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The role of GC/Q-TOF in Exposomics

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Introduction

The exposome is the summation of all exogenous and endogenous exposure events over the course of one's lifetime. Figure 1 show Da Vinci's *Vitruvian Man* and his exposome. Measuring these exposure events through "omics" tools (exposomics) will benefit from the use of GC/Q-TOF simply for the fact that GC and GC/MS are crucial for the evaluation of volatile and semi-volatile compounds in environmental, clinical, food safety, toxicology, forensics and many other disciplines. The ability of GC/Q-TOF to perform high resolution, accurate mass global discovery on biological and non-biological sample types *and* it's ability to perform MS/MS for structural elucidation and confirmation, could prove to be invaluable in exposome monitoring. Herein is presented proof of concept examples of how GC/Q-TOF can be implemented in monitoring the environment and the exposome.

Acute or chronic exposure to persistent organic pollutants, drugs, emerging contaminates and endogenous biochemicals stress the system and influence disease onset. A current example of how and where exposome monitoring could be beneficial is Salt Lake City, UT. In January 2013, a fog containing up to 130 µg/m^3 of soot (more than three times the federal clean air limit) settled over the city for most of the month [1]. The fog was purported to be a shower of combustion particles from tailpipe and other emissions (*sic*) [2]. The short term influence on disease can be measured by increased episodes of asthma and other respiratory maladies but what are the long term effects and how can they be correlated to the relatively acute exposure to this 'toxic' fog? Other examples of exposure and the exposome include exposure to fluorotelomer alcohols in water or upregulation of tryptophan metabolism through the kynurenine pathway as illustrated herein.

Figure 1. The exogenous and endogenous exposome.

Adapted from: Rappaport SM, J Expo Sci Environ Epidemiol, (2011) 21
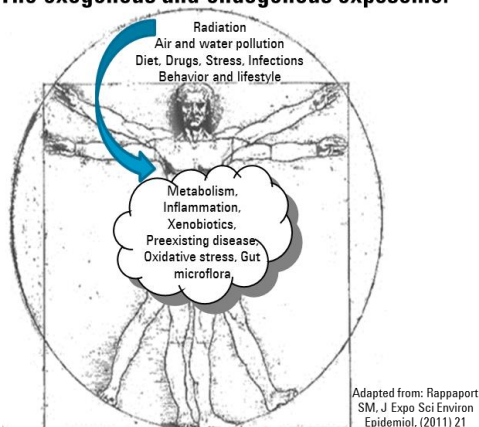
Introduction

Fluorotelomer alcohols (FTOH) are used to synthesize fluorinated polymers. In the environment, FTOH oxidize to fluorinated carboxylic acids, some of which have been found to be toxic. Using GC/Q-TOF in PCI mode to monitor bio-solids from waste water treatment plants, confirmed the presence of five FTOH compounds in native extracts with concentrations ranging from 0.4 ng/mL to 20 ng/mL [3].

Tryptophan is an essential amino acids, which is involved in many different metabolic pathways. One of the key pathways for tryptophan metabolism is the kynurenine pathway. This pathway is significantly modulated in response to inflammation and is linked to a diverse set of diseases. Monitoring of some of they key metabolites (quinolinic acid, abthranilic acid,3-OH-kynurenine, etc) is important due to the neuroprotective properties of kynurenic acid and the excitotoxic quinolinic acid as well as the ability of 3-hydroxykynurenine (3-HK) to scavenge reactive free radicals.

Exposomics

Exposomics was defined in response to genomics failure to identify the etiology of more than 90% of chronic disease that is not directly related to genetic defects. It is generally accepted that the environment has a large impact on the phenotype and this recognition gave rise to epigenetics. The heart of exposomics is epigenetics and the goal is to identify the impact of the exposome on the genome and the etiology of most disease. Exposomics can be viewed as the umbrella under which all other omics tools live. Although not explicitly stated, transcriptomics, proteomics, metabolomics are implied in exposomics as the tools of the trade. Figure 2 shows how exposomics is growing around the world.

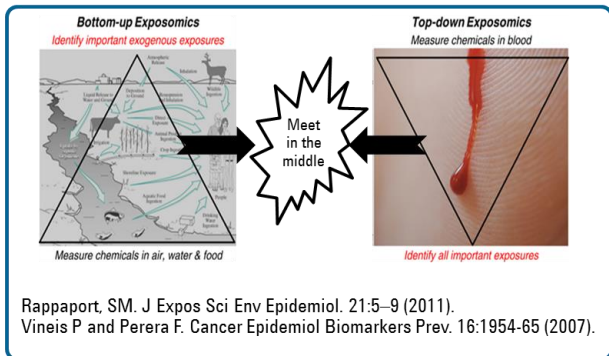**Figure 2. Exposomics is trending.**
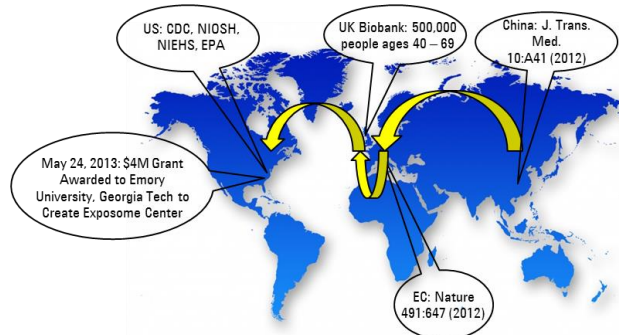
Experimental

### How do we measure the exposome

Rappaport (2011) defined bottom up exposomics as the measure of the land, air, water, etc. and inferring exposure, while top down exposomics measures blood, urine, tissue, etc. to identify biomarkers of disease. The former fails to identify essential features of the internal exposome and the latter fails to identify the source of exposure. Vineis and Perera (2007) defined 'meet in the middle' exposomics to identify the overlap between markers of exposure and markers of disease. Figure 3 illustrates the concepts of the bottom up, top down and meet in the middle exposomic models.

Figure 3. Exposomic models.

Rappaport, SM. J Expos Sci Env Epidemiol. 21:5—9 (2011).
Vineis P and Perera F. Cancer Epidemiol Biomarkers Prev. 16:1954-65 (2007).

Macherone (2013 in press) defines a cyclical exposomics model that combines bottom-up measurement of the environment with bottom-up and top-down exposomics. This model typically begins agnostically and becomes more knowledge driven with each iteration of the loop and allows correlation with true environmental contaminates with markers of exposure and disease identified in the exposome.

Figure 4. Cyclical Exposomics.
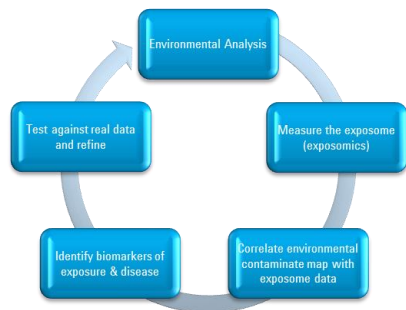
A. Macherone. The Future of GC/Q-TOF in Environmental Analysis. In: Advanced Techniques in Gas Chromatography-Mass Spectrometry (GC-MS-MS and GC-TOF-MS) for Environmental Chemistry. I. Ferrer & M. Thurman (Eds). Elsevier, In Press.

Experimental

Data collection

All data was collected on the 7200 GC/Q-TOF system in PCI or NCI modes. Fifteen meter HP-5MS columns were used in either a two column or single column configuration and all data was collected with backflush enabled through the use a helium purged union. The oven temperature program ranged from 60°C through 320°C. The source temperature was 300°C for PCI and 150°C for NCI. The quadrupole temperature was 150°C for all studies. For chemical ionization, methane was used as the reagent gas. The mass range was 50 to 700m/z with 5Hz acquisition rate. The TOF was calibrated daily. Uncorrected mass accuracy ranged from sub 1 ppm to 7 ppm depending on the ion mass and abundance.

Results and Discussion

Endocrine disruption and fluorotelomer alcohols

FTOH act as xenoestrogens *in vitro*

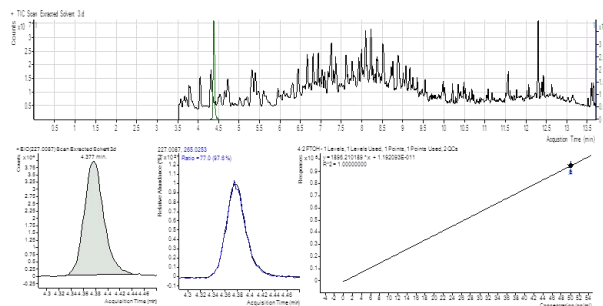Structural similarities of these compounds and 4-noylphenol (reference xenoestrogen)

Ligands for the estrogen receptor

Induce hepatic vitellogenin through activation of the estrogen receptor

Vitellogenin: is a biomarker for estrogen EDC exposure

There is a need to monitor the environment for these compounds to understand exposure risk and correlate with disease biomarkers in the exposome. Figure 5 illustrates the ability of accurate mass and high mass resolution to extract ions of interest out of the heavy bio-solid background. Figure 6 shows how accurate mass can be used in structure elucidation.

Figure 5. 4:2 FTOH identified in bio-solid waste.

Results and Discussion
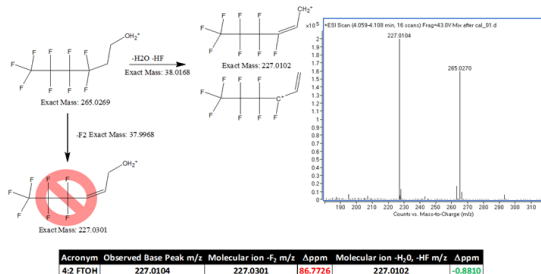
Figure 6. Accurate mass in structure elucidation

Acronym	Observed Base Peak m/z	Molecular ion -F₂ m/z	Δppm	Molecular ion -H₂0, -HF m/z	Δppm
4:2 FTOH	227.0104	227.0301	86.7726	227.0102	-0.8810

Table 1. FTOH uncorrected mass Accuracy

Acronym	Formula	Exact Mass + H	Observed Mass	Δppm
4:2 FTOH	C₆H₅F₅O	265.0269	265.0270	-0.3773
6:2 FTOH	C₈H₅F₁₃O	365.0206	365.0206	0.0000
8:2 FTOH	C₁₀H₅F₁₇O	465.0142	465.0140	0.4301
10:2 FTOH	C₁₂H₅F₂₁O	565.0078	565.0078	0.0000
7:2 sFTOH	C₉H₆F₁₄O	415.0174	415.0190	-3.8553
5:1 FTOH	C₆H₄F₁₁O	301.0081	301.0079	0.6644
6:1 FTOH	C₇H₃F₁₃O	351.0049	351.0050	-0.2849
7:1 FTOH	C₈H₃F₁₅O	401.0017	401.0016	0.2494
8:1 FTOH	C₉H₃F₁₇O	450.9985	450.9985	0.0000
9:1 FTOH	C₁₀H₃F₁₉O	500.9953	500.9956	-0.5988
10:1 FTOH	C₁₁H₃F₂₁O	550.9921	550.9922	-0.1815
11:1 FTOH	C₁₂H₃F₂₃O	600.9889	600.9896	-1.1647
MeFOSE	C₁₁H₈F₁₇N O₃S	558.0026	558.0042	-2.8674
EtFOSE	C₁₂H₁₀F₁₇NO₃S	572.0183	572.0167	2.7971

Tryptophan metabolism

Using GC/Q-TOF in NCI and global screening mode of rat brain samples collected by striatal microdialysis *in vivo* [4], six kynurenines and several amino acids had LODs of 1.0 fmol with RSDs less than 10% over five replicate injections. Raw TOF data was mined for non-targeted compounds such as 5-hydroxy-tryptophan (5-HT) which has potential anti-depressive properties. 5-HT was easily identified as well as other amino acids and endogenous compounds [5].

Figure 7. Kynurenines
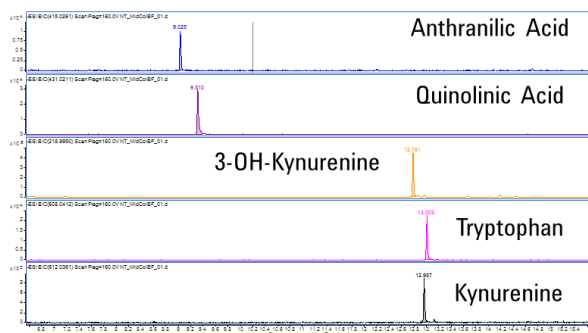
Results and Discussion

Table 2. Kynurenine metabolites

Name	Acronym	Type	Empirical Formula	Mono m/z	Type	Derivative Mono m/z
Quinolinic Acid	Quin	KYN	C7 H5 N O4	167.0213	Di-carboxylic acid	431.0211
3-hydroxy-kynurenine	3HK	KYN	C10 H12 N2 O4	224.0792	Carboxylic acid / Phenol / di-amine	794.0164, 218.9950
Kynurenine	Kyn	KYN	C10 H12 N2 O3	208.0842	Carboxylic acid / Primary & Secondary Amine	632.0423, 612.0361
Kynurenic acid	Kyna	KYN	C10 H7 N O3	189.042	Carboxylic acid / Phenol	453.0418, 320.0320
Tryptophan	Try	Amino Acid	C11 H12 N2 O2	204.0893	Carboxylic acid / Primary & Secondary Amine	628.0474, 608.0412
Anthranilic Acid	AA	Amino Acid	C7 H7 N O2	137.0471	Carboxylic acid / Amine	415.0261
Glutamate	Glu	Amino Acid	C5 H9 N O4	147.0526	di-Carboxylic Acid / Amine	557.0315
Dopamine	DA	Neurotransmitter	C8 H11 N O2	153.0784	Catecholamine	591.0157, 375.9964
Serotonin	Ser	Neurotransmitter	C10 H12 N2 O	176.0944	Phenol / di-amine	614.0317, 594.0197

Table 3. Kynurenine uncorrected mass accuracy

Name	Acronym	Derivative Mono m/z	Observed	Uncorrected d ppm	Resolution (FWHM)	comment
Quinolinic Acid	Quin	431.0211	431.0179	-7.4242	15393	
3-hydroxy-kynurenine	3HK	794.0164, 218.9950	218.9949	-0.4566	7821	EIC = 218.9950
Kynurenine	Kyn	632.0423, 612.0361	612.0349	-1.9607	22668	EIC = 612.0361
Kynurenic acid	Kyna	453.0418, 320.0320	320.0335	4.6870	11036	EIC = 320.0320
Tryptophan	Try	628.0474, 608.0412	608.0405	-1.1512	22520	EIC = 608.0405
Anthranilic Acid	AA	415.0261	415.0241	-4.8190	15371	
Glutamate	Glu	557.0315, 537.0258	537.0228	-0.0006	19179	EIC = 537.0258
Dopamine	DA	591.0157, 375.9964	375.9973	2.3936	14461	EIC = 375.9964
Serotonin	Ser	614.0317, 594.0197	594.0242	7.5755	23761	EIC = 594.0197

Conclusion

The examples of analyzing fluorotelomer alcohols in the environment and tryptophan metabolites in rat brain dialysate given herein illustrate how GC/Q-TOF can play an important role in the cyclical exposomics model. Although not explicitly connected in this proof of concept poster, compounds such as these will be important in monitoring the exposome and environmental risk potential. The inherent capabilities of gas phase systems to analyze a vast array of chemotypes in a multitude of environmental and biological matrices are required in this model to properly map persistent and emerging contaminates in regions where moderate and mega cohort exposome studies are underway. The added knowledge determined by understanding the exposure risks is invaluable when measuring the exposome for both markers of exposure and biomarkers of disease which in many cases are not one and the same. Coupling this information to clinical data will allow researchers to focus their study on the narrowing number of relevant compounds that define the etiology of disease.

References

[1] Foy, P. *Sickening fog settles over Salt Lake City area.* Salt Lake City: Associated Press. (2013)

[2] Hills, S. Retrieved January 29, 2013, from MailOnline.com: http://www.dailymail.co.uk/news/article-2267488/Toxic-fog-hits-Utah-Pregnant-women-children-urged-stay-indoors-pollution-goes-charts.html

[3] Macherone A, Tom Doherty T, Shoji F. Nakayama SF, Aronova S, Tadele, Mills. 31st International Symposium on Halogenated Persistent Organic Pollutants (2011)

[4] Nototangelo, F. M., Wu, H.-Q., Macherone, A. J., Graham, D. R., & Schwarcz, R. Analytical Biochemistry: Methods in the Biological Sciences, 573-81 (2012)

[5] Macherone A, Acosta R, Graham DR. 61st ASMS Conference on Mass Spectrometry and Allied Topics. June 9 - 13, 2013

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