Introduction to Exposomics

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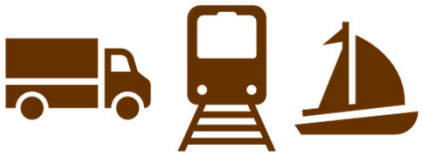
What is the Exposome?

- First defined by Wild in 2005, the exposome includes chemical and non-chemical stressors, from both internal and external sources across all life stages

Diet

Transportation



Products

Habits

Leisure and play

Occupation

General

Location

CP Wild, A Scalbert, and Z Herceg, Environ. Mol. Mutagen. 54:480-499, 2013.

Why Study the Exposome?

1) Understanding causes of disease

"…70-90% of disease risks are probably due to differences in environments"

EPIDEMIOLOGY

Environment and Disease Risks

A new paradigm is needed to assess how a lifetime of exposure to environmental factors affects the risk of developing chronic diseases.

Stephen M. Rappaport and Martyn T. Smith

Although the risks of developing chronic diseases are attributed to both genetic and environmental factors, 70 to 90% of disease risks are probably due to differences in environments (*1*–*3*). Yet, epidemiologists increasingly use genome-wide association studies (GWAS) to investigate diseases, while relying on questionnaires to characterize "environmental exposures." This is because GWAS represent the only approach for exploring the totality of any risk factor (genes, in this case) associated with disease prevalence. Moreover, the value of costly genetic information is diminished when inaccurate and imprecise environmental data lead to biased inferences regarding gene-environment interactions (*4*). A more comprehensive and quantitative view of environmental exposure is needed if epidemiologists are to discover the major causes of chronic diseases.

An obstacle to identifying the most important environmental exposures is the fragmentation of epidemiological research along lines defined by different factors. When epidemiologists investigate environmental risks, they tend to concentrate on a particular category of exposures involving air and water pollution, occupation, diet and obesity, stress and behavior, or types of infection. This slicing of the disease pie along parochial lines leads to scientific separation and confuses the definition of "environmental exposures." In fact, all of these exposure categories can contribute to chronic diseases and should be investigated collectively rather than separately.

To develop a more cohesive view of environmental exposure, it is important to recognize that toxic effects are mediated through chemicals that alter critical molecules, cells, and physiological processes inside the body. Thus, it would be reasonable to consider the "environment" as the body's internal chemical environment and "exposures" as the amounts of biologically active chemicals in this internal environment. Under this view, exposures are not restricted to chemicals (toxicants) entering the body from air, water, or food, for example, but also include chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes (*5*, *6*) (see the figure). This internal chemical environment continually fluctuates during life due to changes in external and internal sources, aging, infections, life-style, stress, psychosocial factors, and preexisting diseases.

The term "exposome" refers to the totality of environmental exposures from conception onwards, and has been proposed to be a

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2) Ensuring chemical safety and human/ecological health

Content from J. Sobus

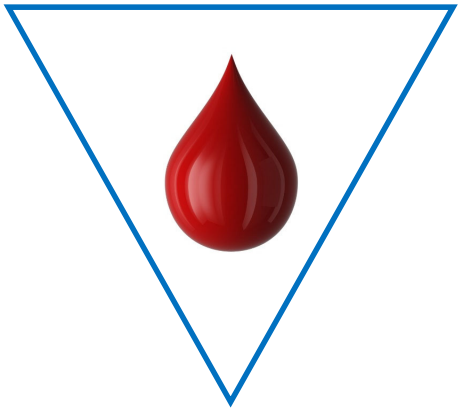
How to Study the Exposome

Bottom-Up

Measure Exposures Within Relevant Media

↑ Exposure surveillance	Present
↑ Exposure forensics	Signature
Chemical prioritization	Relevant
Effect-directed analysis	Active
↓ Biomarker discovery	Predictive

Top-Down

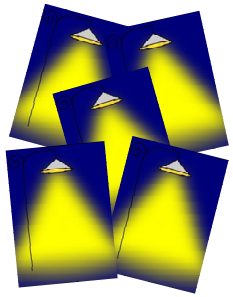


Measure Exposures Within the Receptor

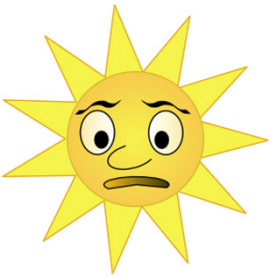
- Need methods that can measure LOTS of chemicals
- Something akin to in vitro toxicity assays but for exposure…

What is Non-Targeted Analysis?

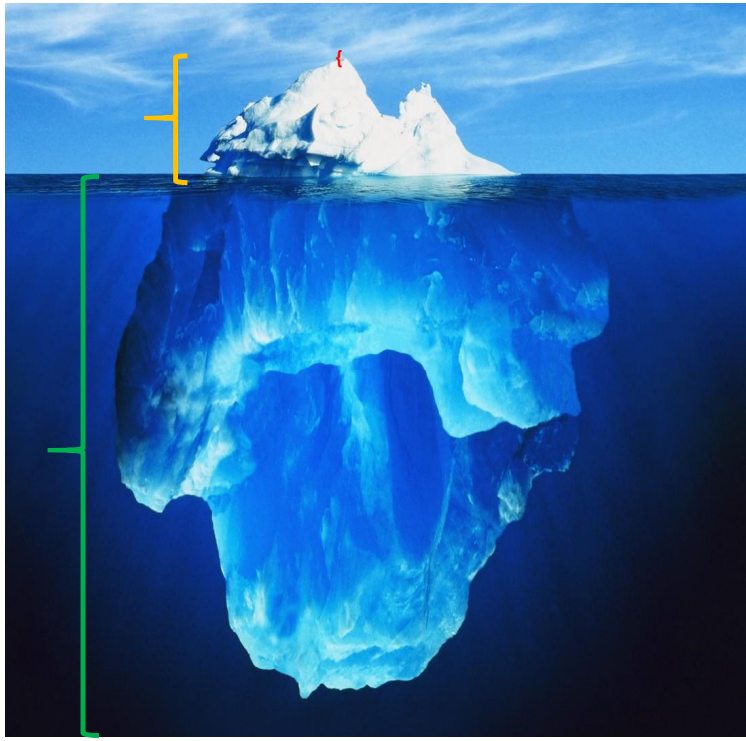
- **Targeted Analysis**
  Standards, calibration curves



- **Suspect Screening Analysis (SSA)**
  Lists of compounds

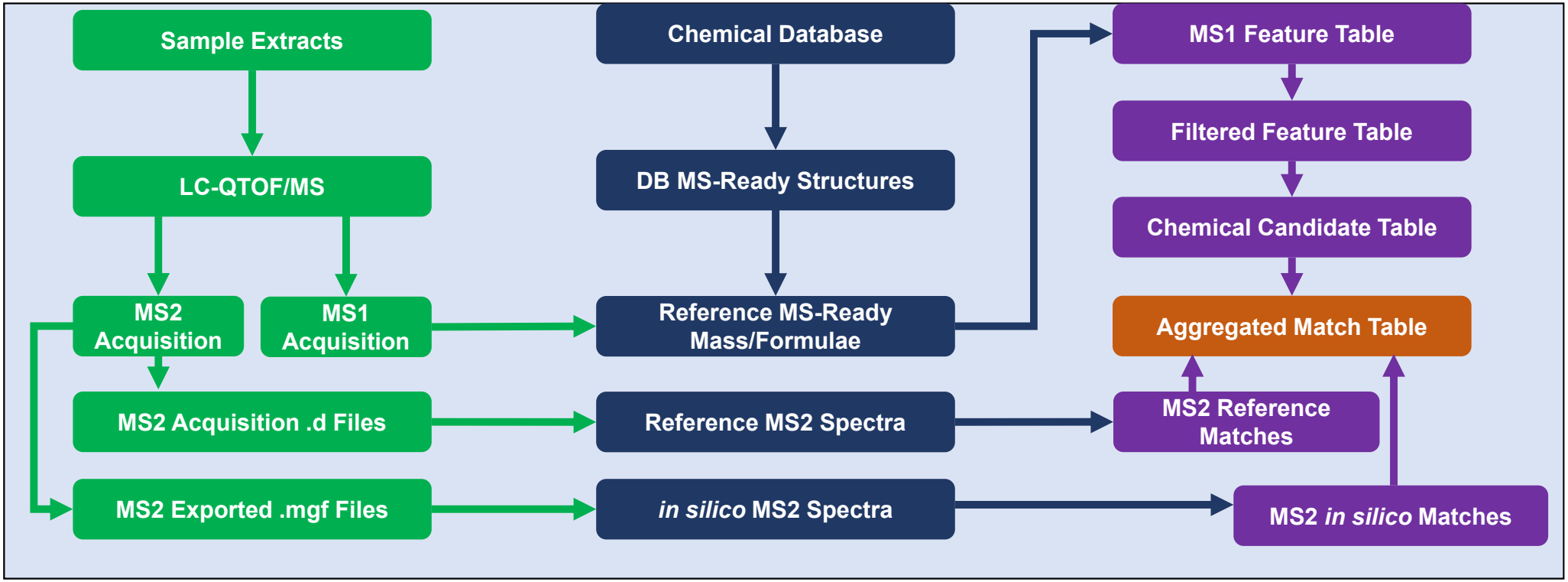


- **Non-Targeted Analysis (NTA)**
  MS first principles



Non-Targeted Analysis Workflow

Experimental Acquisition | Database & Library Matching | Data Analysis & Computational Tools

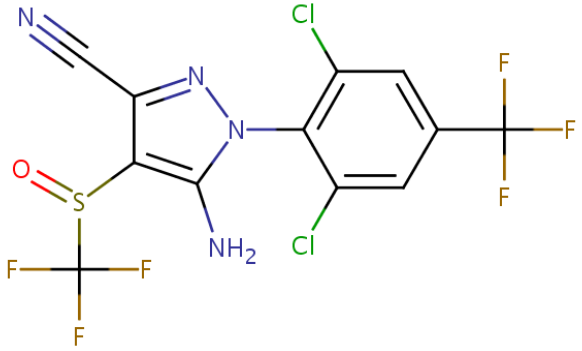


Analytical Instruments	High resolution accurate mass, mass spectrometry (QToF, Orbitrap)
Chemical Databases	CompTox Chemicals Dashboard, MassBank, PubChem
Computational Tools	CPDat, media and retention time prediction, MetFrag, R/Python tools

How does High Resolution MS work?

Atom	Natural Abundance	Exact Mass
^{1}H	99.9885%	1.007825
^{2}H	0.0115%	2.014102
^{12}C	98.93%	12.000000
^{13}C	1.07%	13.003355
^{14}N	99.632%	14.003074
^{15}N	0.368%	15.000109
^{16}O	99.757%	15.994915
^{17}O	0.038%	16.999131
^{18}O	0.205%	17.999159
^{19}F	100%	18.998403
^{32}S	94.93%	31.972072
^{33}S	0.76%	32.971459
^{34}S	4.29%	33.967868
^{36}S	0.02%	35.967079
^{35}Cl	75.78%	34.968853
^{37}Cl	24.22%	36.965903

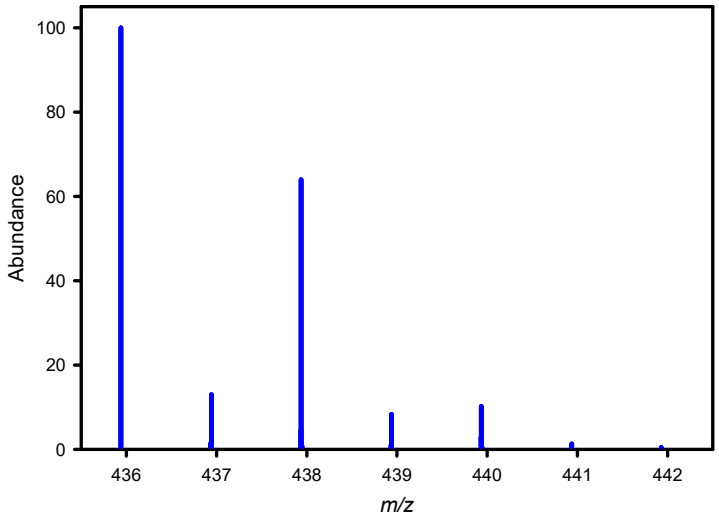
Example: Fipronil



Molecular Formula: $C_{12}H_4Cl_2F_6N_4OS$

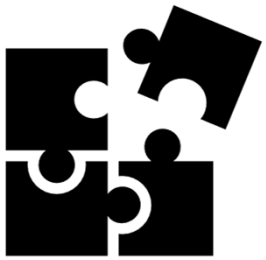
Monoisotopic Mass: 435.938706

= (12.0000*12 Carbon) + (1.007825*4 Hydrogen) + (34.968853*2 Chlorine) + (18.998403*6 Fluorine) + (14.003074*4 Nitrogen) + (15.994915*1 Oxygen) + (31.972072*1 Sulfur)



What do we Learn?

Health



Chemical Safety

- Find chemicals of emerging concern
- Sources contributing to exposure
- Prioritization of chemicals
- Assess toxicity/exposure overlaps
- Predict exposures and risk

Exposomics Experimental Design

Name	Example	Purpose
Tracers	Isotopically labeled standards: $^{13}C_3$-Atrazine, D_3-Thiamethoxam, $^{13}C_4$,$^{15}N_2$-Fipronil	Allows tracking of chromatographic performance and mass accuracy
Replication	Triplicate injections of same sample vial	Removes risk of “one hit wonder”
Run order randomization	8, 3, 7, 4, 2, 1, 10, 5, 8, 6, 9, 2, 5, 4, 1, 9, 4, 7, 3, 8, 1, 6, 10, 9, 6, 7, 5, 3, 2, 10	Minimizes/averages out batch or sample order effects (e.g., carryover, temp & instrument drift)
Pooled QC sample	Combine 5 mg/µL from each of 10 samples (total 50 mg/µL) prior to extract to create pooled QC	Separate confirmation of presence with different matrix, MS2 IDs
Blanks	Solvent, method, matrix, double blanks	Allows identification/subtraction/deletion of interferences introduced in lab processes
Multiple lines of evidence for ID	Retention time prediction/matching, Spectral library/prediction matching, Data source ranking, Functional/product uses, Media occurrence	Improves confidence in identification when chemicals standards are unavailable