The exposome in molecular epidemiology Towards a more global approach to evaluate exposures to disease risk factors

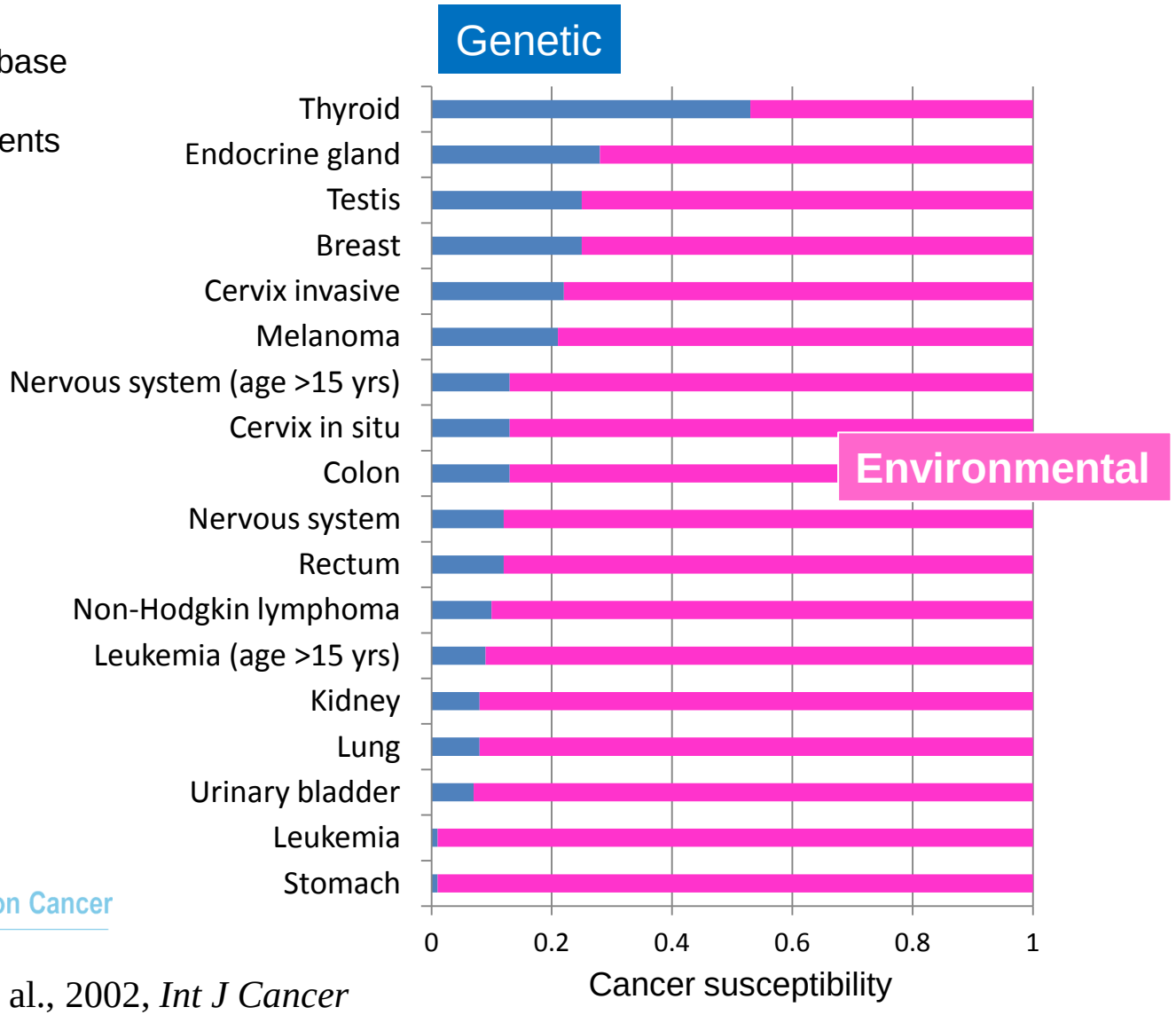
Augustin SCALBERT

Biomarkers Group

International Agency for Research on Cancer
Lyon, France

Causes of cancer are largely environmental

Swedish Family-Cancer Database
(1958–1996)
>600,000 cancers among parents
92,000 among offspring

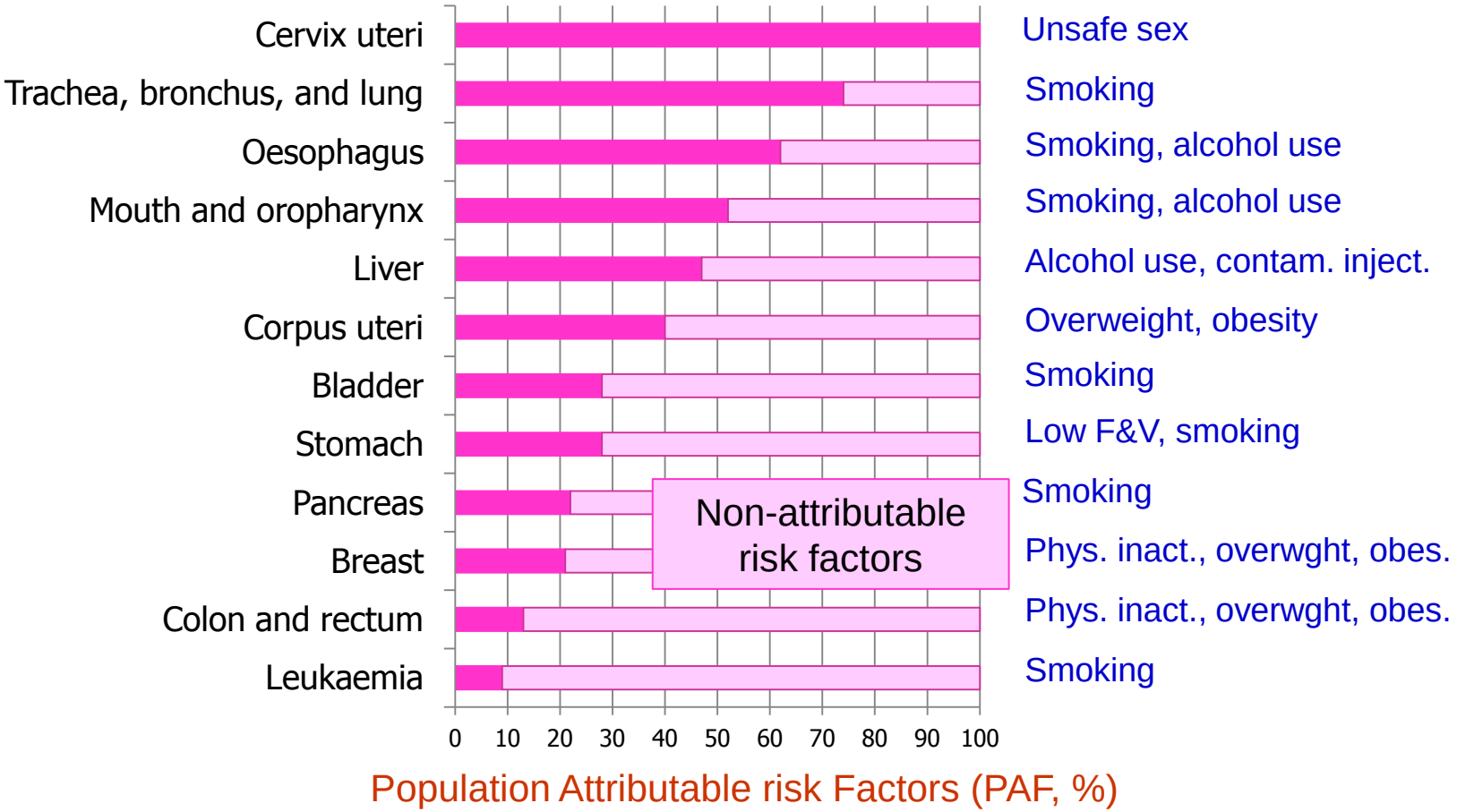


International Agency for Research on Cancer

World Health Organization

Czene et al., 2002, *Int J Cancer*

Known and unknown environmental causes of cancers



- Established causes: infectious agents, smoking, alcohol, diet, lack of physical exercise
- 65% of cancer deaths unexplained

Danaei et al., 2005, *Lancet*

Exposome

Editorial

Complementing the Genome with an "Exposome": The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology

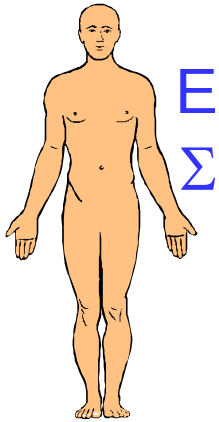
Christopher Paul Wild

Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, Leeds Institute of Genetics, Health and Therapeutics, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom

Wild et al., 2005, *CEBP*

- The totality of environmental exposures received by an individual during life
- 'A collection of environmental factors, such as stress and diet, to which an individual is exposed and which can have an effect on health' (Collins)

Exposome-Wide Association Studies (EWAS)

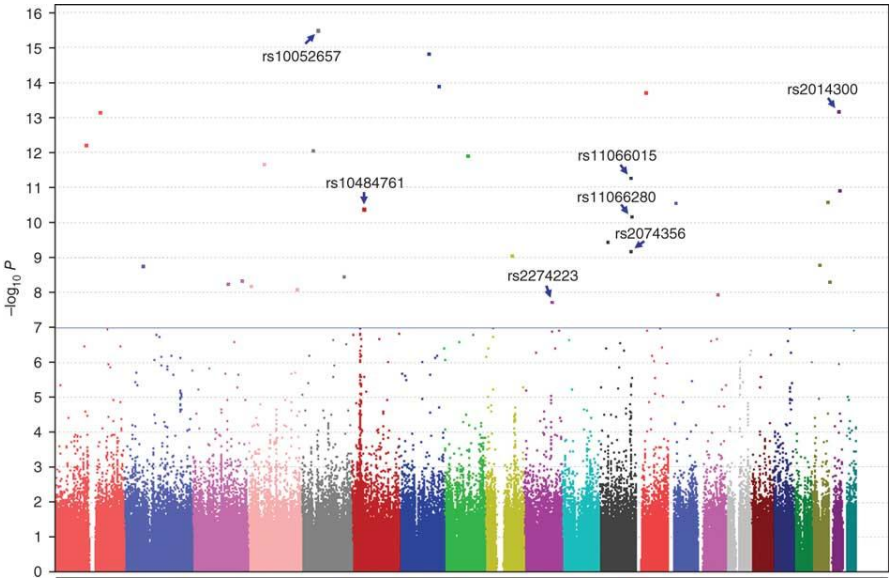


Exposome =
Σ exposures

(Nested)
case-control studies

Disease risk

Association with disease risk

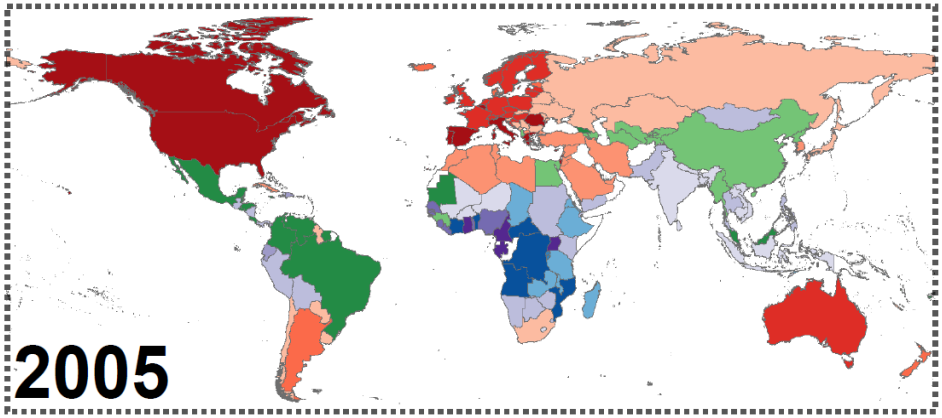


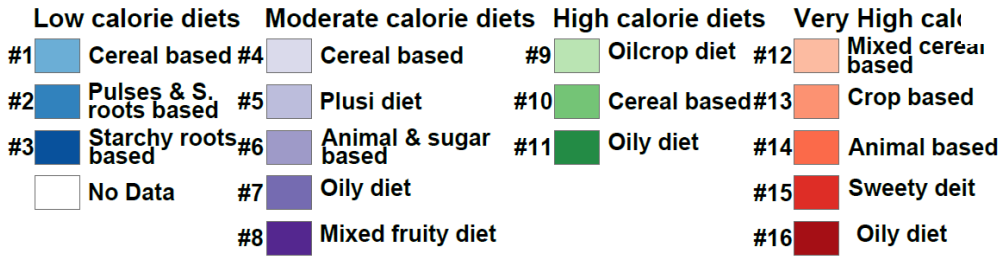
Chemicals, nutrients

- Metabolome-wide, environmental-wide, nutrient-wide association studies (MWAS, EWAS, NWAS)

Dietary patterns and disease risk

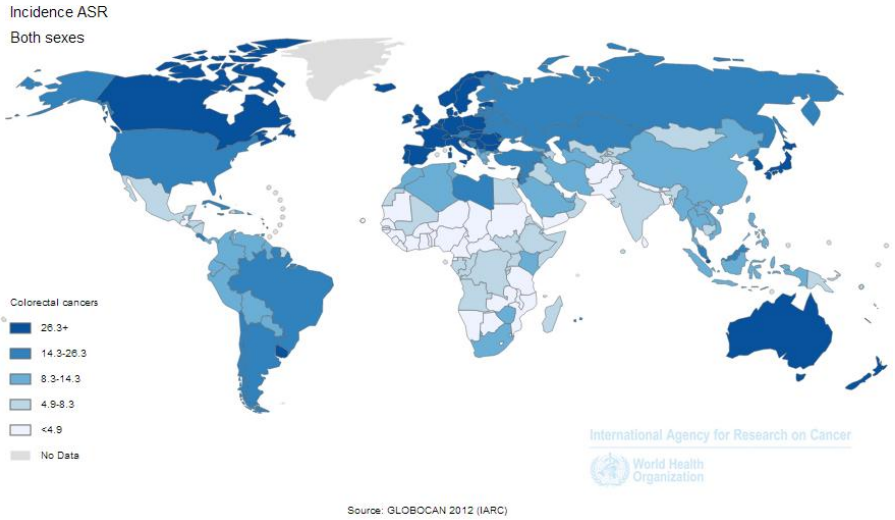
Dietary patterns





Pradhan et al., 2013, *Plos ONE*

Colorectal cancer incidence



GLOBOCAN, 2012

International Agency for Research on Cancer

World Health Organization

The components of dietary patterns

Nutrients

Total fat
SFA
Ratio of MUFA or PUFA to SFA
PUFA
Trans fatty acids
Protein
Carbohydrate
Complex carbohydrates
(Cereal) fibre
Mono- and disaccharides
Sucrose
Cholesterol
Alcohol
Sodium
Calcium
Iron
Vitamin C
Ratio of carbohydrates to protein to fat

Foods

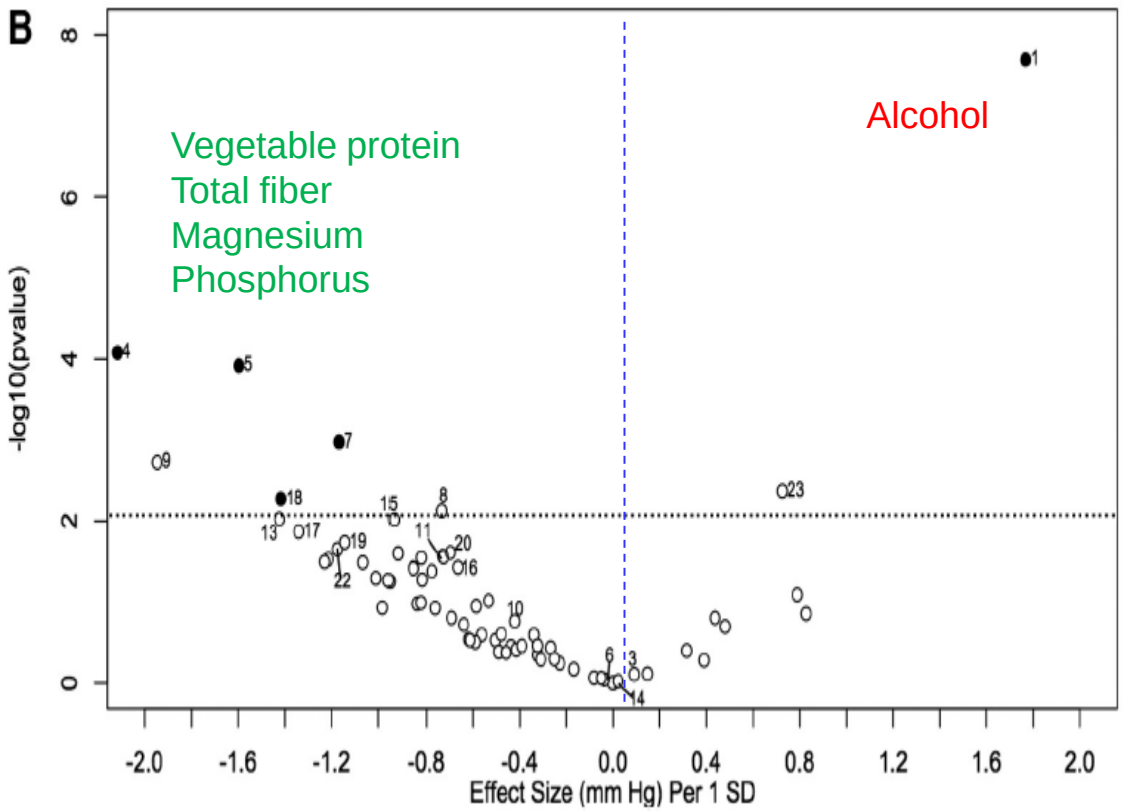
Fruit and vegetables
Fruits (and nuts)
Vegetables
Legumes (and nuts and seeds)
Nuts (and soya)
(Whole) cereals/grains (Coarse) bread
Meat (and meat products)
Ratio of white to red meat
Red and processed meat
Poultry
Fish
Milk (and dairy products)
High fat dairy
Olive oil
Potatoes
Cheese
Red wine
Butter, margarine, animal fat
Sweets/sweet beverages

Waijers et al., 2007, *BJN*

- Traditionally measured with dietary questionnaires
- Number of bias and errors in the measurements

A first nutrient-wide association study

Nutrients and blood pressure associations
in the INTERMAP cohort



1 Alcohol
2 Non-heme Iron (NA)
3 Iron
4 Vegetable Protein
5 Total Fiber
6 Riboflavin
7 Magnesium
8 Vitamin B6
9 Starch
10 Vitamin E
11 Folacin
12 Urinary Sodium to Potassium Ratio (NA)
13 Glutamic Acid
14 Thiamin
15 Copper
16 Retinol
17 Cystine
18 Phosphorus
19 Tryptophan
20 Total Vitamin A
21 Urinary Calcium (NA)
22 Phenylalanine
23 Vitamin B12

Tzoulaki et al., 2013, *Circulation*

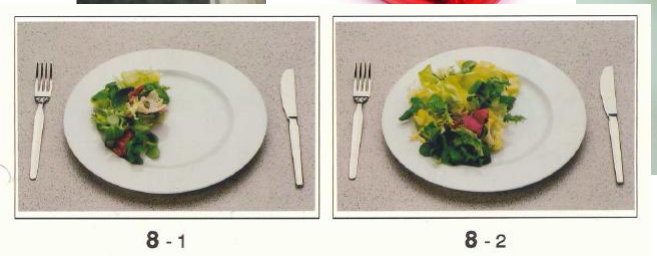
International Agency for Research on Cancer

World Health Organization

- Largely based on nutrient intake data

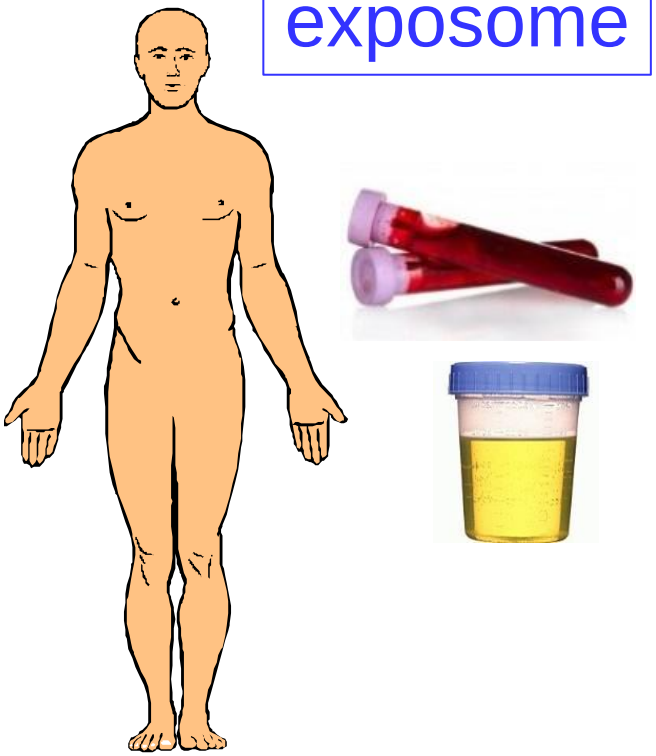
Measurement of the exposome

External exposome



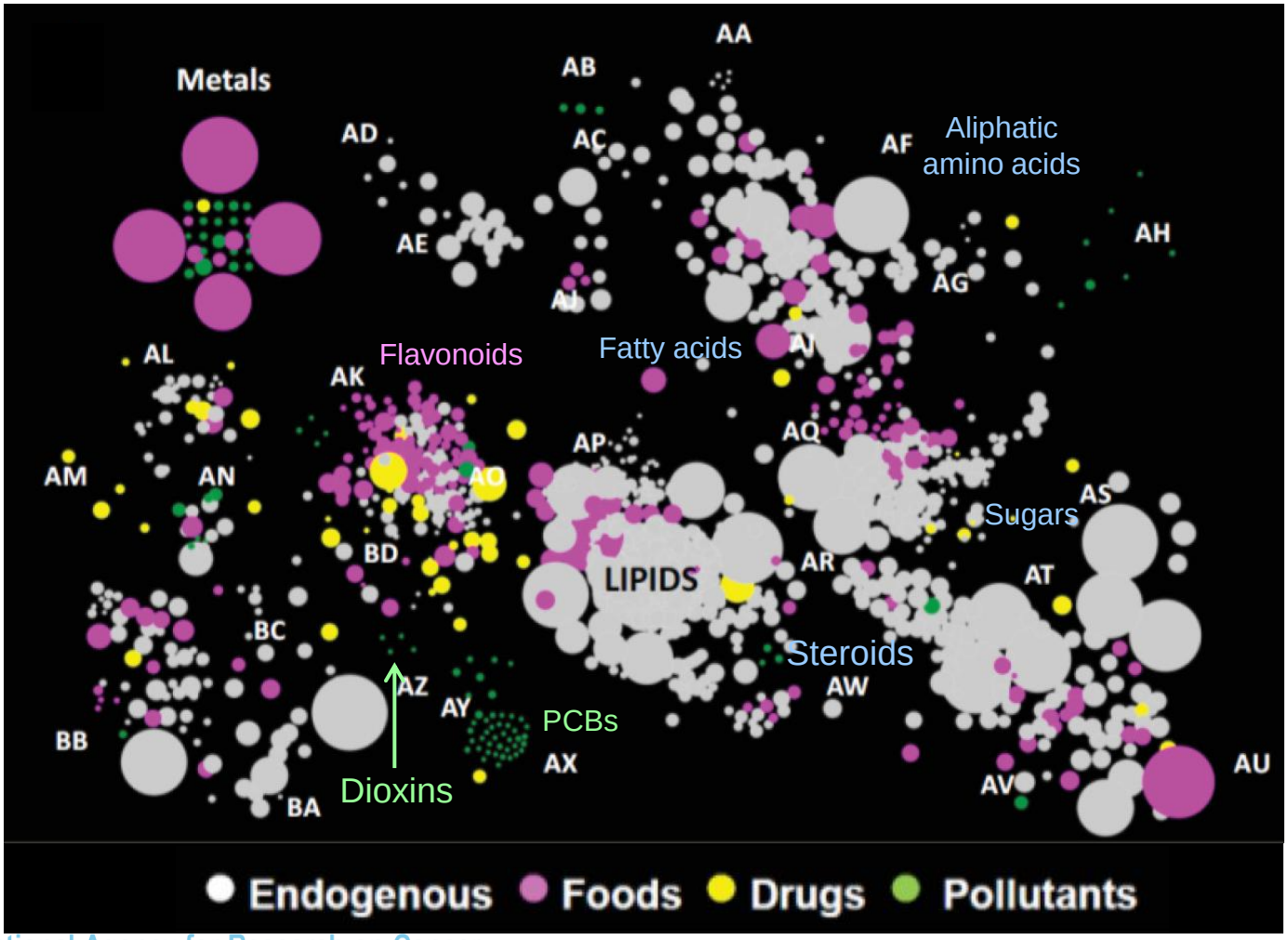
Environmental and personal measurements

Internal exposome



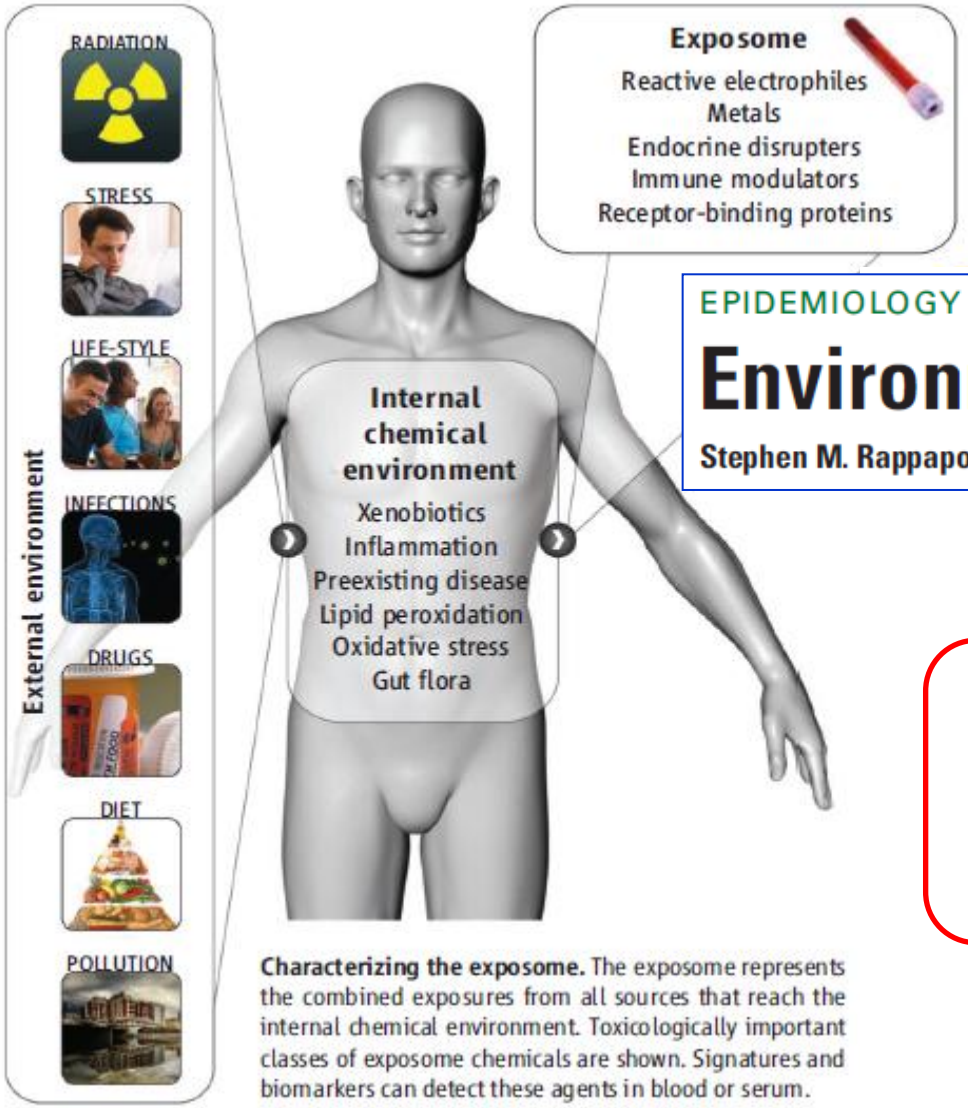
Biomarkers

The blood exposome



Rappaport et al., 2014, *Environ. Health Perspect.*

The internal exposome



Characterizing the exposome. The exposome represents the combined exposures from all sources that reach the internal chemical environment. Toxicologically important classes of exposome chemicals are shown. Signatures and biomarkers can detect these agents in blood or serum.

EPIDEMIOLOGY

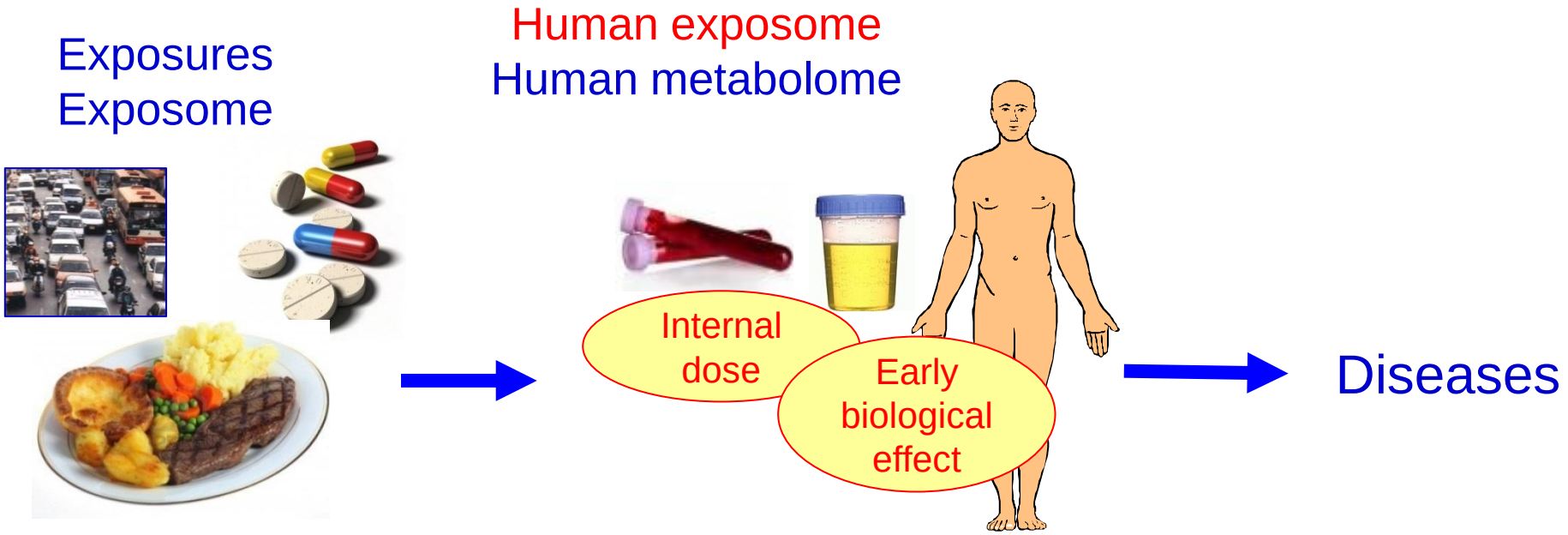
Environment and Disease Risks

Stephen M. Rappaport and Martyn T. Smith

All biologically active chemicals in the internal environment (endogenous and exogenous chemicals in human blood)

Rappaport & Smith, 2010, *Science*

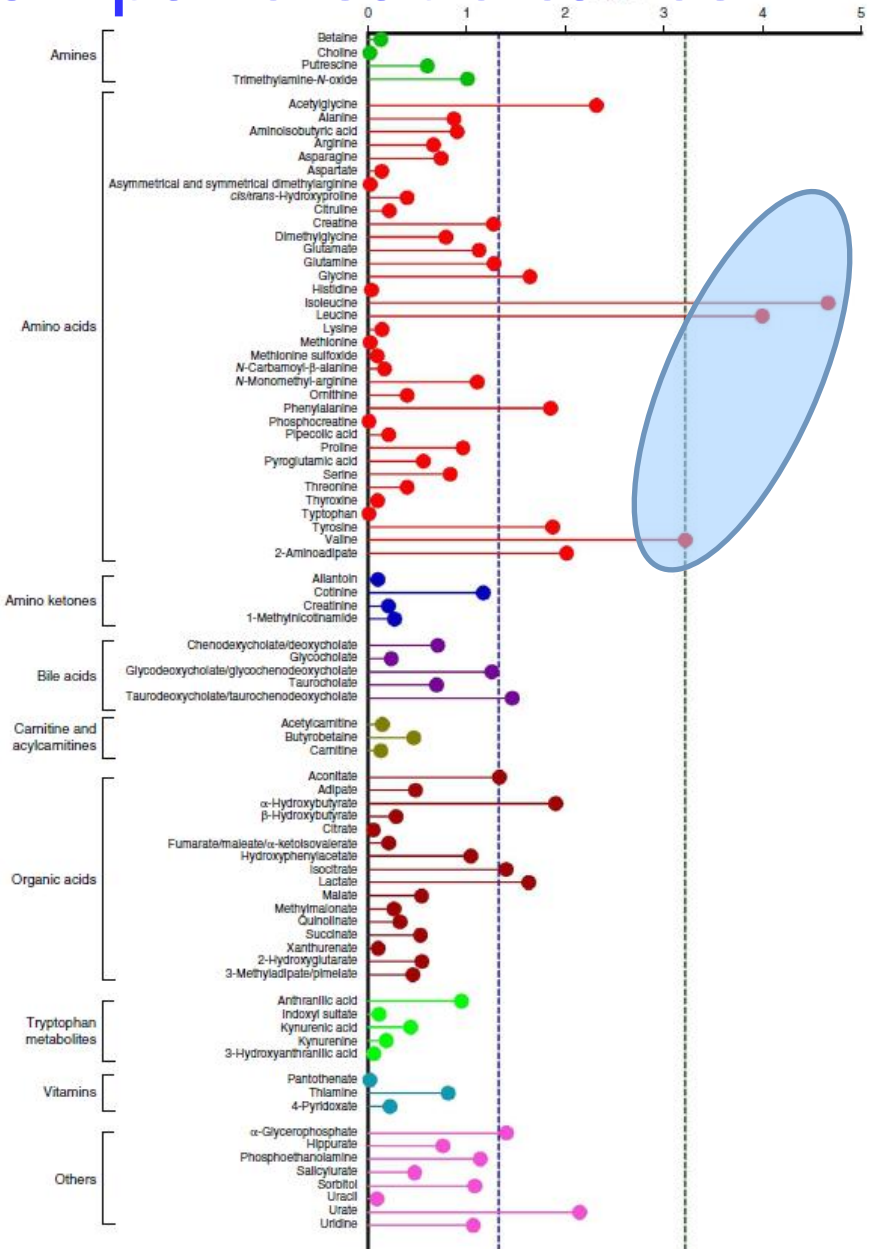
Exposures, exposome and metabolome



- Exposome : The totality of environmental exposures received by an individual during life
- Metabolome : The complete collection of small molecular weight metabolites in a cell, tissue, biofluid or organism

First EWAS in a prospective cohort on pancreatic cancer

Four prospective cohorts (HPFS, NHS, PHS, WHI)
453 cases, 898 controls
Plasma samples
Avg. follow-up time: 8.7 yrs
83 polar metabolites measured by LC-ESI-MS-MS
(central metabolism and amino acid metabolism)



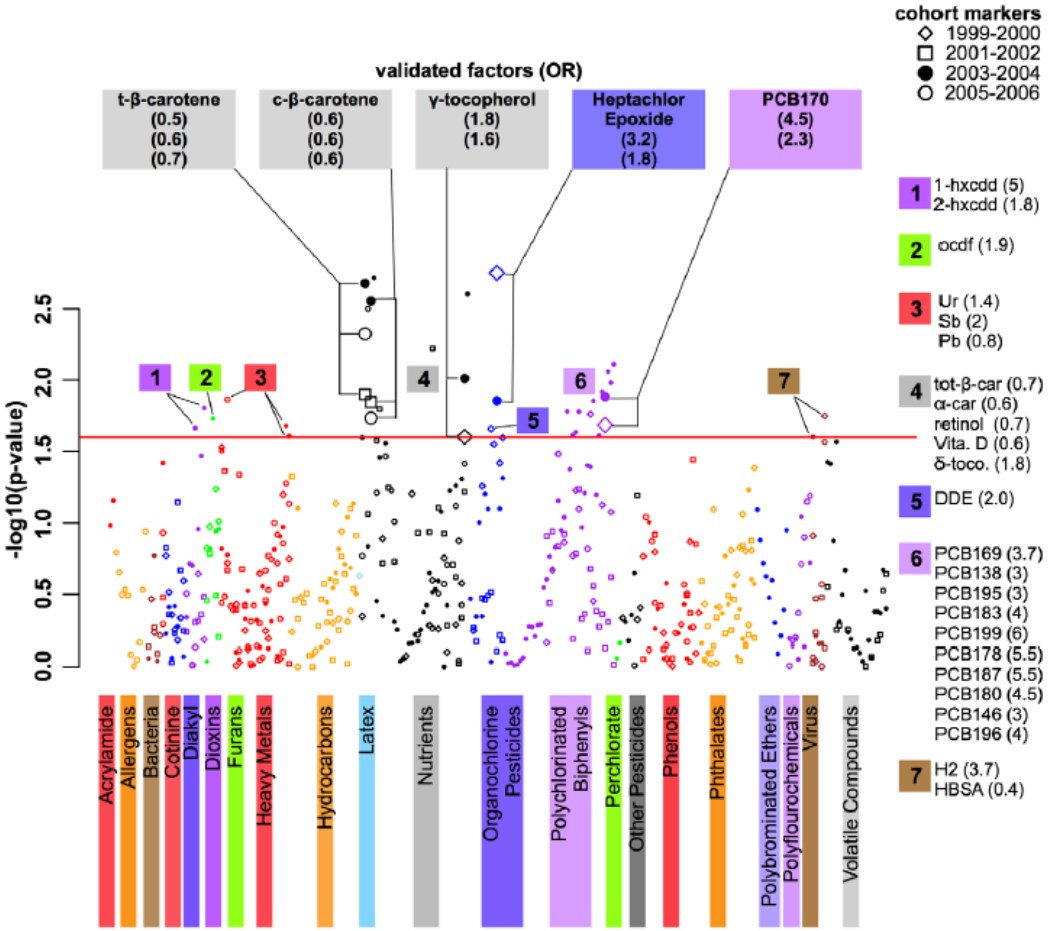
- Isoleucine, leucine and valine significantly increased after correction for multiple-hypothesis testing
- Association independant of intermediate development of diabetes

Mayers et al., 2014, *Nature Med*

Expsome-Wide Association Studies NHANES data

NHANES cohorts
266 environmental factors
in urine or plasma
Fasting glucose

US$ 40,000 per sample

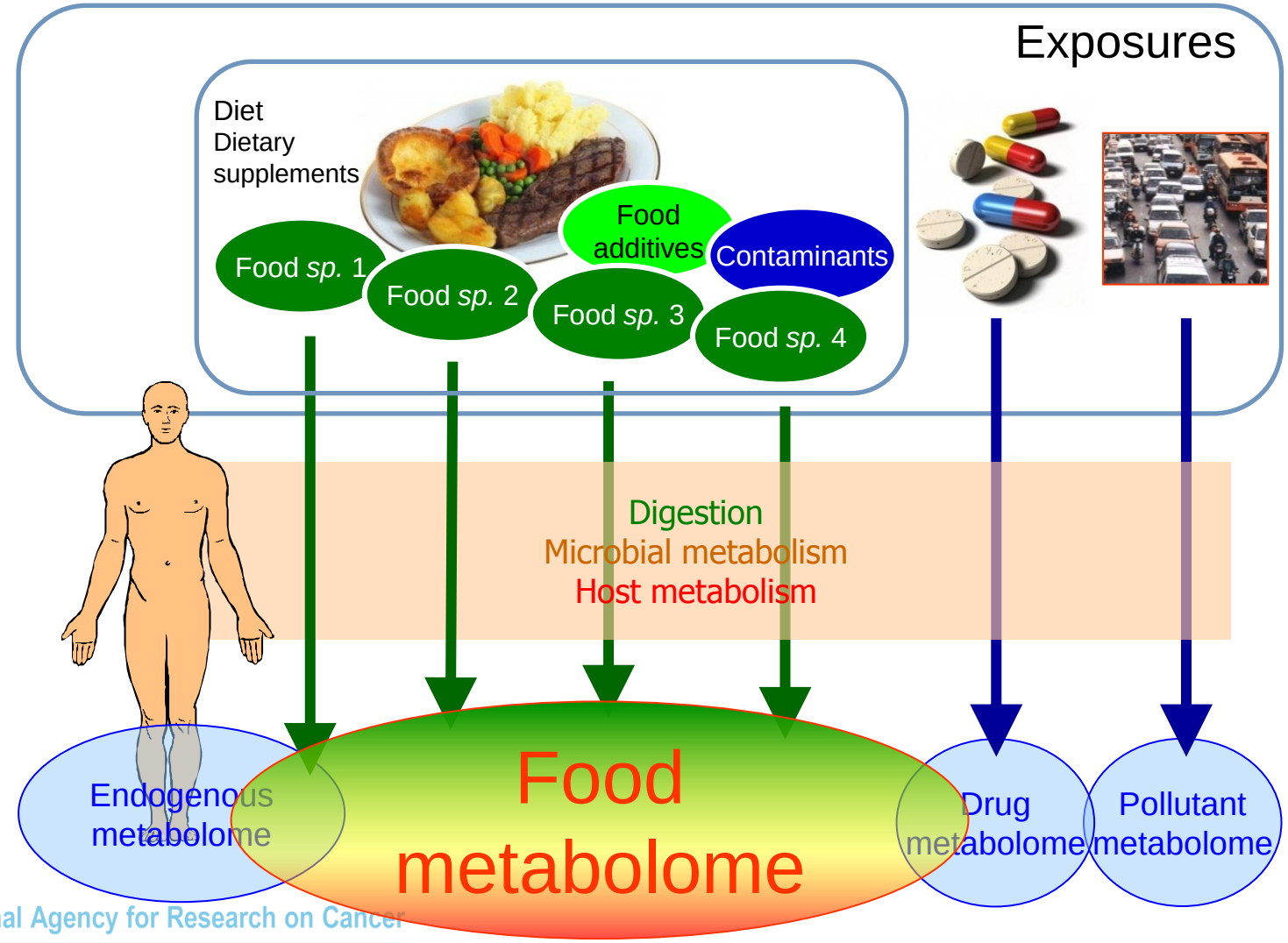


International Agency for Research on Cancer

World Health Organization

Patel et al., 2010, *Plos One*

The food metabolome



International Agency for Research on Cancer

World Health Organization

Scalbert et al., 2014, *Am. J. Clin. Nutr.*

FooDB, the most comprehensive database on food constituents

- 27,509 compounds known in foods

FooDB Foods Compounds Search Downloads About Contact Us

FooDB is the world's largest and most comprehensive resource on food constituents, chemistry and biology. It provides information on both macronutrients and micronutrients, including many of the constituents that give foods their flavor, color, taste, texture and aroma.

Each chemical entry in the FooDB contains more than 100 separate data fields covering detailed compositional, biochemical and physiological information (obtained from the literature). This includes data on the compound's nomenclature, its description, information on its structure, chemical class, its physico-chemical data, its food source(s), its color, its aroma, its taste, its physiological effect, presumptive health effects (from published studies), and concentrations in various foods.

Users are able to browse or search FooDB by food source, name, descriptors, function or concentrations. Depending on individual preferences users are able to view the content of FooDB from the Food Browse (listing foods by their chemical composition) or the Compound Browse (listing chemicals by their food sources).

FooDB Version 1.0

This project is supported by The Metabolomics Innovation Centre (TMIC), a nationally-funded research and core facility that supports a wide range of cutting-edge metabolomic studies. TMIC is funded by Genome Alberta, Genome British Columbia, and Genome Canada, a not-for-profit organization that is leading Canada's national genomics strategy with $900 million in funding from the federal government.

TMIC The Metabolomics Innovation Centre

GenomeAlberta GenomeBritishColumbia GenomeCanada

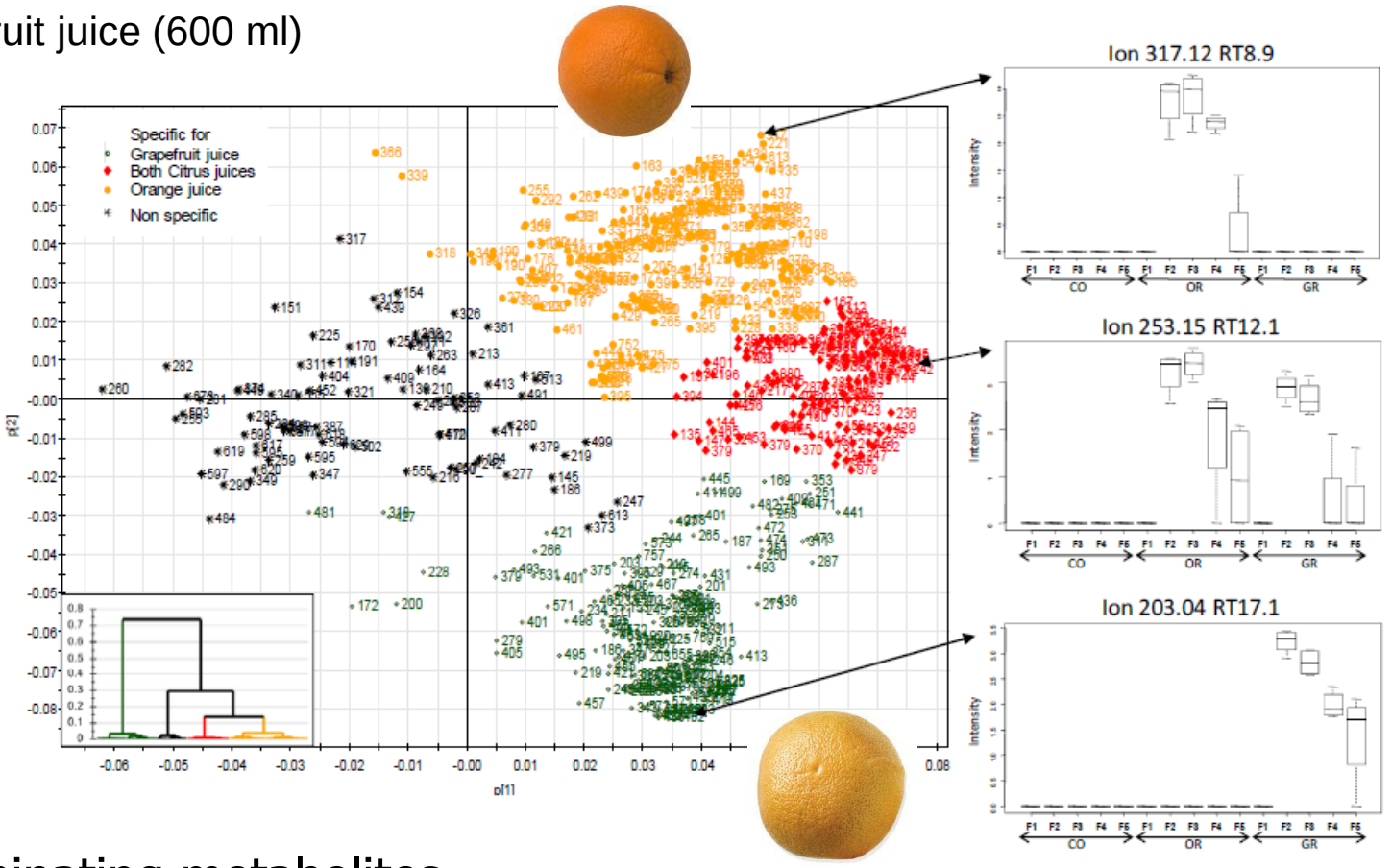
Food Component Database Version **1.0** — Contact Us

World Health Organization

http://foodb.ca/

Biomarkers of citrus fruit intake

4 volunteers
Orange or grapefruit juice (600 ml)
Urine
UPLC-QTof



- 12 discriminating metabolites
  - Proline betaine, hydroxyproline betaine
  - Naringenin 7-O glucuronide, hesperetin 3'-O-glucuronide
  - Limonene 8,9-diol glucuronide, nootkatone 13,14-diol glucuronide

Pujos-Guillot et al., 2013, *J. Proteome Res.*

Metabolomics and the food metabolome in acute intervention studies

Food	Biospe-cimens	Analytical method	Biomarkers	Reference
Citrus fruits	Urine	NMR	Proline betaine	Heinzmann, 2010
	Urine	MS	Proline betaine, 4-hydroxyproline betaine	Lloyd, 2011
	Urine	MS	Proline betaine, 4-hydroxyproline betaine, limonene 8,9-diol-GlcUA, nootkatone 13,14-diol GlcUA, hesperetin GlcUA, naringenin GlcUA, N-methyltyramine sulfate	Pujos-Guillot, 2013
Raspberry	Urine	MS	Caffeoyl sulfate, methylepicatechin sulfate	Lloyd, 2011
Cruciferous vegetables	Urine	NMR	S-Methyl-L-cystein sulfoxide	Edmands, 2011
Wholegrain rye	Urine	MS	3-(3,5-Dihydroxyphenyl)-1-propanoic acid sulfate, enterolactone GlcUA, 2-aminophenol sulfate	Bondia-Pons, 2013
High-fiber diet	Plasma	MS	2-Aminophenol sulfate, 2,6- dihydroxybenzoic acid	Johansson-Persson, 2013
Oily fish	Urine	MS	Anserine, methylhistidine, TMAO	Lloyd, 2011
Cocoa	Urine	MS	Vanilloyl glycine, trigonelline, 3,5-diethyl-2-methylpyrazine, epicatechin sulfate, theobromine	Llorach, 2009, 2010
Coffee	Urine	MS	Caffeoylquinic acid sulfate, caffeoylquinic acid lactone sulfate, caffeic acid sulfate, feruloyl glycine	Stalmach, 2009
	Plasma	MS	Feruloylquinic acid lactone sulfate,	Redeuil, 2011
Tea	Urine	NMR	Hippuric acid, gallic acid, 1,3-dihydroxyphenyl-2-O-sulfate	Daykin, 2005
Nuts	Urine	MS	10-Hydroxy-decene-4,6-diynoic acid sulfate, tridecadienoic/tridecynoic acid GlcUA, dodecanedioic acid	Tulipani, 2011
Wine	Urine	NMR	Tartrate, EtOH, mannitol	Vázquez-Fresno, 2012

Scalbert et al., 2014, *AJCN*

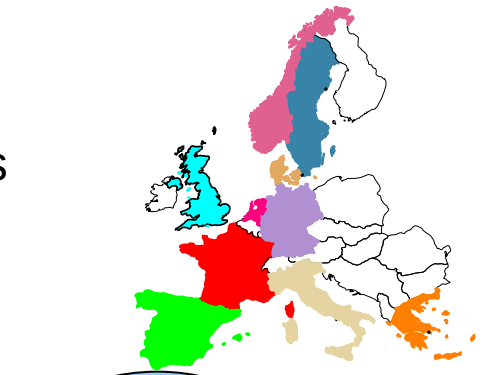
The food metabolome in a cross-sectional study in EPIC

EPIC

- 520,000 participants
- 23 centres in 10 Western European countries
- Areas with varying cancer rates
- Heterogeneity of lifestyle habits
- Food Frequency Questionnaires (FFQ) detailed, validated, country-specific
- Plasma samples

EPIC calibration study

- 36,900 participants
- 24-hr dietary recalls (EPIC-Soft)
- 24-hr urine samples (n=1,103)



24-hr Dietary Recall

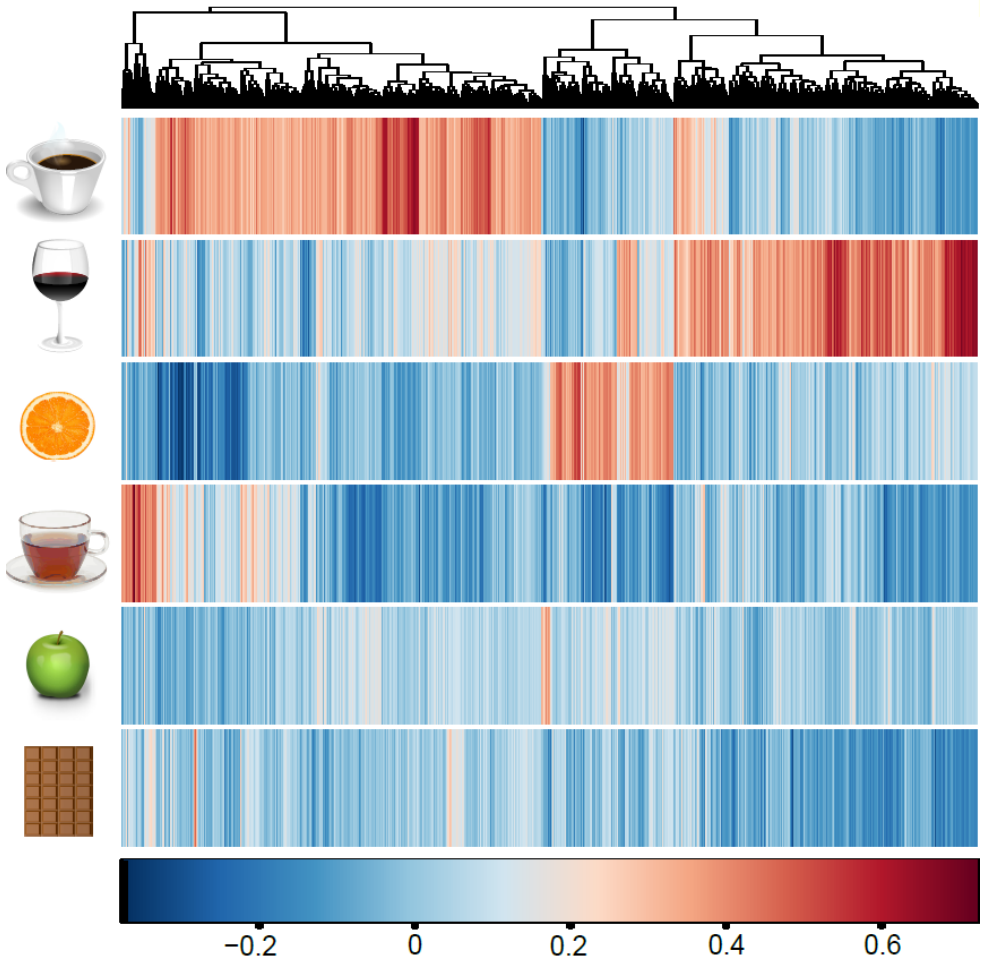
> 10,000 foods

Food Frequency Questionnaire

> 200 foods

Metabolic profiles and food intake

481 subjects from 4 countries
24-hr Dietary recalls
24-hr Urine samples
High-resolution mass spectrometry
(UPLC QTof, neg ionization)
Iterative regression analyses

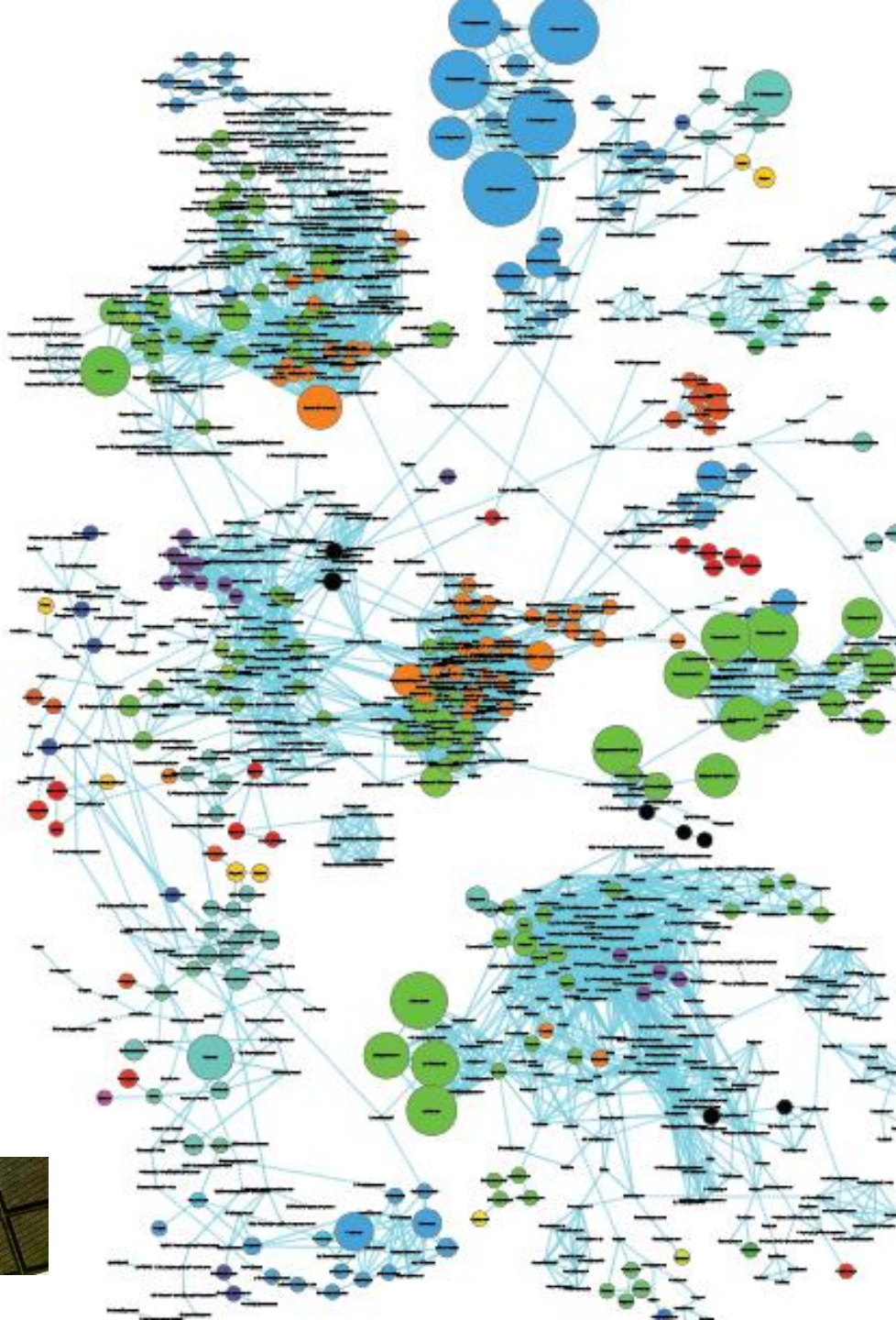


- 14,000 mass spectrometry feature detected
- 2,272 features correlated to intake of six different foods

Edmands et al., 2014, *Bioinformatics*
Edmands et al., 2014, *Anal Chem*
Edmands et al., submitted

Dietary polyphenols

- > 500 polyphenols in >450 foods
- Often specific of a particular food or food group
- 60,000 content values in foods



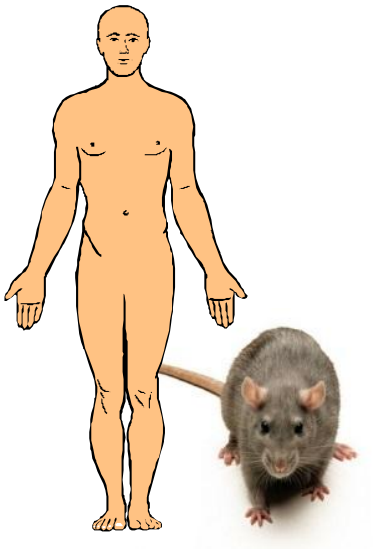
Phenol-Explorer
Database on polyphenol content in foods

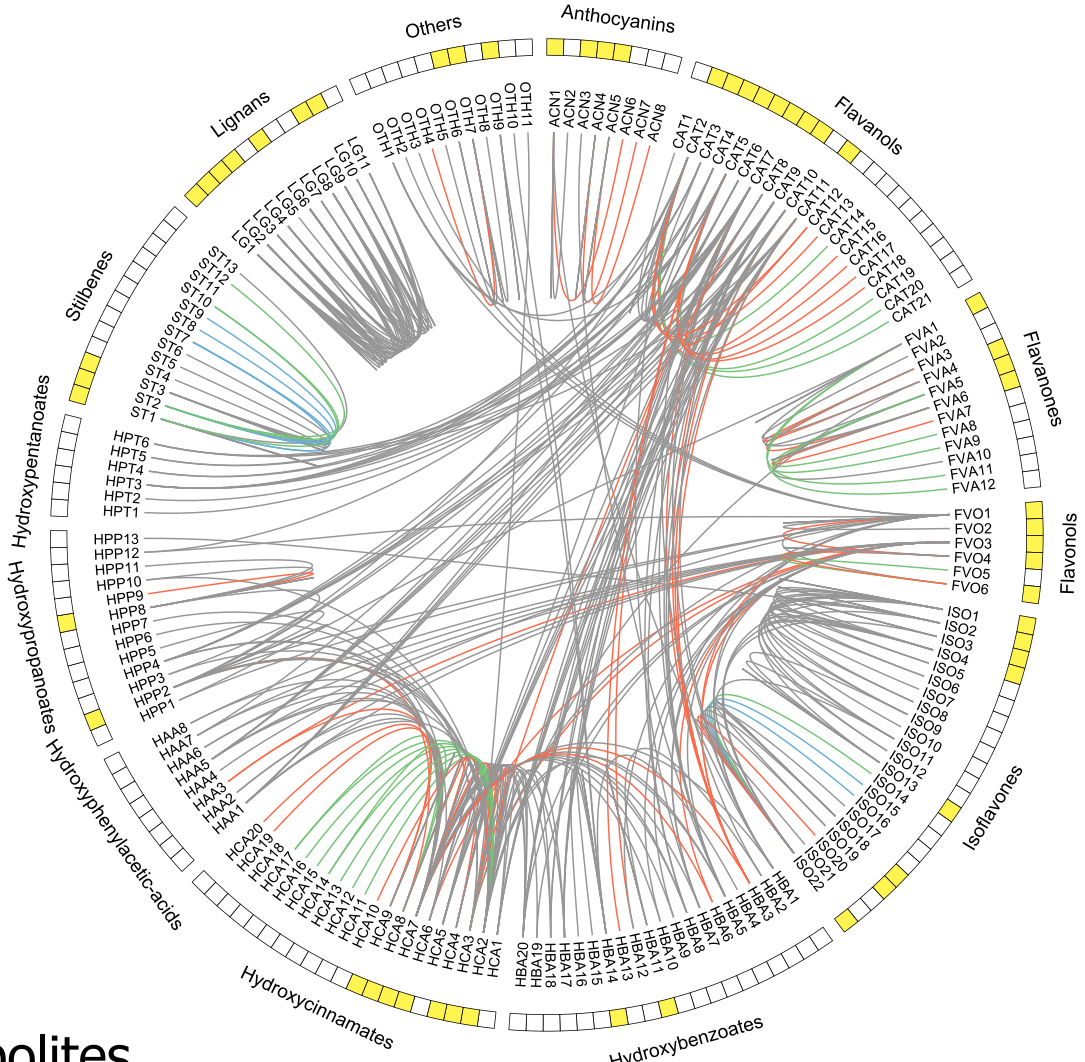
http://www.phenol-explorer.eu

Neveu et al., 2010, *Database*
Perez-Jimenez et al., 2010, *JAFC*
Rothwell et al., 2014, *Database*

Phenol-Explorer - Polyphenol metabolites

Rothwell et al., 2012, *Database*

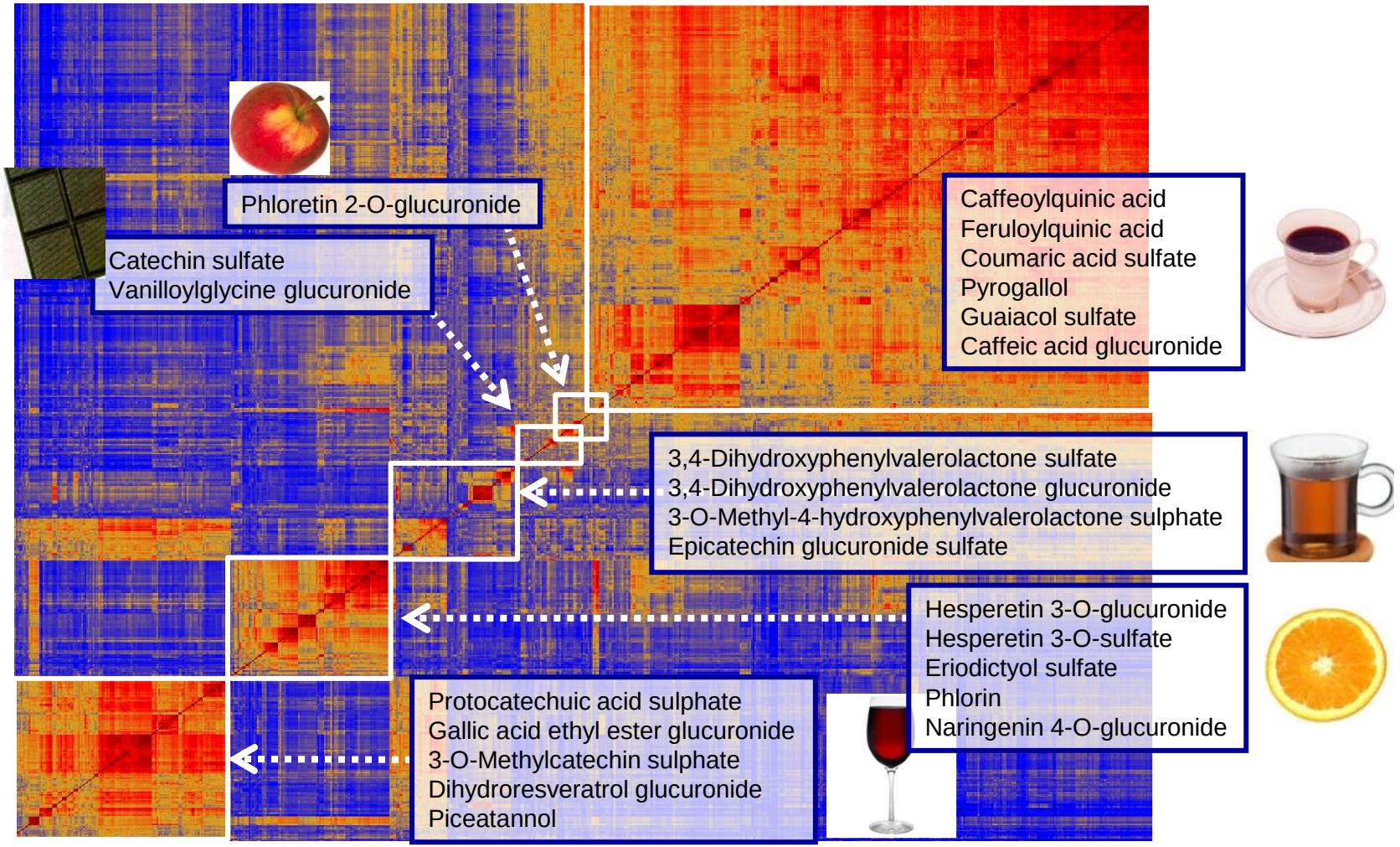




Rothwell et al., in preparation

- 375 polyphenol metabolites
- Human subjects and experimental animals
- Filiation between parent polyphenols and metabolites documented

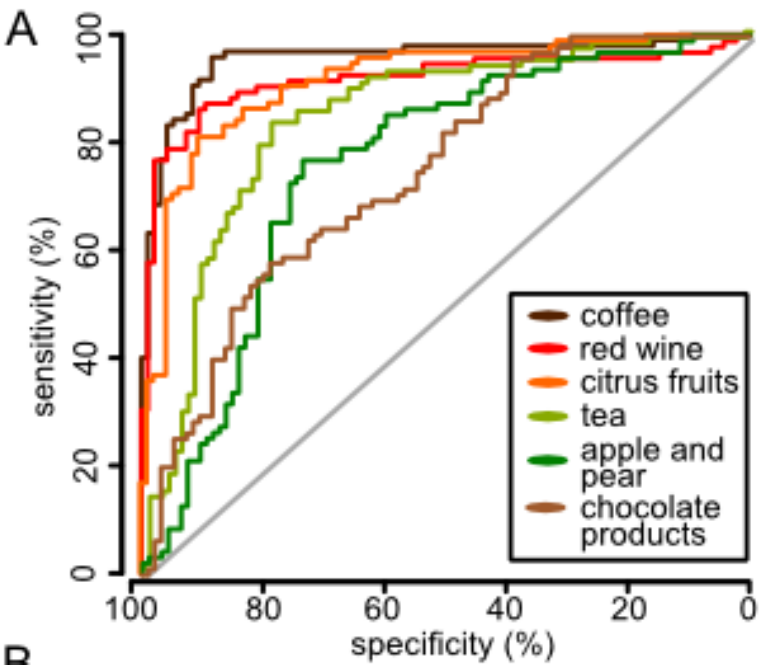
Polyphenol metabolites as dietary biomarkers in EPIC

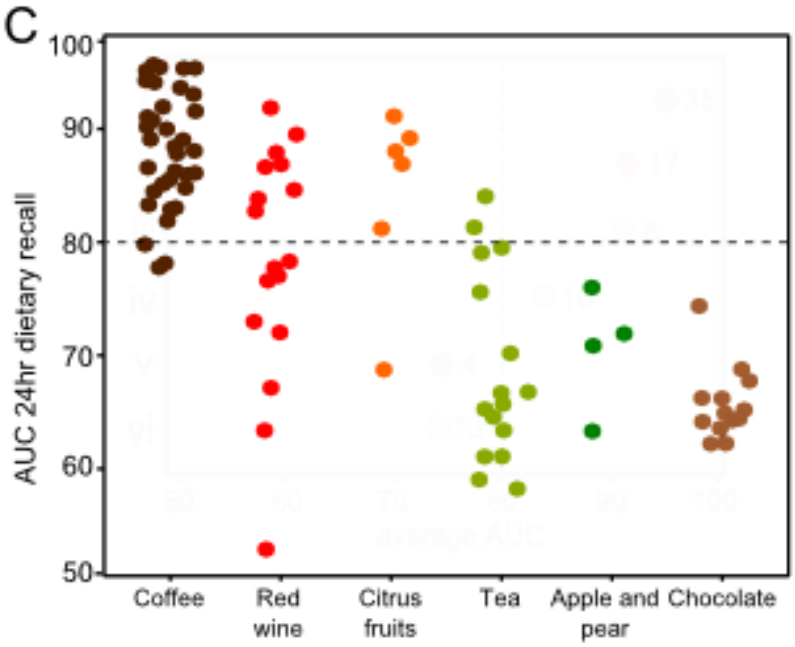


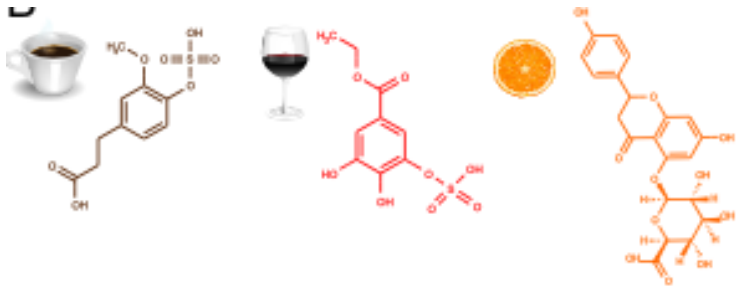
- 83 metabolites associated to the consumption of six polyphenol-rich foods

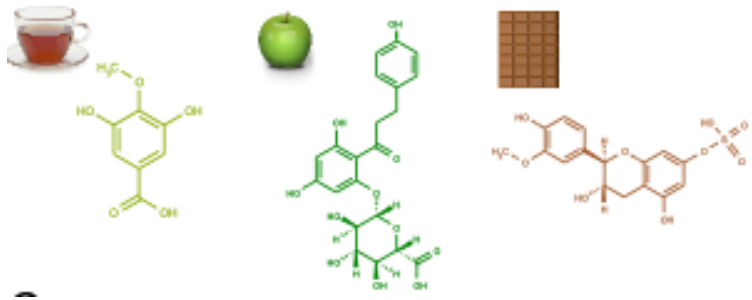
Edmands et al., submitted

Selection of best exposure biomarkers









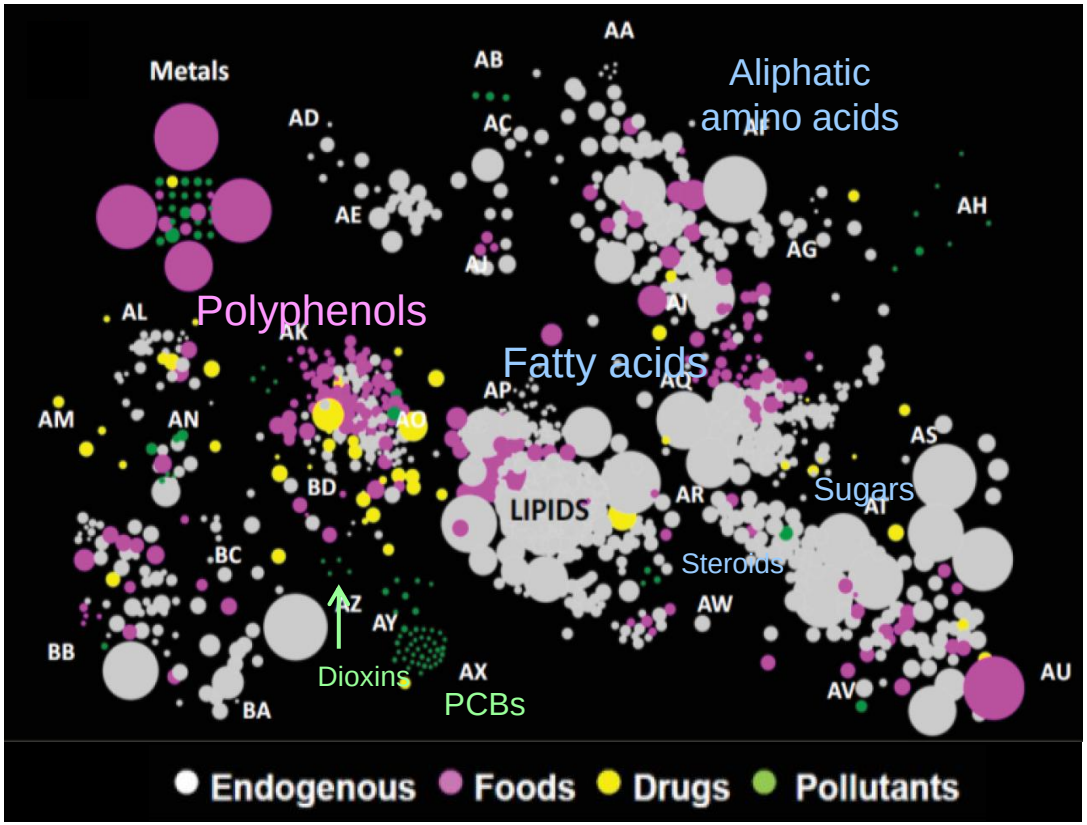
- 6 polyphenol metabolites selected as best predictors of food intake

Edmands et al., submitted

Different metabolomic approaches

	Targeted metabolomics	Untargeted metabolomics
Quantitative analyses*	50-200 metabolites	--
Semi-quantitative analyses		Up to 15,000 MS features

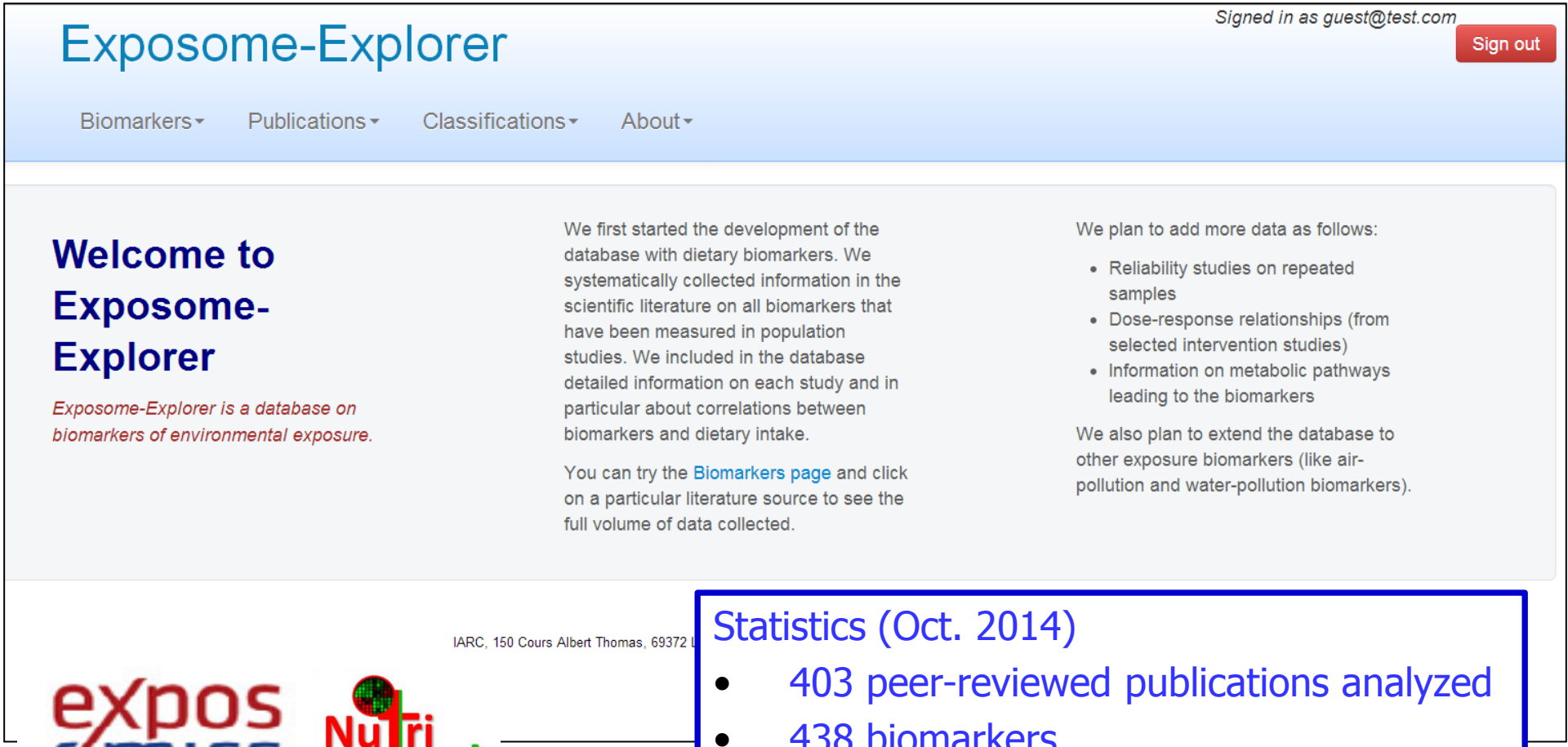
* Requires calibration with appropriate chemical standards



Tandem mass spectrometer (triple quadrupole, QqQ)

Time of flight (Tof) Orbitrap

Exposome-Explorer



Statistics (Oct. 2014)

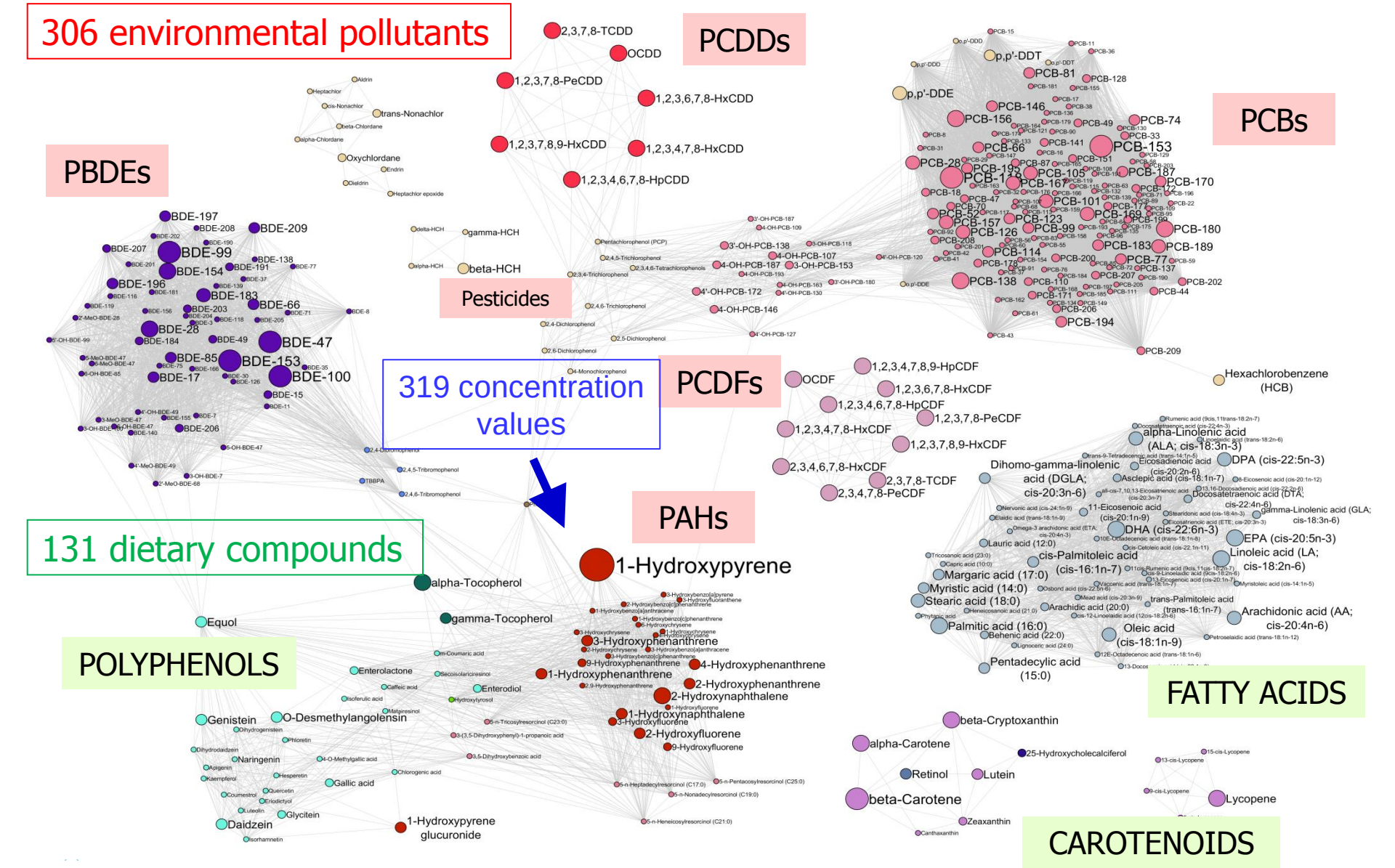
- 403 peer-reviewed publications analyzed
- 438 biomarkers
- 10,474 concentration values
- 8,090 correlation values with dietary exposures

Exposome-Explorer
438 biomarkers of exposures (Oct 2014)

306 environmental pollutants

131 dietary compounds

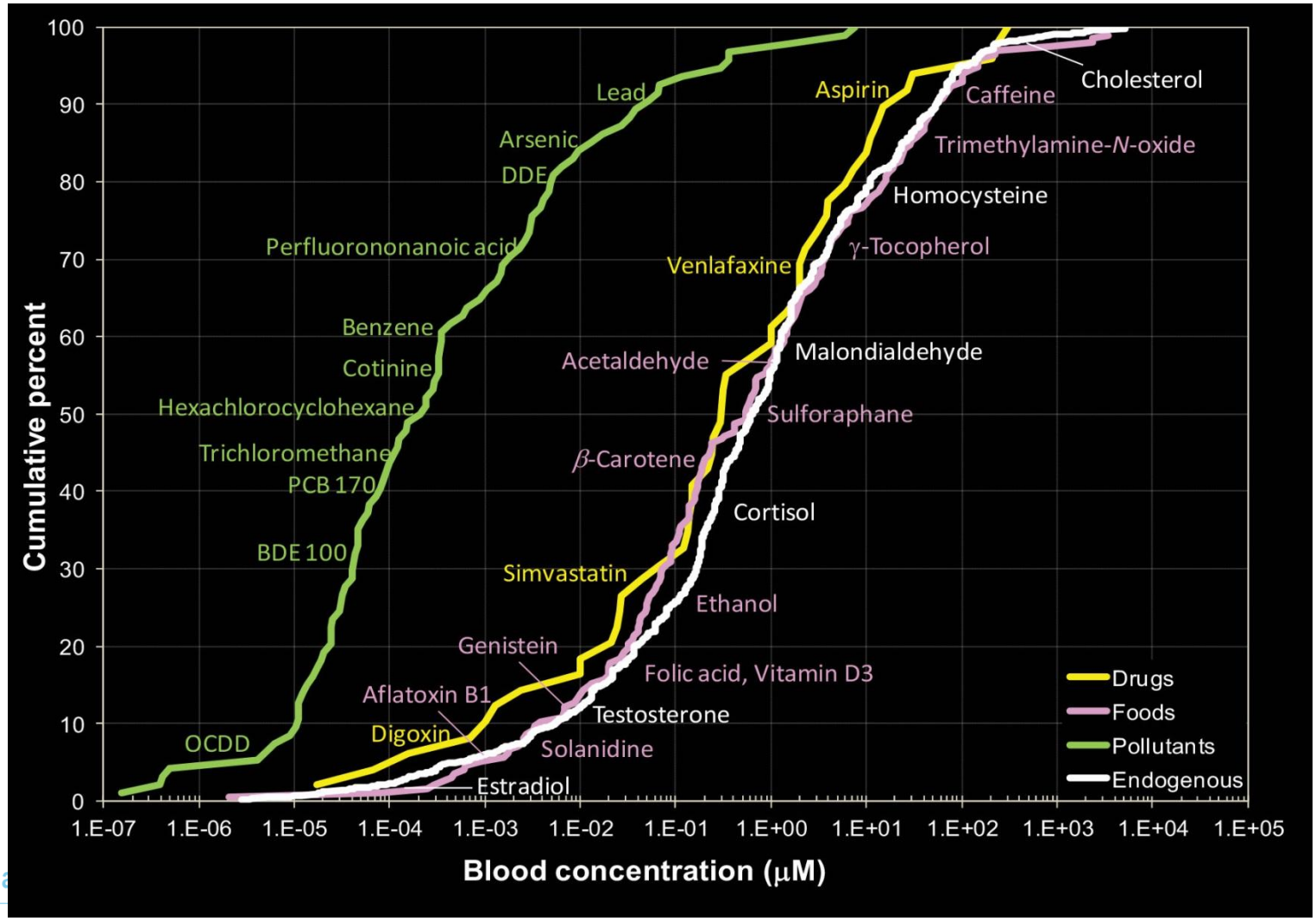
319 concentration values



A panel of dietary biomarkers for targeted nutrient-wide association studies

Chemical class	Biomarkers (#)	Food/nutritent intake	Example
Amino acids	2	Proteins, meat, fish	1-Methylhistidine
Organic acids	1	Proteins	Taurine
Aliphatic acyclic compounds	1	Proteins	Urea
Fatty acids	35	Fish, milk, milk products, beef meat	DHA
Vitamins	11	Fruits, vegetables, citrus fruits	Vitamin C
Inorganic compounds	6	Milk	Iodine
Carotenoids	6	Fruits, vegetables, carrot, tomato, citrus fruits	lycopene
Flavonols	4	Apple, tea, fruits, fruit & vegetables	Quercetin
Flavanones	3	Orange, grapefruit, citrus fruits	Hesperetin
Dihydrochalcones	1	Apple	Phloretin
Isoflavones	7	Soy milk, soy solid foods, soy proteins	Daidzein
Phenolic acids	5	Tea, coffee, wine, orange, apple	Gallic acid
Lignans	2	Vegetables, dietary fibre	Enterolactone
Stilbenes	1	Wine	Resveratrol
Alkylresorcinols	6	Wholegrain cereals, dietary fibre	5-Heptadecylresorcinol
Food contaminants	3	Fish	Mercury
Cooking products	2	Grilled meat	1-hydroxypyrene glucuronide
Enzymes	3	Alcoholic beverages	ALAT

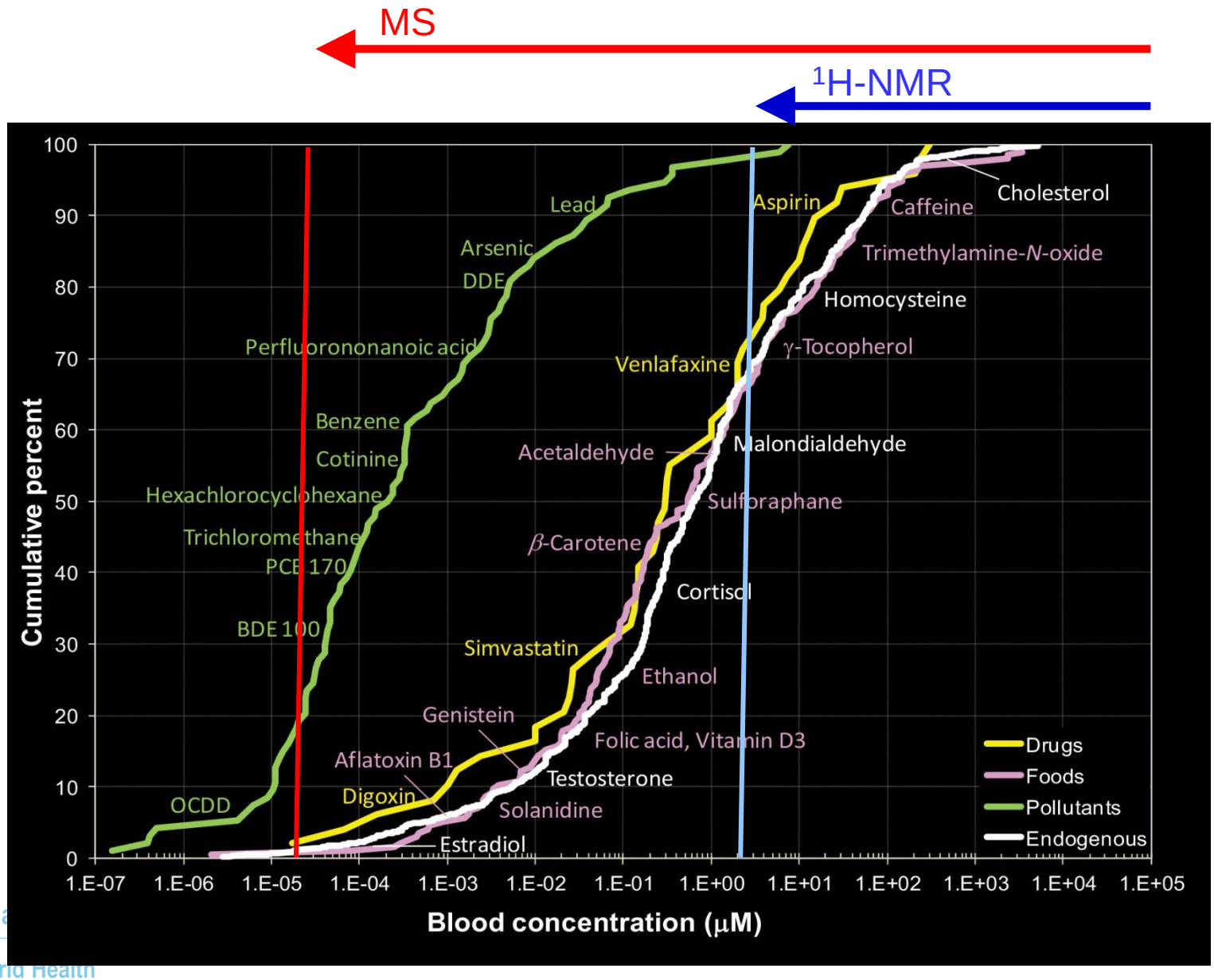
Environmental pollutants present at (very) low concentrations in blood



International
World Health Organization

Rappaport et al., 2014, *Environ. Health Perspect.*

Sensitivity of analytical instruments

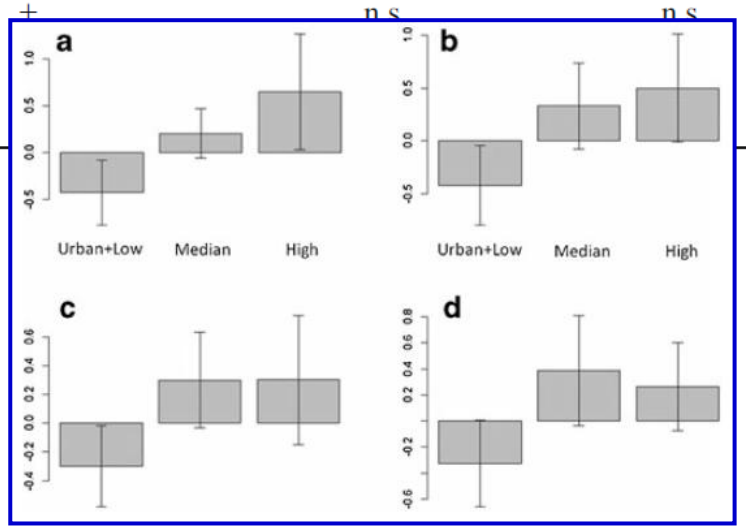


Rappaport et al., 2014, *Environ. Health Perspect.*

Metabolomics and pesticide exposures

Table 3 Variation of signals as a result of exposure

Metabolite	*p*-value	(Urban + low)/medium	(Urban + low)/high	Medium/high
Methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate sulfate	6.5×10^{-6}	+	+	n.s.
2-Methyl-2-phenylpropanoic acid	2.2×10^{-5}	+	+	n.s.
Methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (1)[a]	9.6×10^{-5}	+	+	n.s.
Methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (2)[a]	6.3×10^{-5}	+	+	n.s.
3,3-Dimethyl-2,3-dihydro-1-benzofuran-7-ol sulfate	0.0197	n.s.	+	n.s.
3,3-Dimethyl-2,3-dihydro-1-benzofuran-7-ol glucuronide	0.0409	+	n.s.	n.s.
7-Hydroxy-2,2-dimethyl-1-benzofuran-3(2*H*)-one glucuronide	0.0033			
2-(4-Hydroxyphenoxy)propanoic acid sulfate (2)[a]	0.0404			



- 7 pesticide metabolites identified in urine and associated to proximity of cereal crop land

Jamin et al., 2014, *Anal. Bioanal. Chem.*

Environmental contaminants and untargeted metabolomics

Environmental chemical	Detected *m/z*	Delta
Meta-tyrosine (herbicide)[a]	182.0805	0.0007
Carbendazim; mercarzole (carcinogen)[a]	192.0741	0.0026
N-butyl-benzenesulfonamide (plasticizer)[ab]	214.0892	0.0004
Diethyl phthalate (plasticizer)[ab]	223.0937	0.0028
Pirimicarb (insecticide)[a]	239.1482	0.0021
Diisopropyl phthalate (plasticizer)[ab]	251.1270	0.0008
Dibutyl phthalate (plasticizer)[ab]	279.1574	0.0017
Butylbenzyl phthalate (plasticizer)[ab]	313.1417	0.0018
Triphenyl phosphate (flame retardant)[ab]	327.0766	0.0015
Di-n-hexyl phthalate (plasticizer)[ab]	335.2176	0.0041
Chlorsulfuron (herbicide)[a]	358.0372	
Imazalil nitrate[ab]	359.0407	
Di-*n*-heptyl phthalate (plasticizer)[ab]	363.2503	0.0026
Di(2-ethylhexyl) adipate (plasticizer)[ab]	371.3134	0.0022
Surfentrazone (herbicide)[ab]	386.9885	0.0007
Diisooctyl phthalate (plasticizer)[ab]	391.2818	0.0025
Macluraxanthone (insecticide)[ab]	395.1526	−0.0037
Endosulfan (insecticide)[a]	404.8213	0.0028
Diisononyi phthalate (plasticizer)[ab]	419.3134	

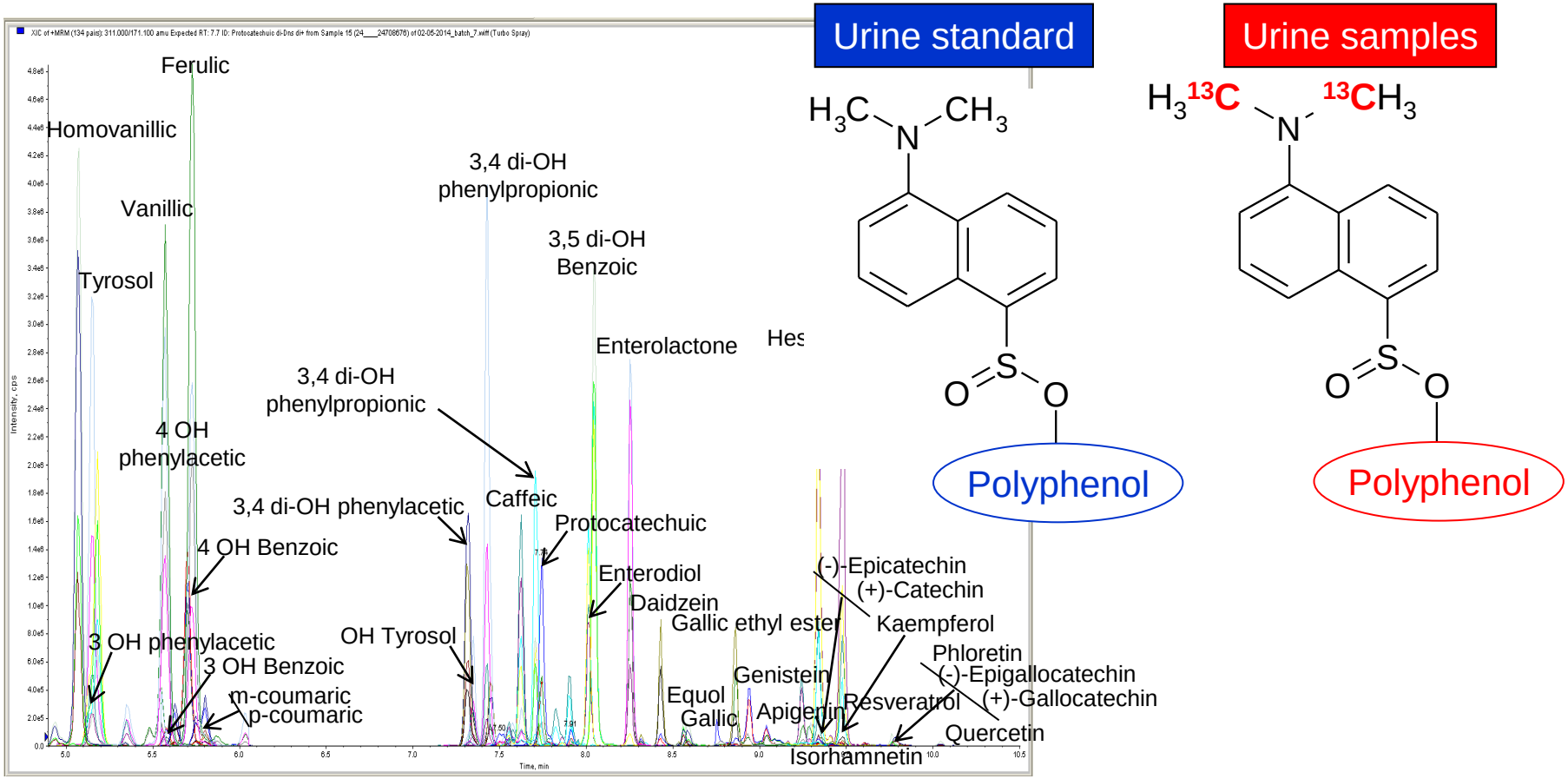
Human plasma samples
LC-FTICR-MS

Soltow et al., 2011, *Metabolomics*

Measuring the exposome
Analytical challenges

Characteristics	Requirements
High complexity of the exposome	Improved annotation of the exposome (human metabolites)
Low concentrations of metabolites	Higher sensitivity
Low sample volumes	Miniaturization of sample processing
Intra-individual variations along time	Repeated samples Biomarkers with longer half-life
Large cohorts	Lower analytical run time
	High robustness of the analytical workflow
	Quantitative measurements

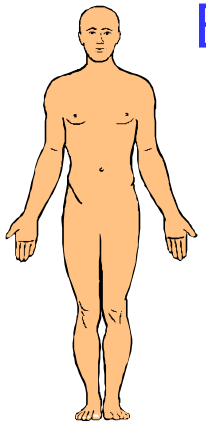
Quantifying the exposome with differential isotopic labelling



- Dansylation of phenols and amines
- First application to a cohort study (38 dietary polyphenols)

Achaintre et al., in preparation

Exposome-Wide Association Studies (EWAS)

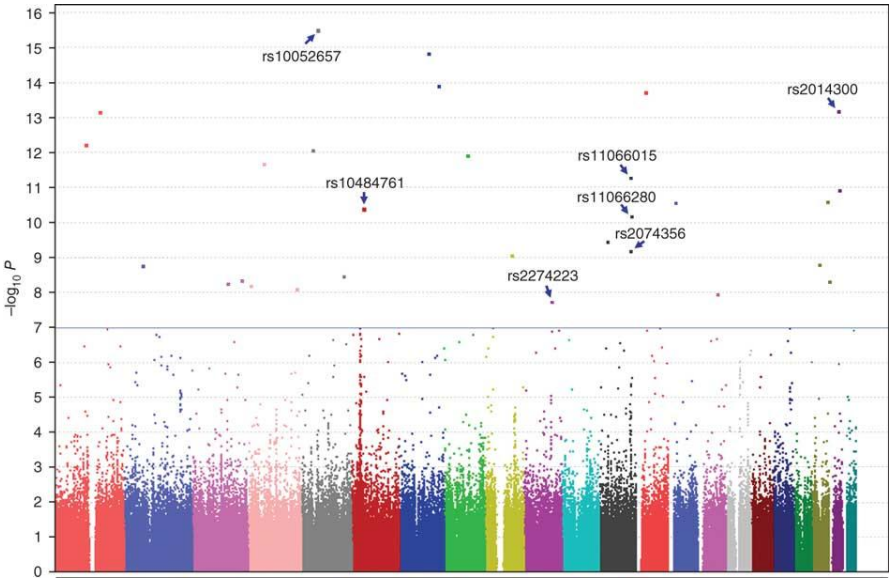


Blood exposome

(Nested)
case-control studies

Disease risk

Association with disease risk



Chemicals, nutrients

- Success for a truly agnostic approach in discovering novel disease risk factors will depend on our capacity to reliably measure the exposome

Metabolomics on various biospecimens

	References
Plasma (heparin, citrate, EDTA)	
Serum	
Urine (spot, 24hr)	
Red blood cells	Darghouth et al., 2011, *Blood*
Dried blood spots	Kong, S.T. et al., 2011, *Anal. Chem.*
Saliva	Takeda et al., 2009, *NMR Biomed.*; Sugimoto et al., 2010, *Metabolomics*
Hair	Sulek et al., 2014, *Theranostics*

Biomarkers and biospecimens in Exposome-Explorer

PBDEs

PCDDs

PCBs

Pesticides

PCDFs

PAHs

Adipose tissue
Hair
Breath…

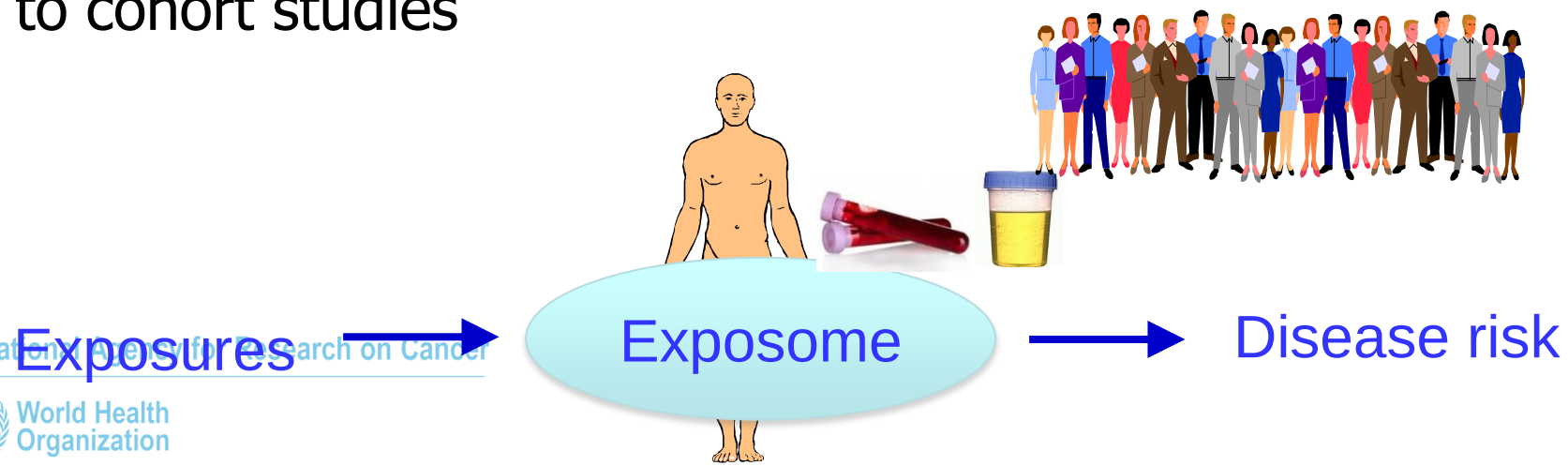
POLYPHENOLS

FATTY ACIDS

CAROTENOIDS

Measuring the exposome
Conclusions

- A minor fraction of the exposome so far measured
- To replace or complement classical methods of assessment of exposure to disease risk factors
  - Missing data in questionnaires
  - Improved measurements
- To identify new risk factors for cancer and other diseases
- Methodological progress still needed to apply metabolomics to cohort studies



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