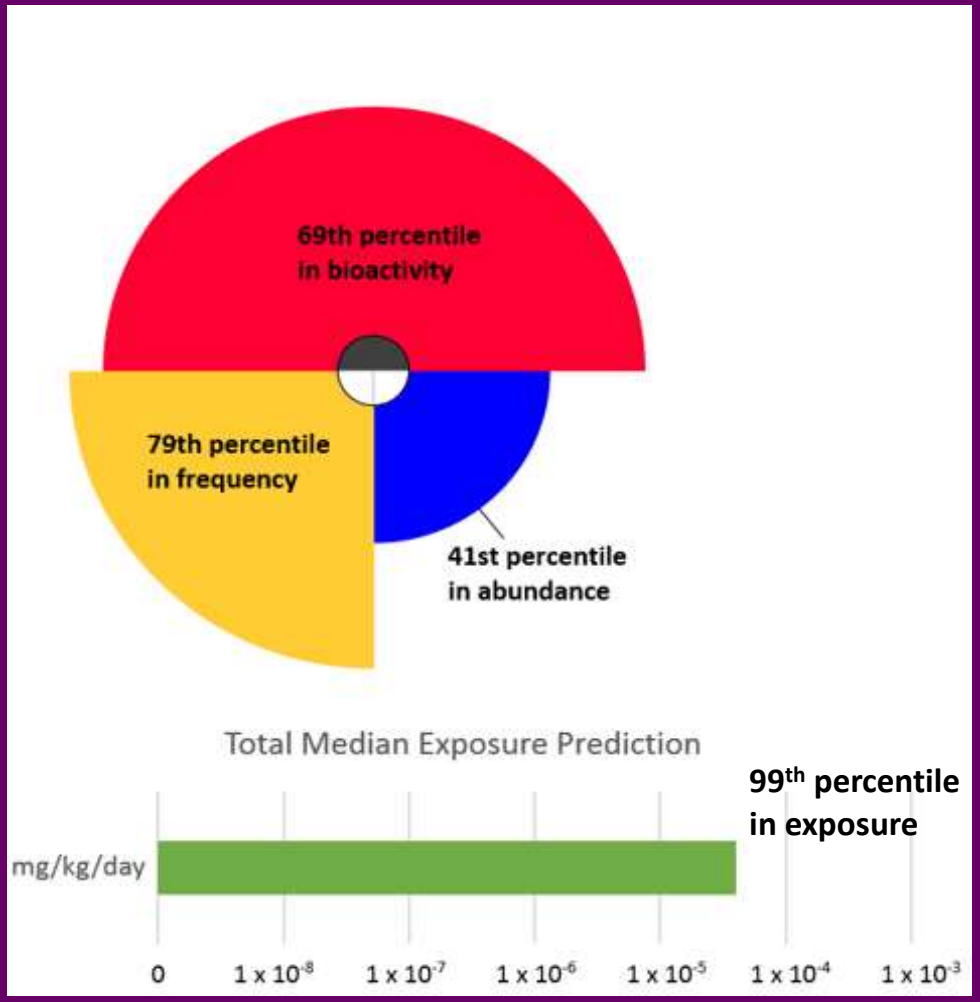
Diethyl Phthalate



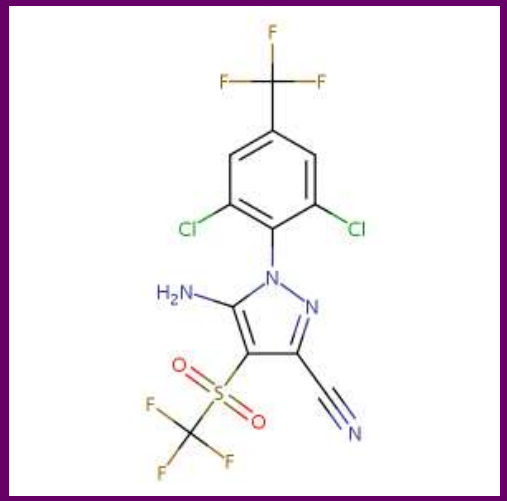
$C_{12}H_{14}O_4$ detected in 8 samples

ToxPi score = 1.84 out of 4

Sources: manufacturing, food production, fragrances, paint, pesticides, automotive care, cleaning fluids



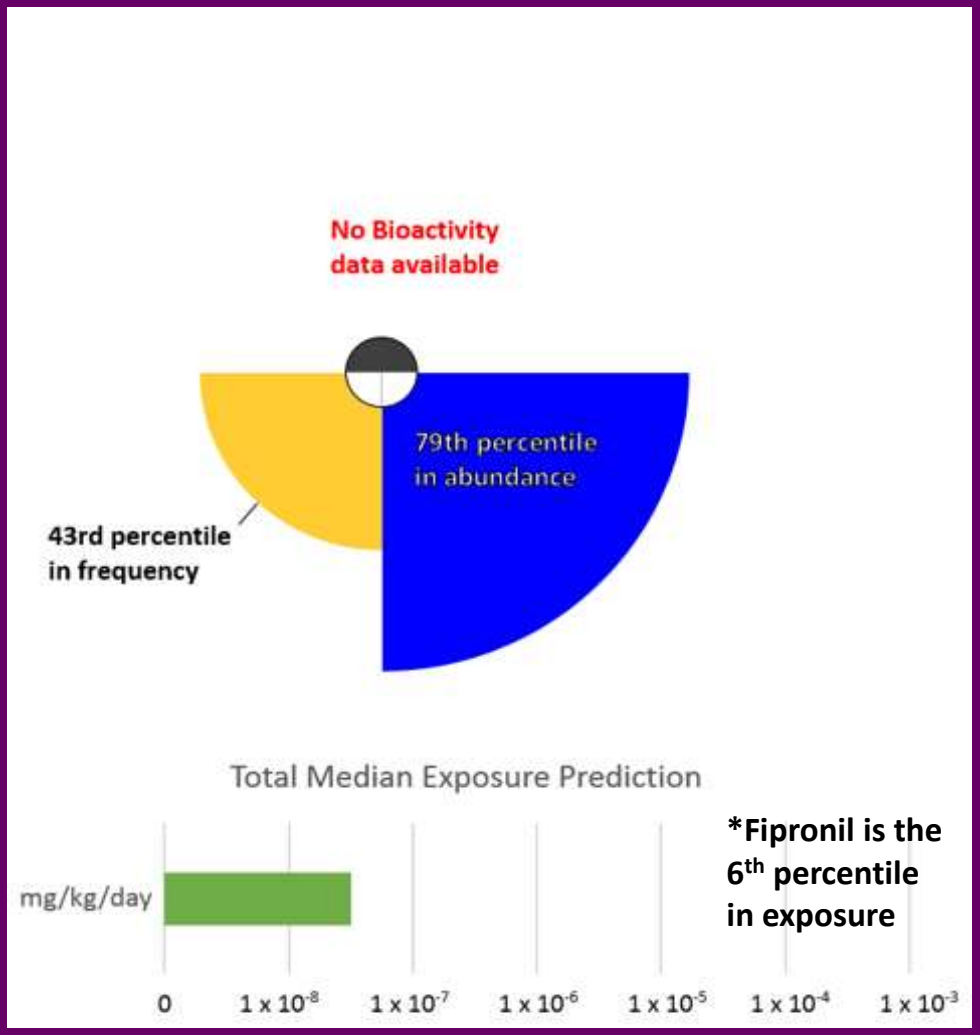
Fipronil Sulfone



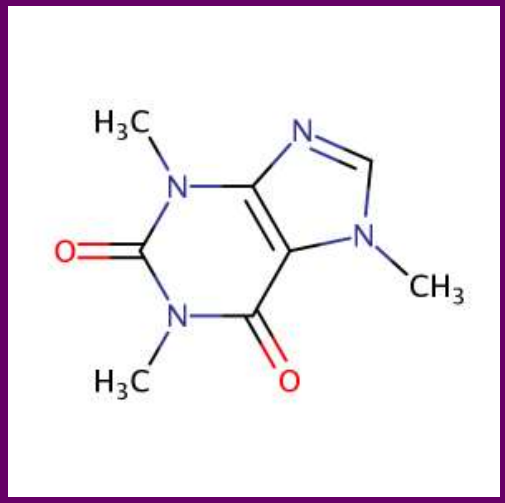
$C_{12}H_4Cl_2F_6N_4O_2S$ detected in 2 samples

ToxPi score = 0.96 out of 2

Sources: pesticides, metabolite of Fipronil (a phenylpyrazole insecticide used to control termites, fleas, roaches, ants, and other pests)



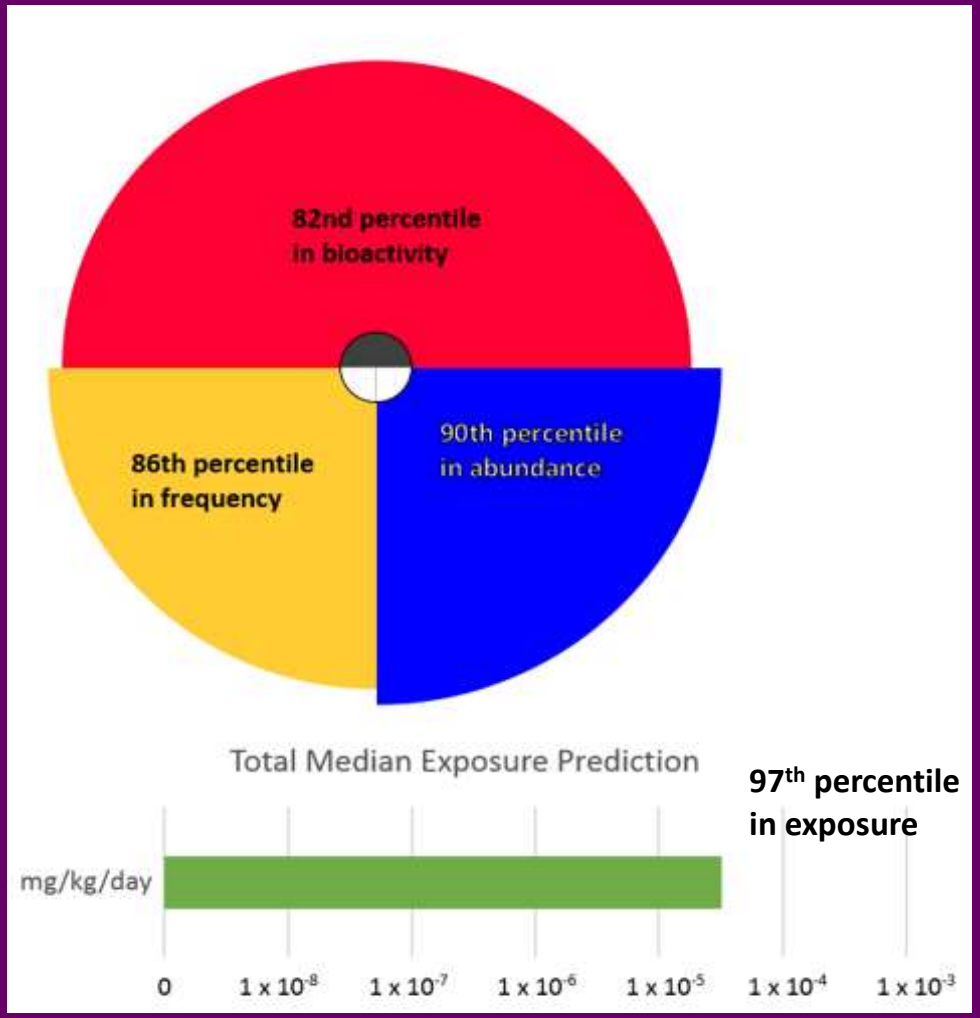
Caffeine



$C_8H_{10}N_4O_2$ detected in 11 samples

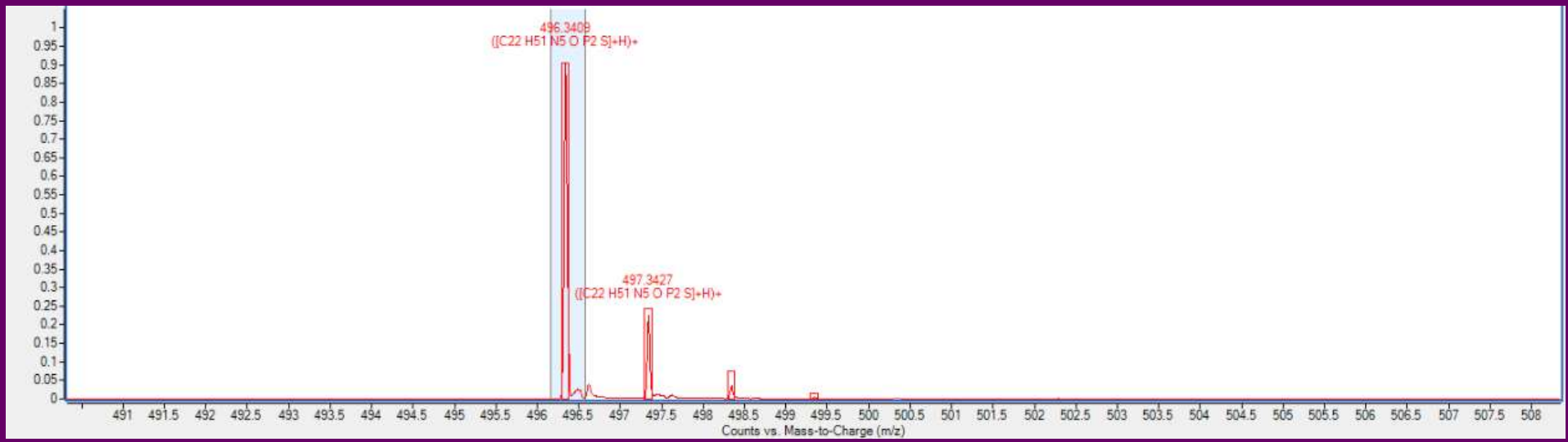
ToxPi score = 2.44 out of 4

Sources: food & beverages, pesticides, animal feed



Non-targeted Approach

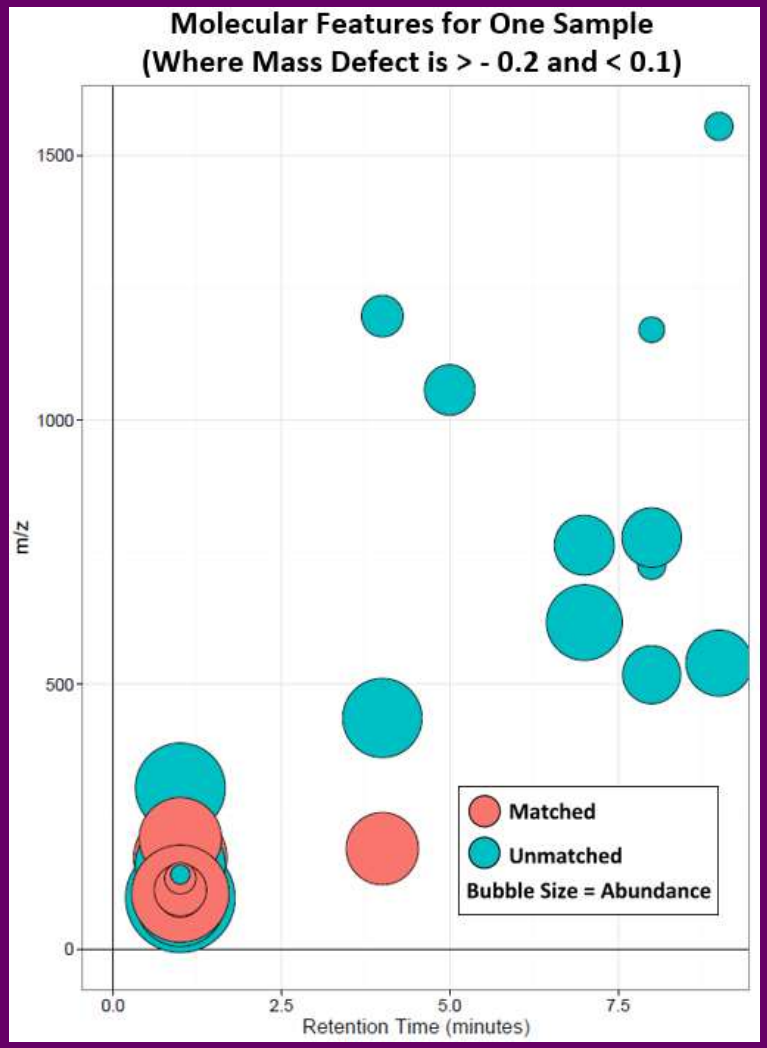
- To identify the features that did not match to DSS-Tox, Agilent MassHunter Qualitative Analysis software is used to generate candidate formulas.
- Possible formulas are ranked based on how well they match the mass spectrum of the molecular feature.
- Information about a compound's structure can be obtained through fragmentation using a QTOF instrument.



Matched Features

12 out of 32 features matched, including:

Endothal ($C_8H_{10}O_5$), Benzocaine ($C_9H_{11}NO_2$), Surinabant ($C_{23}H_{23}BrClN_4$), Phensuximide ($C_{11}H_{11}NO_2$), and Dimepranol ($C_5H_{13}NO$)

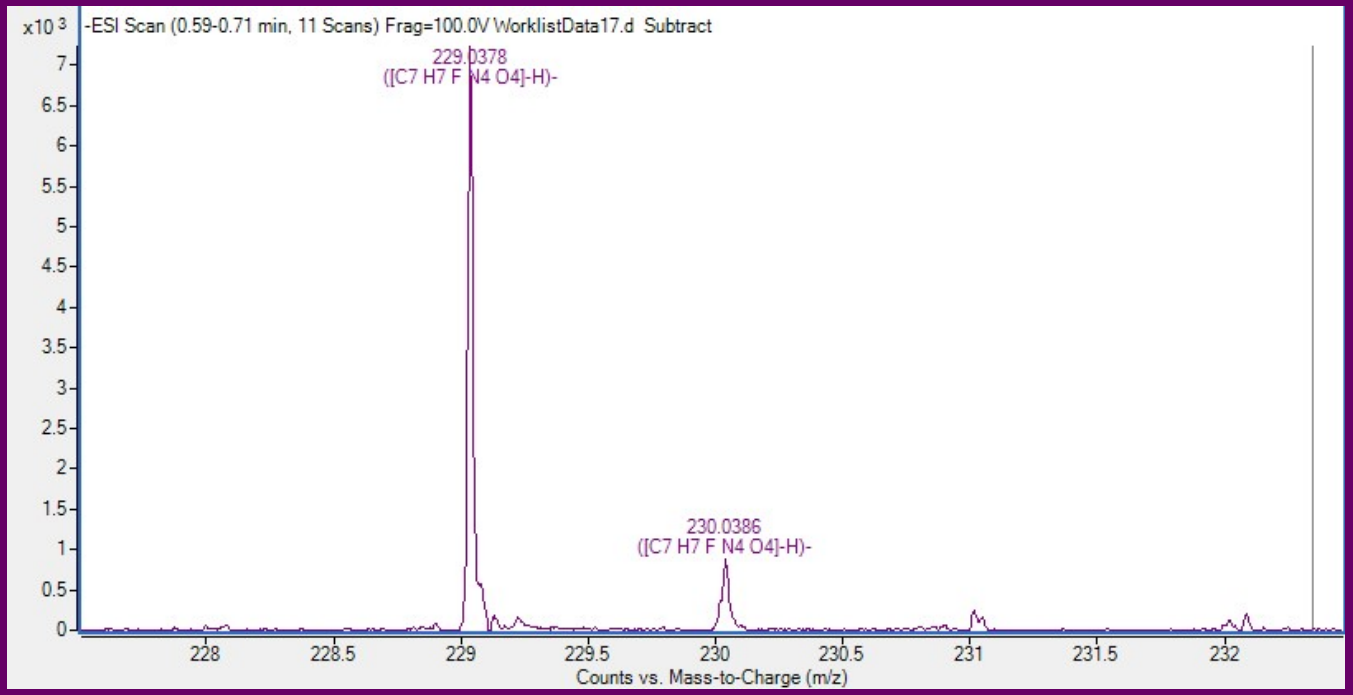


Unmatched Features

20 out of 32 features will be investigated via non-targeted screening.

Compound found through non-targeted screening: $C_7H_7FN_4O_4$

This formula does not correspond to any chemicals in the EPA's Chemistry Dashboard, suggesting that it may be a novel compound.



References:

Davidson, G. and D. Plumb (2003). Veterinary Drug Handbook: Client Information Edition.

Idowu, S. "ToxPi GUI." Retrieved July 22, 2016, from http://comptox.unc.edu/toxpi.php.

Little, J. L., et al. (2012). "Identification of "known unknowns" utilizing accurate mass data and ChemSpider." J Am Soc Mass Spectrom 23(1): 179-185.

McMahen, R. L., et al. (2015). "Identification of fipronil metabolites by time-of-flight mass spectrometry for application in a human exposure study." Environ Int 78: 16-23.

McMahen, R. L., et al. (2016). "Comparison of fipronil sources in North Carolina surface water and identification of a novel fipronil transformation product in recycled wastewater." Sci Total Environ.

Rager, J. E., et al. (2016). "Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring." Environ Int **88**: 269-280.

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US Environmental Protection Agency. "Chemistry Dashboard." Retrieved July 22, 2016, from https://comptox.epa.gov/dashboard.

Questions?

Photo Source:
https://noruffdays.com/category/dogs/german-sheppard/