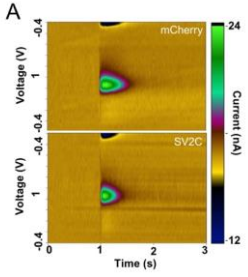
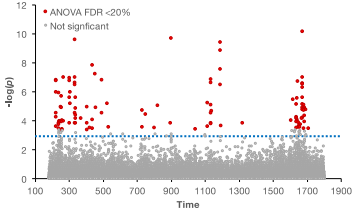
The exposome: 21st century challenges





Gary W. Miller, Ph.D.
Vice Dean for Research Strategy and Innovation
Professor of Environmental Health Sciences
Mailman School of Public Health
Columbia University, New York, NY
gary.miller@columbia.edu

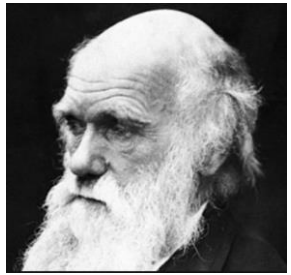
@exposome
@garywmiller3

Disease causation/ exacerbation = $\sum$ Genetic factors + $\sum$ External factors

Health/disease phenotype = Genome + Exposome

Exposome – a systematic, unbiased, and omic-scale examination of external factors contributing to disease or health status

An imbalanced equation



$$G \times E = P$$

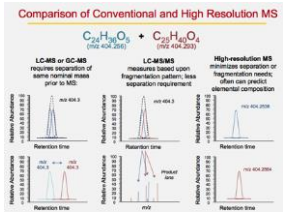
If our phenotype is a result of our genetics and environment, why then do we spend a disproportionate amount of time, money, and energy on genetics?

Exposome: the cumulative measure of the environmental influences and corresponding biological responses throughout the lifespan.

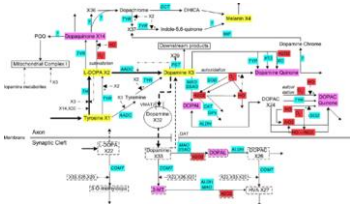
Miller GW, Jones DP. The Nature of Nurture: Refining the Definition of the Exposome. Toxicological Sciences, 137:1-2, January, 2014.

“Derived from the term exposure, the exposome is an omic-scale characterization of the nongenetic drivers of health and disease.”

MM Niedzwiecki, DI Walker, R Vermeulen, M Chadeau-Hyam, DP Jones, and GW Miller. The Exposome: Molecules to Populations. Annual Reviews of Pharmacology and Toxicology. 59:107-127, 2019



HERCULES
Exposome Research Center



Awarded in 2013 (NIH P30-ES019776), Funded through 2022

administration (Miller 2013-2018; now Marsit)

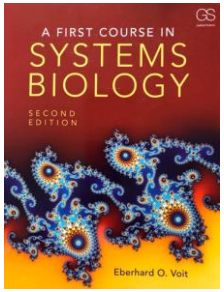
analytical chemistry-targeted (Barr, Ryan)

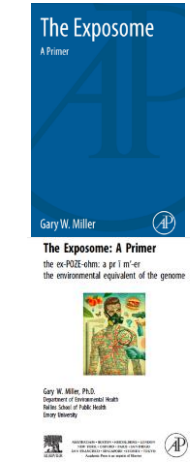
metabolomics/exposomics-untargeted (Jones, Li)

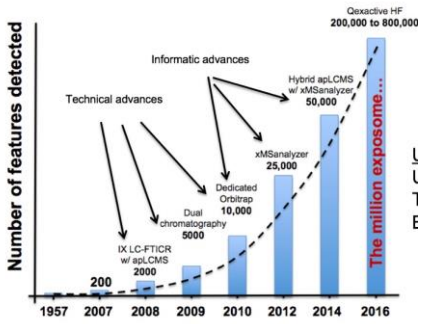
pilot awards (Morgan) and patient studies (Ziegler, Marsit)

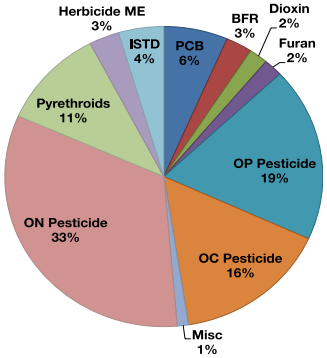
community engagement (Kegler/Pearson)

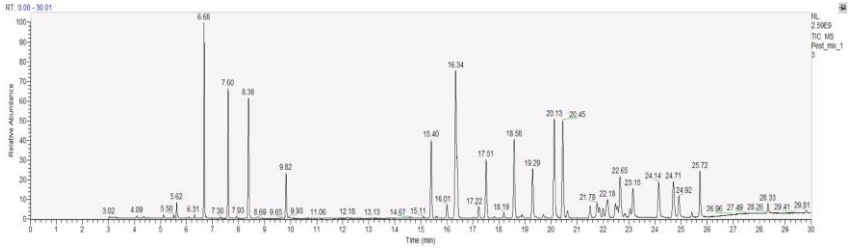
data science/systems biology (Waller, Voit, Clifford, Li, Qiang, Kemp)











Replicating the high-resolution metabolomics LC or GC HRMS at Columbia as part of the Irving Institute CTSA

Capturing exogenous chemicals and endogenous metabolites

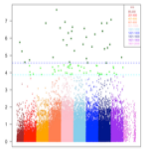
ESI LC-MS

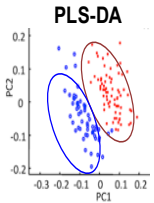
Ultra-high resolution MS
LTQ-FT
LTQ-Velos Orbitrap
Q-Exactive HF
Thermo Fusion

Data extraction

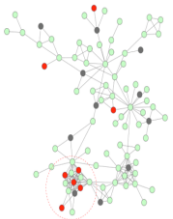
Data Table
***m/z*, RT, Intensity**
Quality metrics

Biostatistics
Bioinformatics





Metabolites of interest



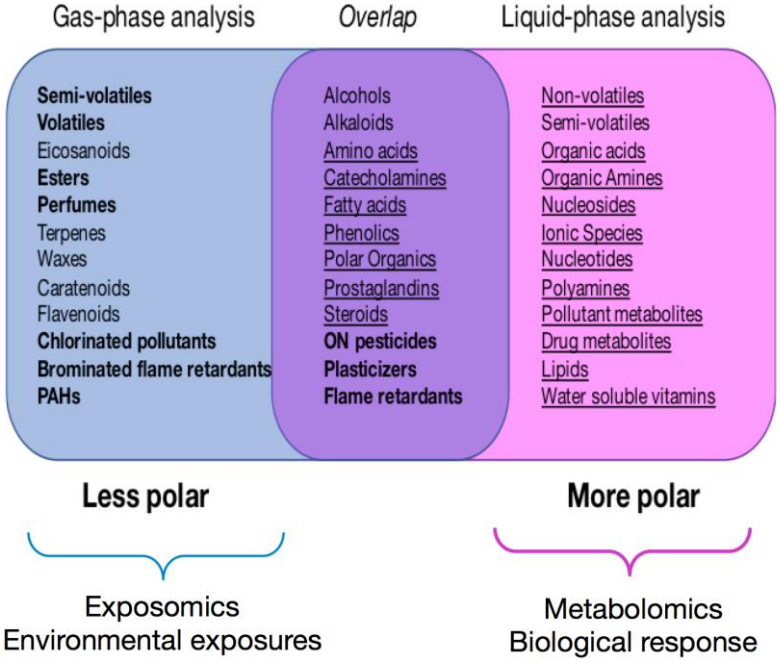
Network and pathway analysis: *Mummichog*, MetabNet, others

Metabolite forensics

Unequivocal Identification
MS^n; UHRAM MS; 1-D, 2-D NMR; ion Mobility; Chemical Reactivity, etc

T Yu et al 2009 Bioinformatics 25:1930-6
JM Johnson et al 2010 Analyst 135: 2864-2870
Q Soltow et al 2013 Metabolomics 9:S132–S143
T Yu et al 2013 J Proteome Res 12:1419-27
S Li et al 2013 Plos Comp Biol 9:e1003123
K Uppal et al 2013 BMC Bioinformatics 14:15
K Uppal et al 2015 Frontiers Bioeng Biotech 3:87

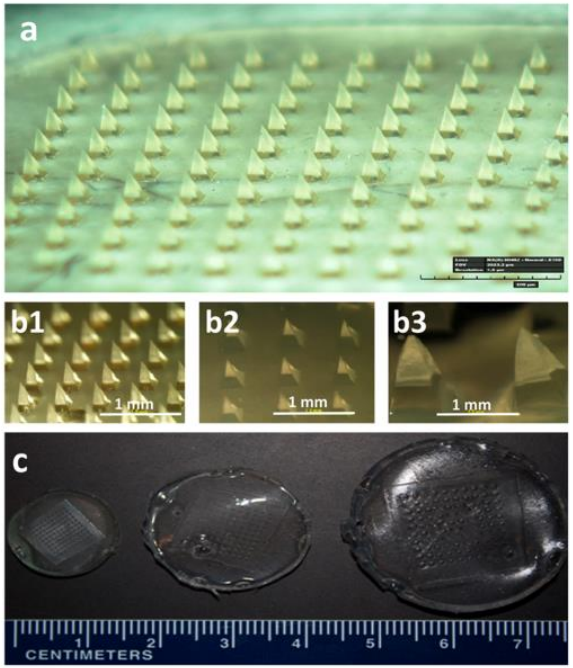
DP Jones 2016 *Tox Reports* **3**: 29-45



analytical chemistry
Letter
pubs.acs.org/ac

Collection of Analytes from Microneedle Patches

Andrey V. Romanyuk,[†] Vasiliy N. Zvezdin,[‡] Pradnya Samant,[†] Mark I. Grenader,[†] Marina Zemlyanova,[§] and Mark R. Prausnitz*[,†]

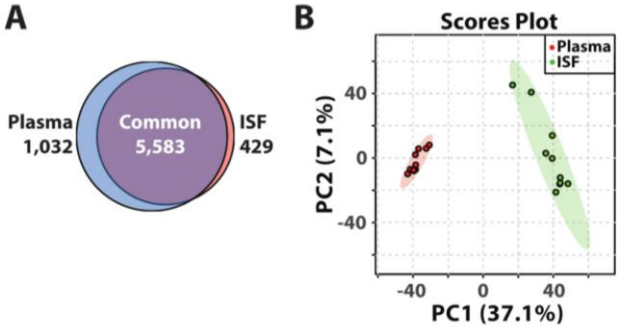


analytical chemistry
ACS AuthorChoice
Article
Cite This: Anal. Chem. 2018, 90, 3786–3792
pubs.acs.org/ac

Human Suction Blister Fluid Composition Determined Using High-Resolution Metabolomics

Megan M. Niedzwiecki,[†,∥] Pradnya Samant,[‡,∥] Douglas I. Walker,[§] ViLinh Tran,[§] Dean P. Jones,[§] Mark R. Prausnitz,*[,‡] and Gary W. Miller*[,†]

Figure 1. High-resolution untargeted metabolomic profiles of ISF and plasma.



Samant, Niedzwiecki, Raviele, Tran, Mena-Lapaix, Walker, Felner, Jones, Miller, Prausnitz. Science Translational Medicine-Under revision

Mega-scale identification of xenobiotic metabolites. Compound ID Core. Jones, Morgan, Li, Miller (MPI)

A **Xenobiotic chemical with known structure**

B Predicted products from array of known xenobiotic metabolism systems

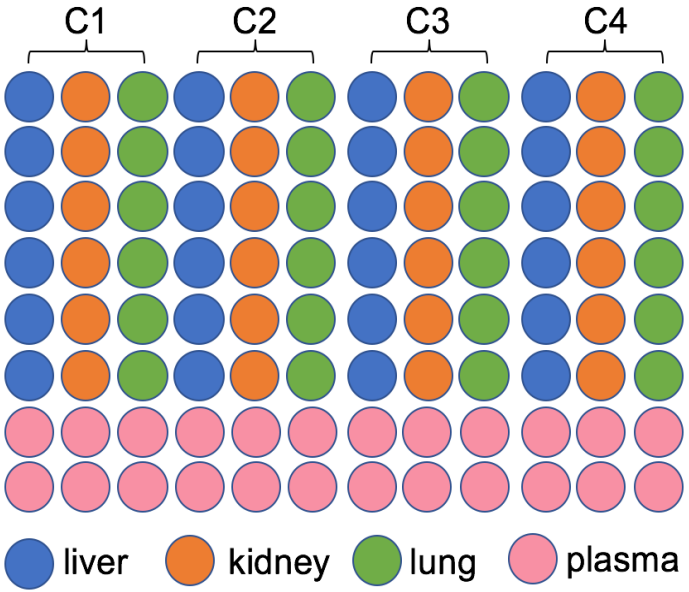
C Predicted MS/MS ions for each xenobiotic metabolite

D Transformation of xenobiotic by array of xenobiotic metabolizing systems

E MS programmed to fragment ions corresponding to predicted xenobiotic products

F **Algorithm to score quality of match of detected MS/MS ions with predicted MS/MS ions**

First generation 96-well plate assay, 4 tissues:
4 chemicals, 6 concentrations or
8 chemicals, 3 concentrations



96-well plate with human plasma, liver, kidney and lung S9 fractions to study metabolites of four chemicals, C1-C4.

Commercial S9 fractions (microsomes + cytosol) pooled from 50 human liver, kidney, and lung, are supplemented with necessary cofactors for oxidation, reduction, glucuronidation, sulfation, methylation and acetylation

A recent example from a collaboration with the Mayo Clinic

- ***Primary sclerosing cholangitis (PSC)***
- US prevalence: 13.6/100,000 (0.014%)
- Average age of diagnosis: 41 years
- Transplant free survival: 12 years
- Only treatment: Liver transplant
- Outcomes: malignancy, liver failure
- Mayo sees 5% of all U.S. patients with disease
- ~70% of cases have inflammatory bowel disease

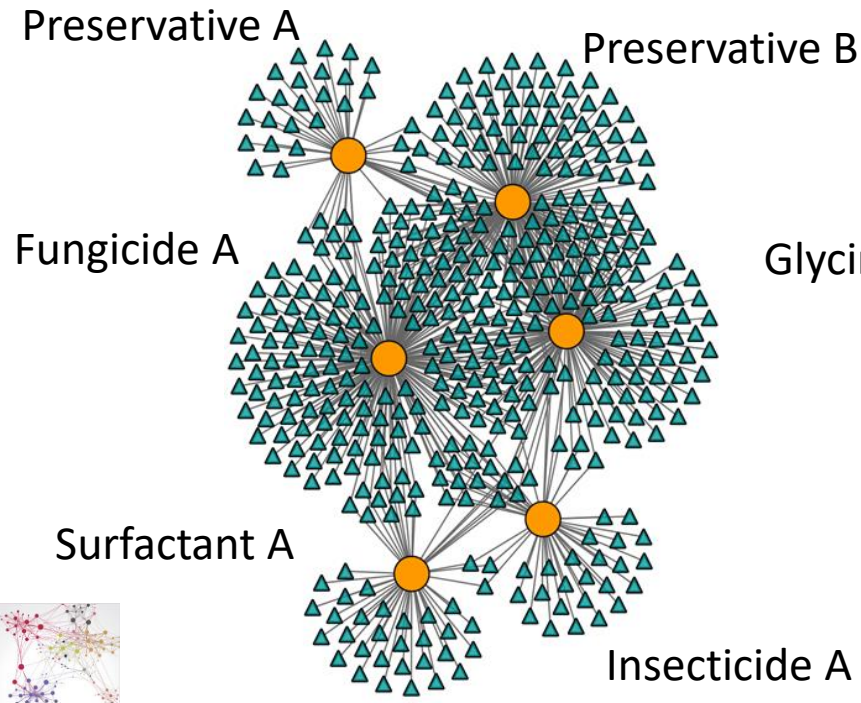
EWAS reveals altered levels of environmental pollutants
MWAS identified bile acid alterations

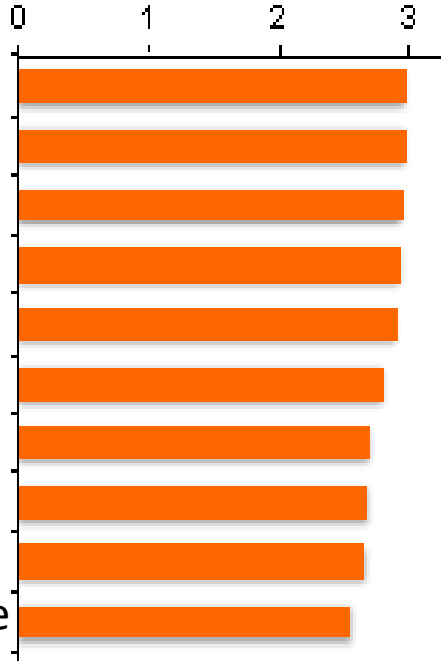
Chemical	*p-value*	Regression Coefficient	Odds ratio , IQR (95% Confidence interval)
Fenpropimorph	0.012	2.05	7.78 (2.47, 82.22)
Nonylphenol	0.002	1.04	2.84 (1.55, 5.89)
Protocatechuic acid	0.003	1.01	2.75 (1.53, 5.75)
Aldicarb sulfone/Acetamiprid	0.012	0.75	2.13 (1.28, 4.19)
Ethyl paraben	0.014	0.63	1.87 (1.22, 3.33)
Chlorthiophos	0.078	0.49	1.63 (0.97, 2.89)
Terbutylazine	0.056	0.48	1.62 (1.01, 2.75)
Fenvalerate	0.063	0.44	1.56 (1.00, 2.58)
Triclocarban	0.076	-0.24	0.79 (0.59, 1.02)
Anthraquinone	0.093	-0.37	0.69 (0.44, 1.05)
Perfluorooctanoic acid	0.084	-0.38	0.68 (0.44, 1.05)
Diphenamid	0.012	-0.40	0.67 (0.47, 0.9)
Diphenamid	0.052	-0.41	0.66 (0.43, 0.99)
Dimethachlor	0.049	-0.45	0.64 (0.40, 0.98)
Monocrotophos	0.030	-0.46	0.63 (0.41, 0.94)
Thiabendazole	0.062	-0.59	0.55 (0.29, 1.02)
Perfluorooctanesulfonic acid	0.007	-0.65	0.52 (0.31, 0.79)
Fenobucarb/Promecarb	0.019	-0.76	0.47 (0.24, 0.87)
Carbaryl	2.6E-05	-0.97	0.38 (0.23, 0.57)

>205 environmental chemical biomarkers identified in PSC, PBC and control population. Each was tested for association with disease status using logistic regression.

	Metabolite	OR
PSC	Glycochenodeoxycholic acid	>10
	Taurochenodeoxycholic acid	>10
	Taurine	2.8
PBC	Glycocholic acid	6
	Cholic acid	3
	Taurine	3.7

Exposomics-Metabolomics Networks Reveal Top Pathways Associated with PSC



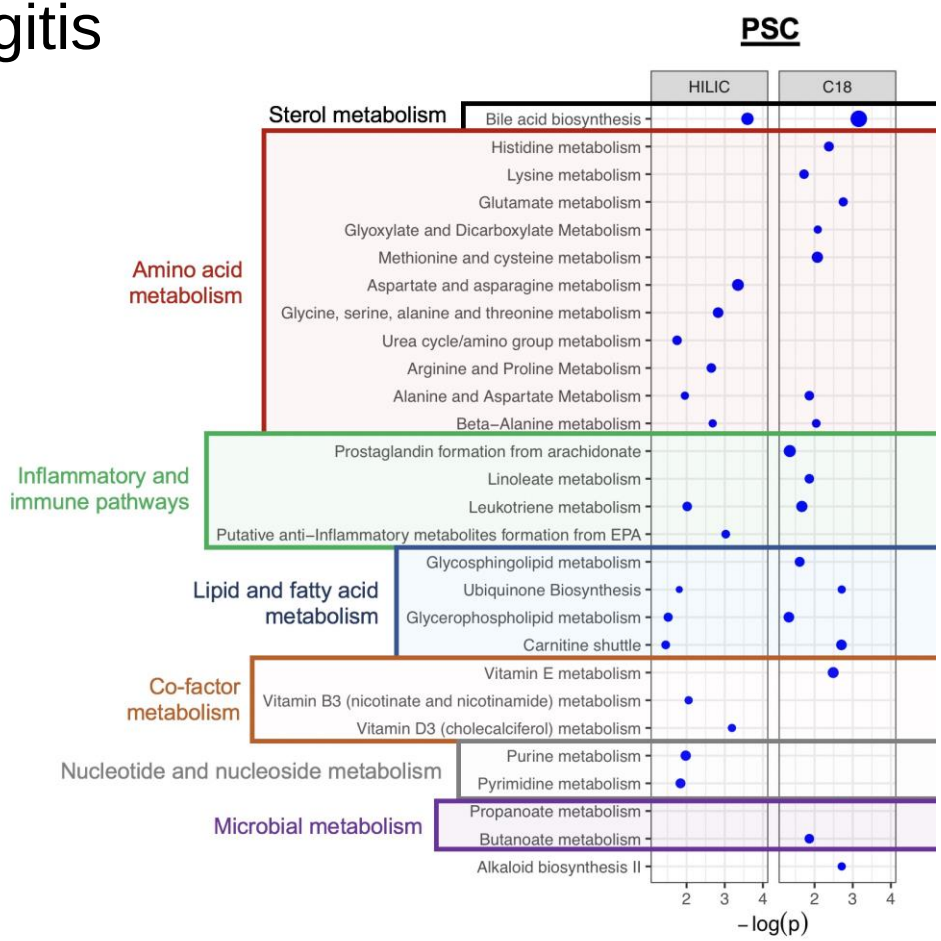


*** Only top 10 pathways shown**

Primary sclerosing cholangitis

Dissecting the pathogenesis an outcomes of PSC using multi-omics by studying the exposome and genome.
NIDDK RC2 $8M

>800 patients per group



Alzheimer's disease (93), Mild cognitive impairment (50), controls (59)
APOEgentoype, CSF (AB42, pTau)

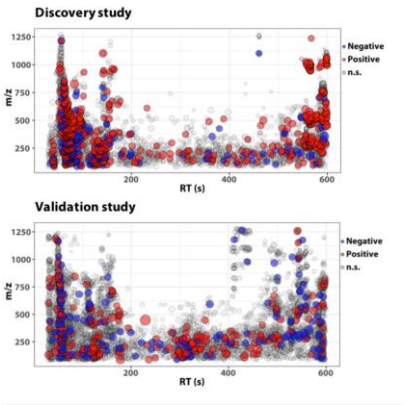


Table 4. Putative compound identification of plasma features from MWAS.

m/z	RT	Change in AD	Putative compound(s)	Predicted adduct	ID level[a]	Notes
129.0661	89	Higher	Glutamine (2 ppm)	-H_2O+H	1	--
231.1205	211	Higher	5S,6S-epoxy-15R-hydroxy-ETE (+Na, 0 ppm)	--	3	--
246.9550	127	Higher	Numerous database matches	-H_2O+H	--	Contains halogen (Cl and/or Br)
334.1410	86	Lower	Piperettine (1			--
349.1515	80	Lower	Piperine (1 ppm)	+ACN+Na	4	--
386.8946	61	Higher	1,1-Dichloro-2-(dihydroxy-4'-chlorophenyl)-2-(4'-chlorophenyl)ethylene (9 ppm)	+K	2	Contains halogen (Cl and/or Br)
662.0933	158	Higher	GDP-D-mannuronate (+ACN+H [M+1], 0 ppm); Chaetocin (-2H_2O+H [M+1], 8 ppm); Blighinone (+H [M+1], 9 ppm)	[M+1] isotope	4	--
663.4524	36	Higher	Lipid A-disaccharide-1-P (+2H, 2 ppm); Aluminium dodecanoate (+K, 2 ppm)	--	4	--

Hydroxyated metabolite of DDE

[a]ID level indicates annotation confidence: 1, *m/z* and retention time confirmed with MS^2, 2: Multiple/isotopes present; 3: *m/z* matched single adduct mass within 10 ppm mass error, 4: *m/z* matched adduct mass of multiple isobaric species, probable identifications listed.

Niedzwiecki, Walker, Howell, Watts, Jones, Miller, Hu preliminary data

Metabolic, halogenated environmental chemical, dietary constituent, Alzheimer's medication

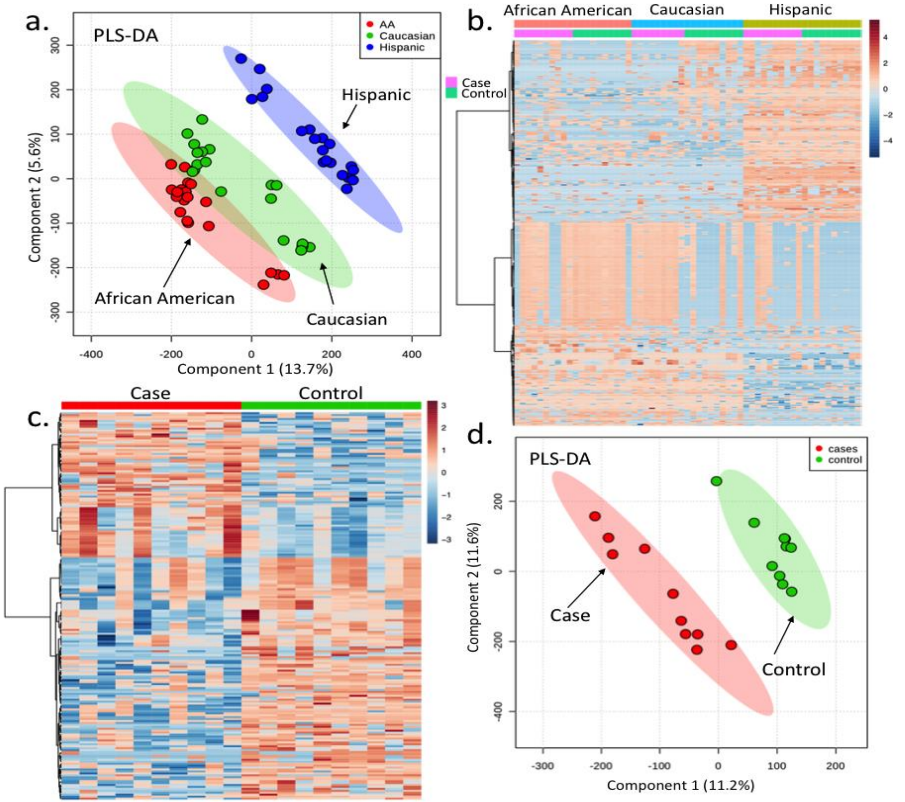
Table 2. Non-medication plasma metabolite features reproducibly associated with AD from MWAS

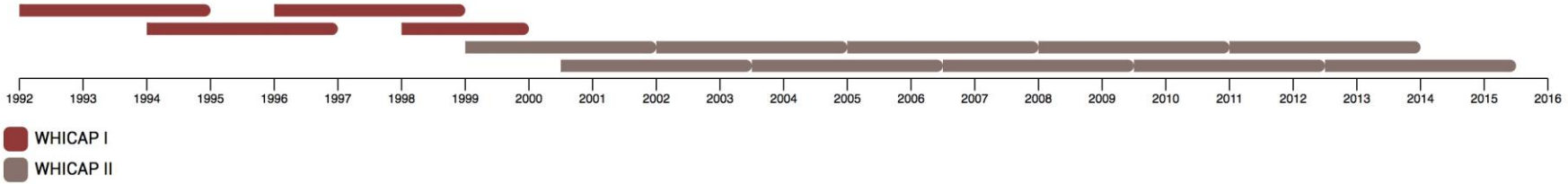
Feature			Study 1		Study 2		Meta-analysis		
m/z[a]	RT[a]	Metabolite	Est (SE)	*p*	Est (SE)	*p*	Est (SE)	*p*	FDR
129.0661	89	Glutamine	0.22 (0.11)	0.04	0.31 (0.13)	0.02	0.25 (0.08)	0.002	0.07
246.9550	127	Unknown	0.41 (0.17)	0.02	0.38 (0.21)	0.07	0.40 (0.14)	0.003	0.08
349.1515	80	Piperine	-0.59 (0.31)	0.06	-0.89 (0.49)	0.07	-0.68 (0.27)	0.01	0.18

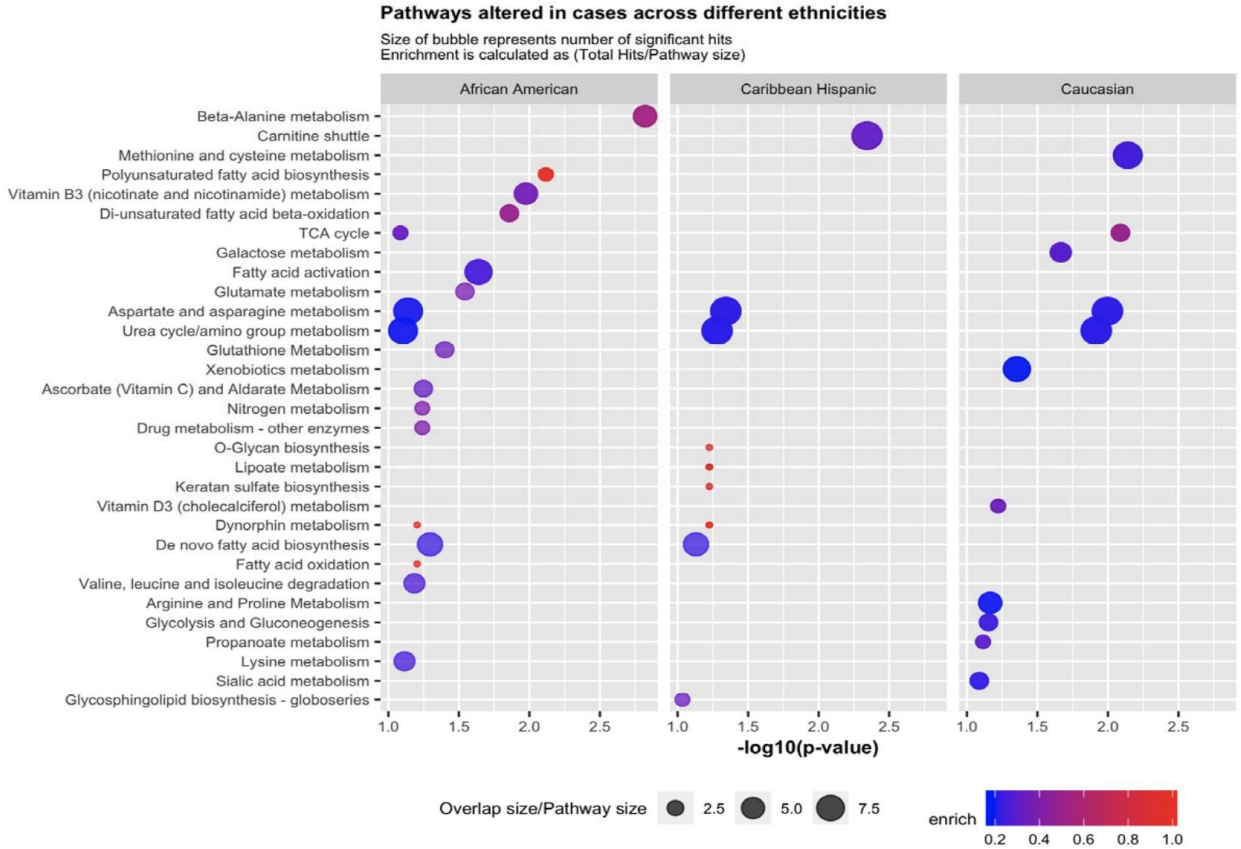
*Adduct of rivastigmine strongest feature associated with AD

Niedzwiecki et al., 2019 Annals of Clinical and Translational Neurology

WHICAP study of Alzheimer's disease, Richard Mayeux, PI Columbia University







Vardarajan et al. Differences in plasma metabolites related to Alzheimer's disease, APOE-ε4 status and ethnicity. medRxiv (PrePrint) posted January 20, 2020

Study	Diagnostic groups	Replication cohort
Orešič et al. 2011[48]	MCI (n=143), AD (n=47), Control (n=46)	None
Ibáñez et al. 2012[10]	AD (n=25), MCI-AD (n=13), MCI-SNAP (n=24), Control (n=23)	None
Trushina et al. 2013[73]	MCI (n=15), AD (n=15), Control (n=15)	None
Motsinger-Reif et al. 2013[55]	AD (n=40), Control (n=38)	None
Cui et al. 2014[49]	AD (n=46), Control (n=37)	AD (n=63), Control (n=67)
Graham et al. 2015[74]	MCI (n=16), MCI-AD (n=19), Control (n=37)	None
Morris et al. 2018[75]	AD (n=64), Control (n=62)	None
Pena-Bautista et al. 2019[76]	MCI-AD (n=29), Control (n=29)	None
Habartová et al. 2019[77]	AD (n=20), Control (n=13)	None

Study	Diagnostic groups	Replication cohort
Orešič et al. 2011[48]	MCI (n=143), AD (n=47), Control (n=46)	None
Ibáñez et al. 2012[10]	AD (n=25), MCI-AD (n=13), MCI-SNAP (n=24), Control (n=23)	None
Trushina et al. 2013[73]	MCI (n=15), AD (n=15), Control (n=15)	None
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Pena-Bautista et al. 2019[76]	MCI-AD (n=29), Control (n=29)	None
Habartová et al. 2019[77]	AD (n=20), Control (n=13)	None

Table 2. Number of blood samples	SA1		SA 2a	All Aims
	Controls	Incident AD	Prevalent AD	Metabolomes
2 +	724	247	375	3,692
3 (or more) ++	760	529	260	4,647
Totals	1484	776	635	8,339

R. Mayeux, B. Vardarajan, G. Miller, Y. Gu., I. Ionita-Laza

The Irving Institute for Clinical and Translational Science

The Irving Institute for Clinical and Translational Research, funded by a National Institutes of Health Clinical and Translational Science Award (CTSA), serves as the cornerstone of translational science for the Columbia Precision Medicine Initiative.

All of Us RESEARCH PROGRAM

The Precision Medicine Initiative

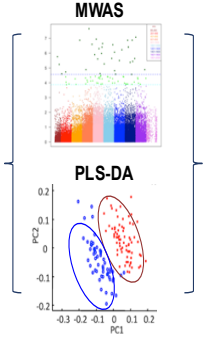
Ultra-high resolution MS
LTQ-FT
LTQ-Velos Orbitrap
Q-Exactive HF
Thermo Fusion

Unequivocal Identification
MS^n; UHRAM MS; 1-D, 2-D NMR; ion Mobility; Chemical Reactivity, etc

Data extraction

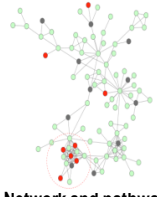
Data Table
m/z, RT, Intensity
Quality metrics

Biostatistics
Bioinformatics



Metabolites of interest

Metabolite forensics



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T Yu et al 2009 Bioinformatics 25:1930-6
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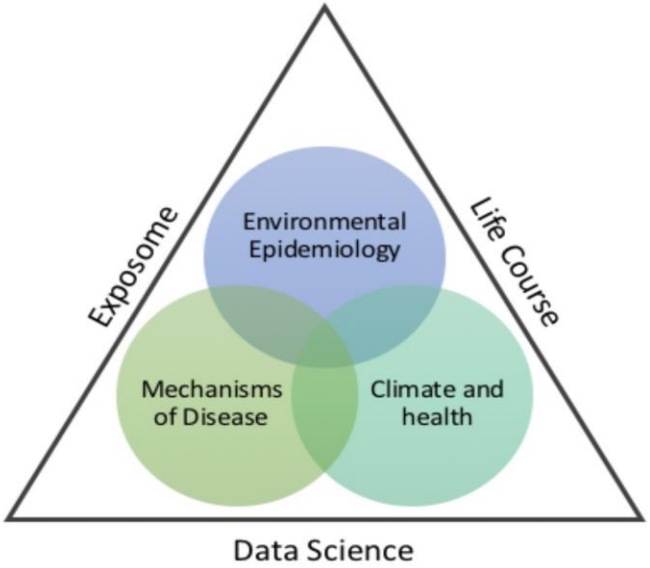
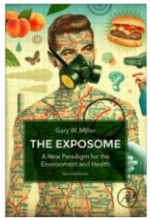
DP Jones 2016 *Tox Reports* **3**: 29-45

Exposome Training Efforts





The Exposome

2nd Edition

A New Paradigm for the Environment and Health

Write a review

Authors: Gary W. Miller

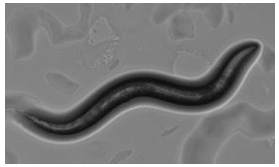
Paperback ISBN: 9780128140796

Imprint: Academic Press

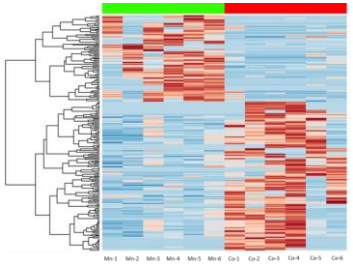
Published Date: 1st July 2020

Page Count: 320

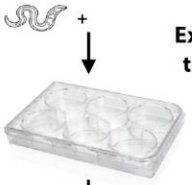
These fellows will enhance their leadership skills by facilitating our workshops, bootcamps, and mini-courses **(machine learning, data visualization, network science)** for the predoctoral trainees



The metabolomic/exposomic analysis works in as few as 500 worms



Metabolomics sample preparation



Exposure to chemical mixtures that represent the exposome

Aliquots of 500 worms stored at -80℃
Metabolites extracted in organic solvent using a bead-beater

Samples run on a high-resolution metabolomics platform

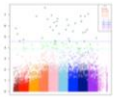
Metabolomics data processing

Data Table, *m/z*, RT, Intensity Quality metrics, Peak grouping/deconvolution

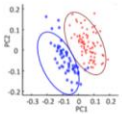
Biostatistics and bioinformatics

Database of m/z, time, and intensity

MWAS



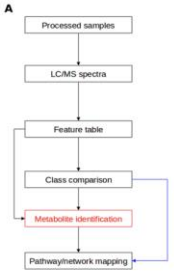
PLS-DA

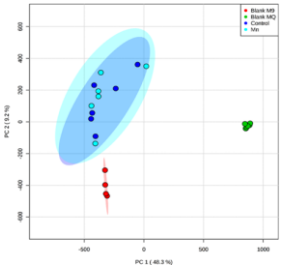


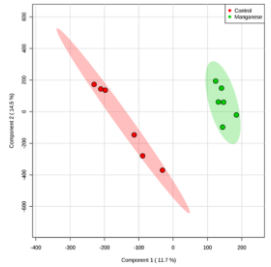
Metabolites of interest

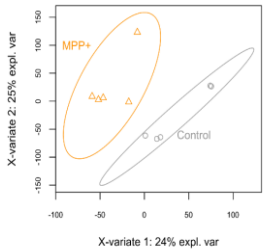
Network and pathway analysis

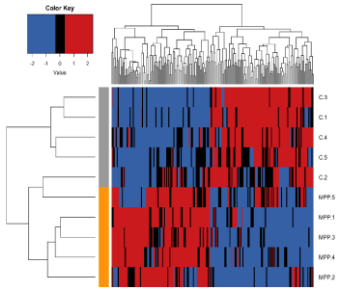
Metabolite forensics:
Spectral databases, Isotope matching, Mass defect searching, Fragments/sub-structures

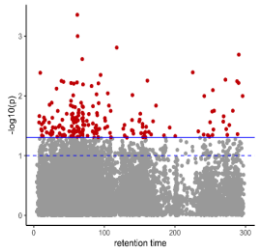


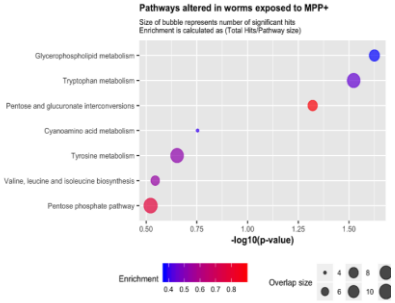




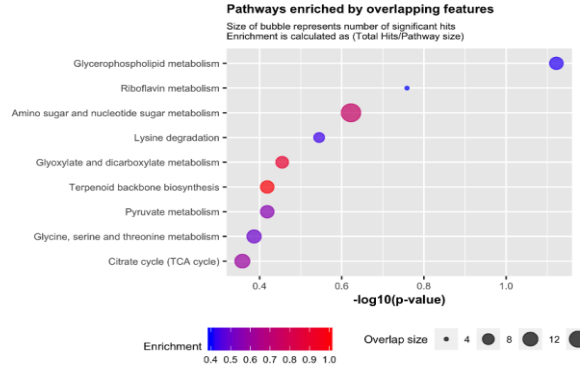


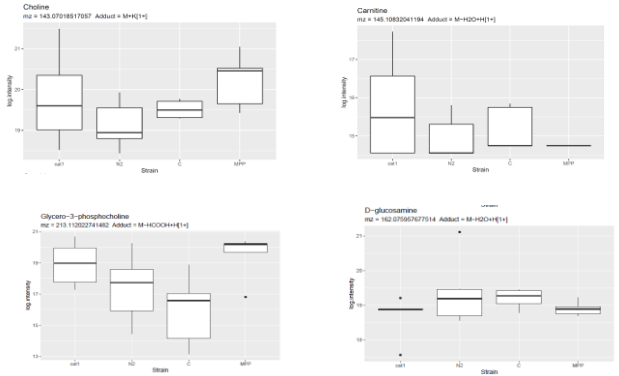






A) PLS-DA analysis comparing N2 worms with N2 worms treated with 1mM MPP+ **B)** Manhattan plot shows features higher (red) and lower (gray) in 1mM MPP+ treated N2's compared to untreated N2 worms **C)** Hierarchical clustering of features detected **D)** Top pathways altered in 1mM MPP+ treated N2 worms, using Mummichog





A) Mummichog analysis of overlapping patterns of pathway enrichment between *cat-1* (*ok411*) worms and N2 worms treated with MPP+

Global Exposome Harmonization Project

Validation inter/intra laboratory
Harmonization of exposome measures
Standardization of operating procedures
Radical transparency
Shared pooled standards
Shared bioinformatic platforms

Columbia, Mt. Sinai, Emory, Mayo Clinic, Yale, Brown (open to other participants)

Inserm (France), Masaryk Univ (Czech Repub), Utrecht (Netherlands), Antwerp (Belgium), Helmholtz (Germany), Univ of Vienna (Austria), Imperial Univ (UK)
Human Biomonitoring for the European Union (HBM4EU)

European Commission Human Exposome Network

EXPANSE: Exposome powered tools for healthy living in urban settings - Prof Roel Vermeulen, Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

EQUAL LIFE: Early Environmental quality and life-course mental health effects – Dr Irene van Kamp, Senior Researcher, National Institute for Public Health and the Environment (RIVM), The Netherlands

LONGITOOLS: Dynamic longitudinal exposome trajectories in cardiovascular and metabolic non-communicable diseases – Dr Sylvain Sebert, University of Oulu, Finland

ATHLETE: Advancing tools for human early lifecourse exposome research and translation - Prof Martine Vrijheid, Barcelona Institute for Global Health, Spain

EXIMIOUS: Mapping exposure-induced immune effects: connecting the exposome and the immunome – Prof Peter Hoet, Catholic University of Leuven Belgium

HEDIMED: Human exposomic determinants of immune mediated diseases – Prof Heikki Hyöty, University of Tampere, Finland

HEAP: Human Exposome Assessment Platform - Prof Joakim Dillner, Karolinska Institute, Sweden

REMEDIA: Impact of exposome on the course of lung diseases – Dr Sophie Lanone, Research Director, French National Institute of Health and Medical Research (INSERM), France

EPHOR: Exposome project for health and occupational research – Dr Anjoeka Pronk, Senior Scientist, Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands

$\sum$ Human Exposome Network and other European partners + $\sum$ Other global partners in the Americas, Japan, China, and India = Human Exposome Project…

….that rivals the Human Genome Project

$$\sum \text{Human Genome} + \sum \text{Human Exposome} = \text{True nature of health and disease}$$

Possible steps and principles

Commitment of collaboration to advance the field

Shared pooled reference material

Shared data and bioinformatic platforms

Standardized confidence levels for identification

Validation among laboratories (instrument-specific)

Investigator exchange program

Standardized operating procedures for harmonized projects

Establishment of a steering/leadership committee

Conclusions

High-resolution mass spectrometry has become the *de facto* machinery for the **exposome (in biological and environmental matrices: plasma, urine, water, dust, air, passive samplers)**

Current technologies, computational workflows, throughput, and costs/sample are insufficient for the needs of the biomedical, clinical, and environmental research communities

As such, a concerted effort to create fit-for-purpose, instrumentation, automation (technical and informatic), and computational systems is essential to balance G x E = P

Organizers

Jarod Grossman
Agilent Technologies

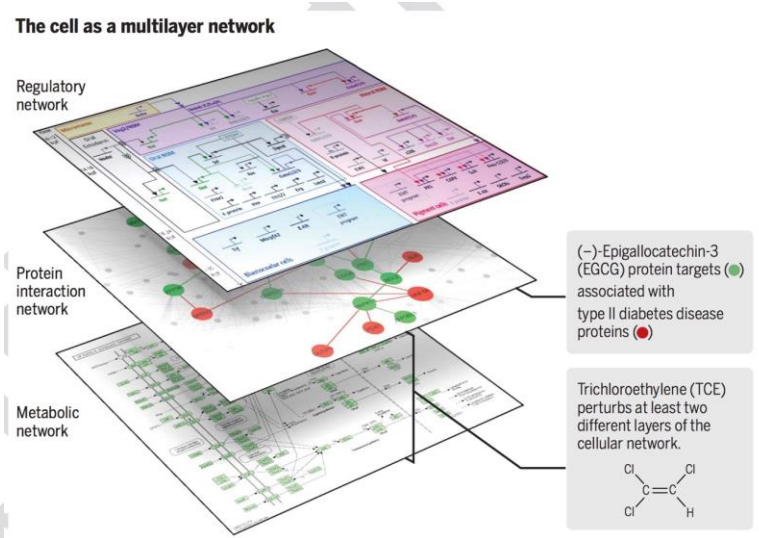
Anthony Macherone
Agilent Technologies &
Johns Hopkins University
School of Medicine

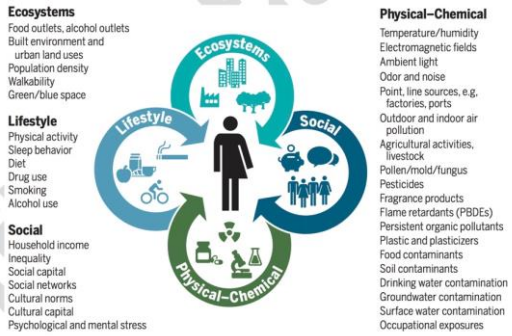
REVIEW

The exposome and health: Where chemistry meets biology

Roel Vermeulen[1,2]*, Emma L. Schymanski[3], Albert-Laszlo Barabási[4,5,6], Gary W. Miller[7]*

Despite extensive evidence showing that exposure to specific chemicals can lead to disease, current research approaches and regulatory policies fail to address the chemical complexity of our world. To safeguard current and future generations from the increasing number of chemicals polluting our environment, a systematic and agnostic approach is needed. The "exposome" concept strives to capture the diversity and range of exposures to synthetic chemicals, dietary constituents, psychosocial stressors, and physical factors, as well as their corresponding biological responses. Technological advances such as high-resolution mass spectrometry and network science have allowed us to take the first steps toward a comprehensive assessment of the exposome. Given the increased recognition of the dominant role that nongenetic factors play in disease, an effort to characterize the exposome at a scale comparable to that of the human genome is warranted.





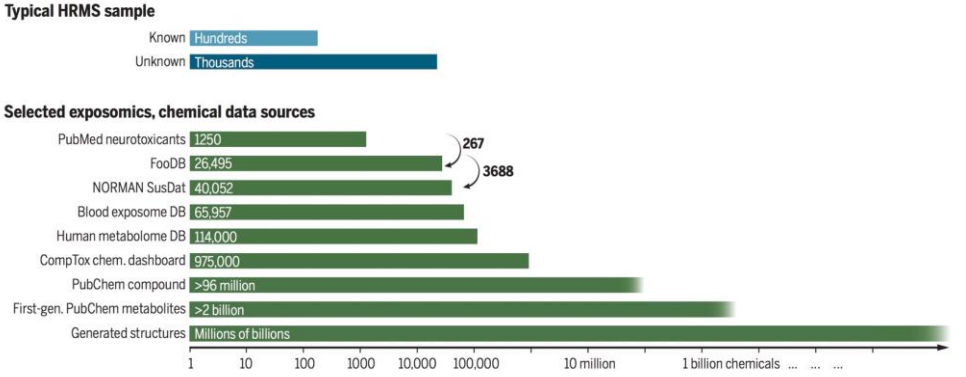


Fig. 2. Chemical complexity of HRMS and the exposome. Top: Known versus unknown features in a typical HRMS measurement [data from (*7*)]. Bottom: Selected data sources relevant to the chemical exposome (*10*–*14*, *19*). Arrows show the overlap of potential neurotoxicants in FooDB (http://foodb.ca/) and FooDB components in NORMAN SusDat (www.norman-network.com/nds/susdat/) (prioritized chemicals of environmental interest).

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