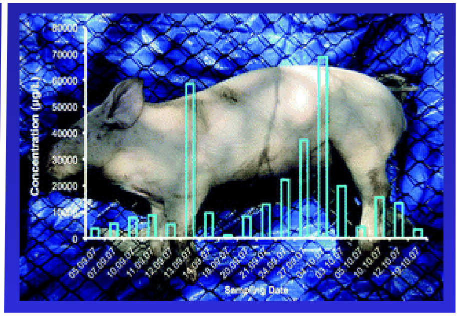
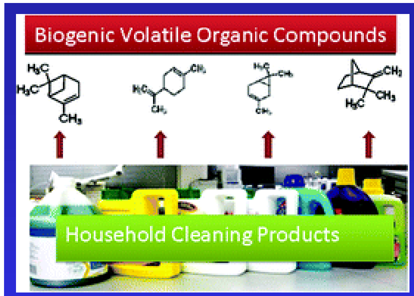
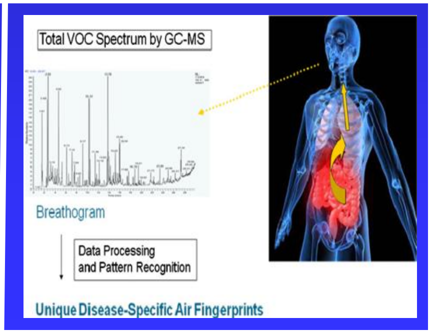
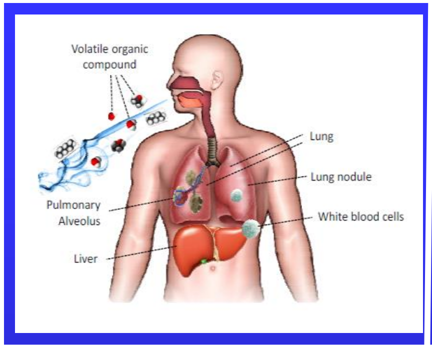


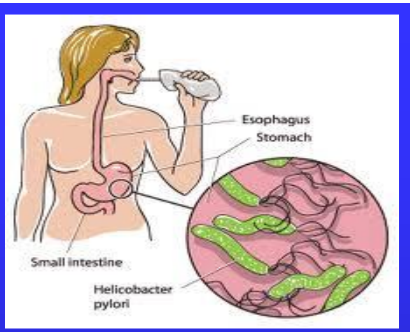


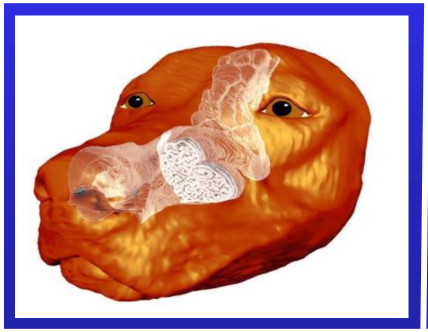
Agapios Agapiou
Lecturer





The importance of human Volatilome





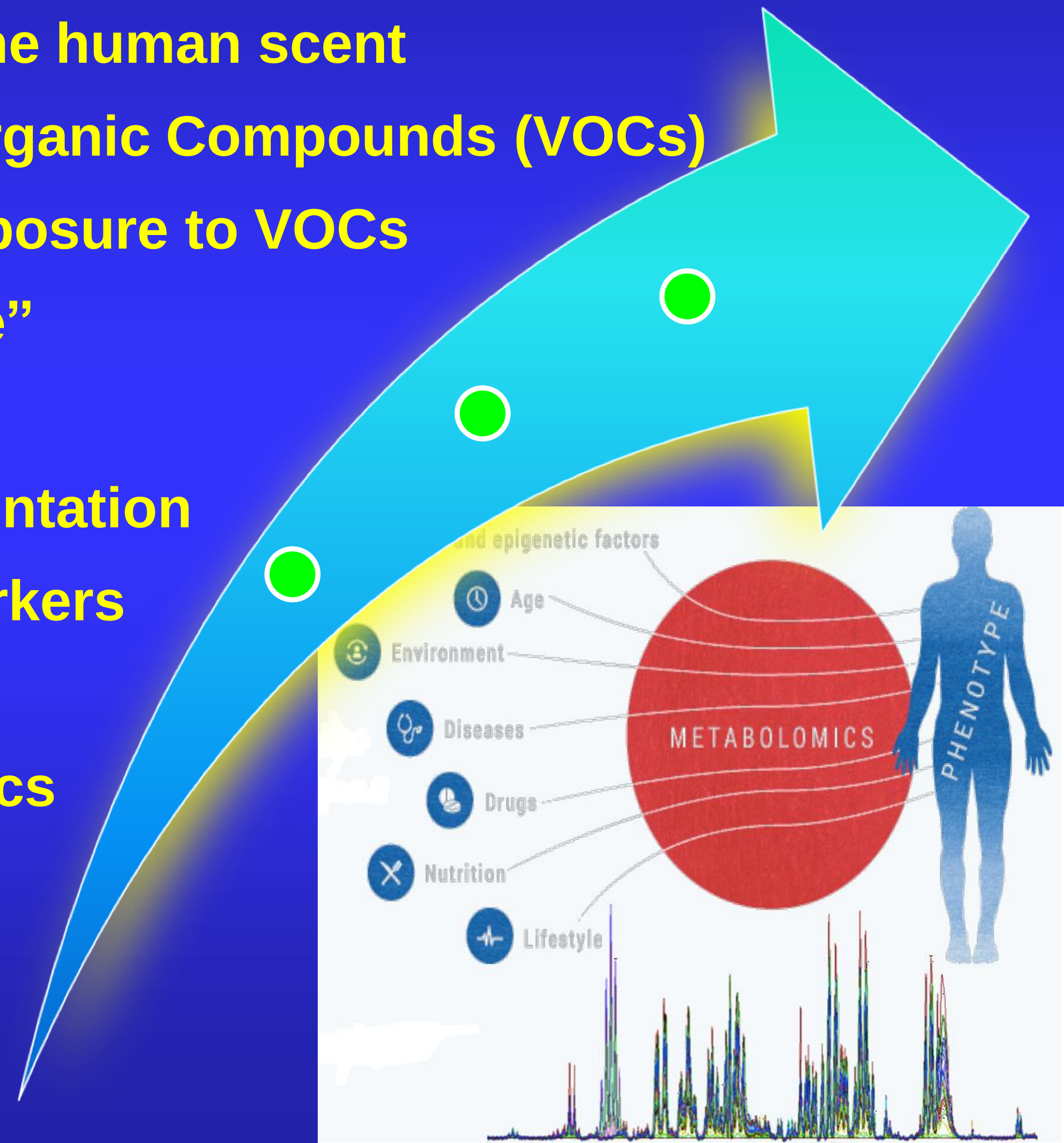
4th Metabolomics Workshop

Thessaloniki, Greece

17 - 19 / 04 / 2016

Outline

1. The sense of smell and its importance
2. The human nose and the human scent
3. Definition of Volatile Organic Compounds (VOCs)
4. Origin, impacts and exposure to VOCs
5. The human "Volatilome"
6. "Breathomics"
7. The analytical instrumentation
8. Diseases ad breath markers
9. Novel applications
10. The future of brethomics



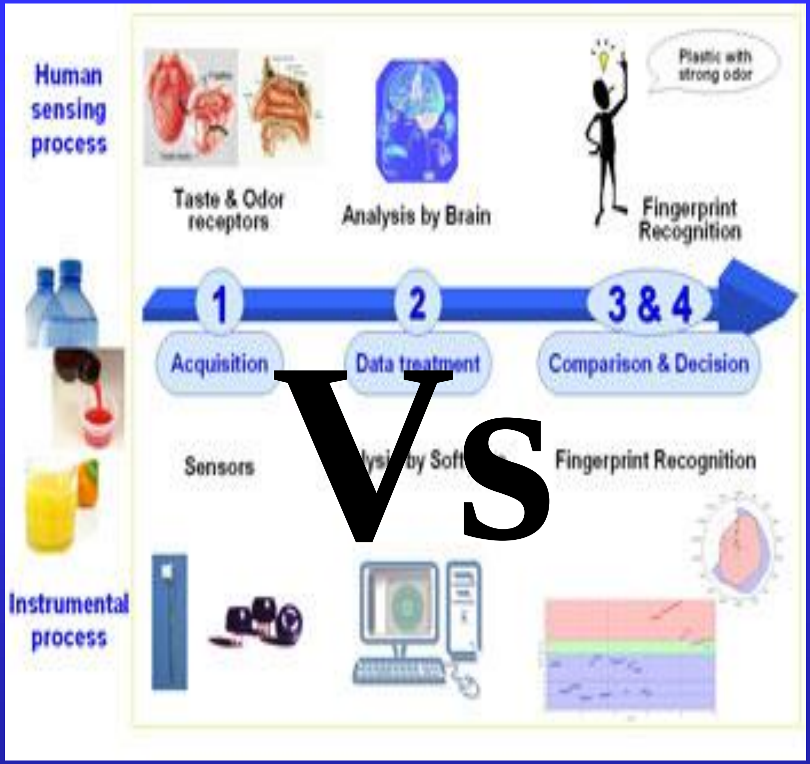
" Our sensens shape our lives
in ways we are only begining to understand..."

[http://www.monell.org/]

Human nose: a unique bio-sensor

- ✓The human nose can distinguish over 10,000 different smells using 350 receptors
- ✓Smell molecule (odorants) interact with the receptors to create an overall 'fingerprint' that is recognised by the brain

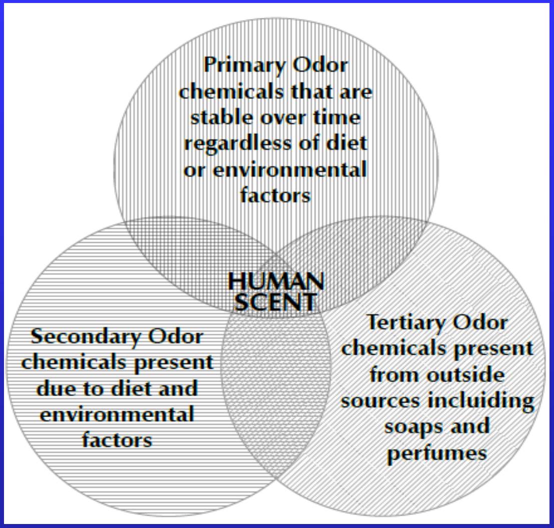
The sensing sequence Human smell

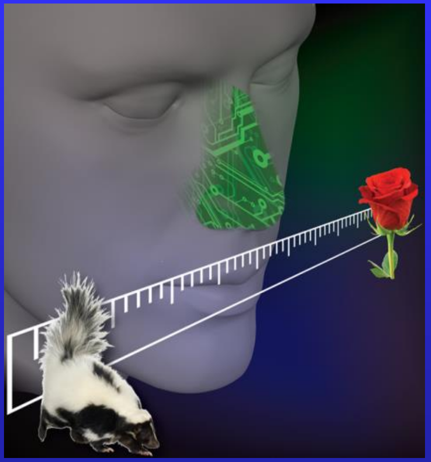


Analytical instrumentation

Human Scent

- ✓ Human scent is actually a smell pattern of VOCs evolved from breath, urine, blood, skin etc.
- ✓ Humans evolve hundreds of volatile organic compounds (VOCs) as a result of their daily metabolic activities (endogenous)
- ✓ At the same time, others are inserted in the human body through food and beverages or as pollutants (exogenous)
- ✓ Tracking of volatile scent is interesting for social, survival, medical and security applications





What are the Volatile Organic Compounds (VOCs)?

Volatile Organic Compounds (VOCs) – "VOCs are ground-water contaminants of concern because of very large environmental releases, human toxicity, and a tendency for some compounds to persist in and migrate with ground-water to drinking-water supply well … In general, VOCs have high vapor pressures, low-to-medium water solubilitites, and low molecular weights. Some VOCs may occur naturally in the environment, other compounds occur only as a result of manmade activities, and some compounds have both origins." [Zogorski and others, 2006].

Volatile Organic Compounds (VOCs) – "Volatile organic compounds released into the atmosphere by anthropogenic and natural emissions which are important because of their involvement in photochemical pollution." [Lincoln and others, 1998].

Volatile Organic Compounds (VOCs) – "Hydrocarbon compounds that have low boiling points, usually less than 100ºC, and therefore evaporate readily. Some are gases at room temperature. Propane, benzene, and other components of gasoline are all volatile organic compounds." [Art, 1993].

Volatile Organic Compounds (VOCs) – "VOCs are organic compounds that can be isolated from the water phase of a sample by purging the water sample with inert gas, such as helium, and, subsequently, analyzed by gas chromatography. Many VOCs are human-made chemicals that are used and produced in the manufacture of paints, adhesives, petroleum products, pharmaceuticals, and refrigerants. They often are compounds of fuels, solvents, hydraulic fluids, paint thinners, and dry-cleaning agents commonly used in urban settings. VOC contamination of drinking water supplies is a human-health concern because many are toxic and are known or suspected human carcinogens." [U.S. Geological Survey, 2005].

Definition of VOCs [EPA]:

Any compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates, and ammonium carbonate, which participates in atmospheric photochemical reactions (As of 3/31/2009, 40 CFR 51.100(s), US EPA).

In a nutshell:

VOCs are a group of chemicals that contain organic carbon, and readily evaporate - changing from liquids to gases when exposed to the air:

- ✓ **High vapor pressure (P^s > 0.1 Torr at 25 ^{0}C and 760 mmHg)**
- ✓ **Low-to-medium water solubilities (< 12 atoms of Carbon); low molecular weights (MW < 350 amu)**
- ✓ **Low boiling points (300 ^{0}C)**

Origin:

- **Anthropogenic (xenobiotic, man-made)**

(e.g. in paints, adhesives, petroleum products, pharmaceuticals, refrigerants, as compounds of fuels, solvents, hydraulic fluids, paint thinners, dry-cleaning agents etc.)

- **Biogenic**

(e.g. from living organisms, vegetation, decomposition of organic matter etc.)

Impacts (short and long term effects):

✓**Malodorous**

✓**Toxic and hazardous properties**

✓**Potential air and ground water pollutants**

✓**Contribute to global warming, stratospheric ozone depletion and tropospheric ozone formation**

✓**Human-health concern; some are known or suspected human carcinogens**

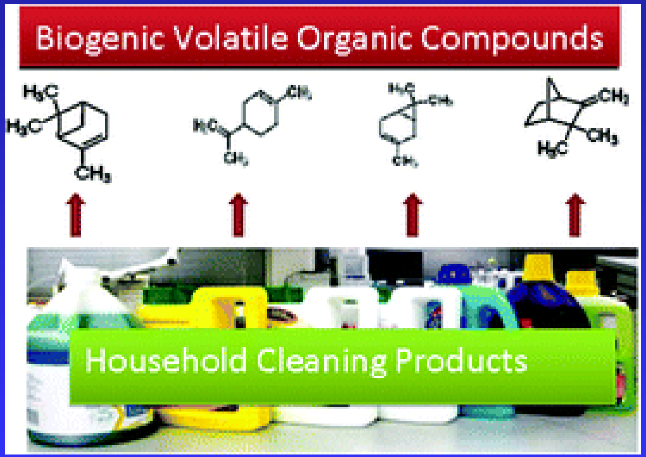
Exposure to VOCs:

Three 3 ways to be exposed:

✓by **ingesting / swallowing**

✓through **respiration / breathing**

✓direct **contact with the skin**



A number of health effects:

✓**eye/nose/throat irritation**

✓**headaches**

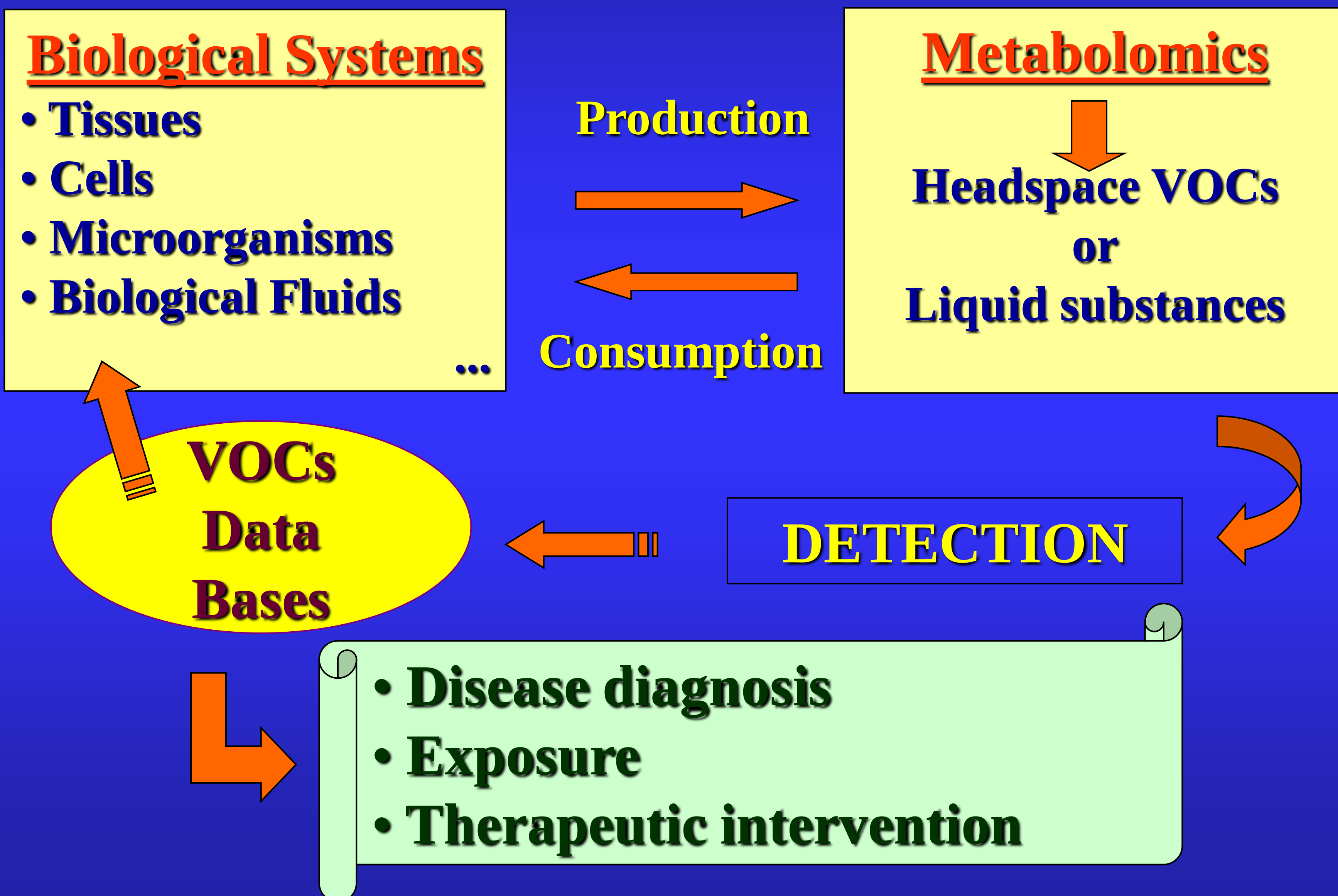
✓**loss of coordination**

✓**nausea**

✓**damage to liver, kidney, and central nervous system**

*** Level of exposure: depends on the chemical, the amount, the time of exposure**

Relationship between biological systems and VOCs



The Human Volatilome

 # Exhaled breath
872 compounds

 # Skin emanations
532 compounds

 # Urine headspace
279 compounds

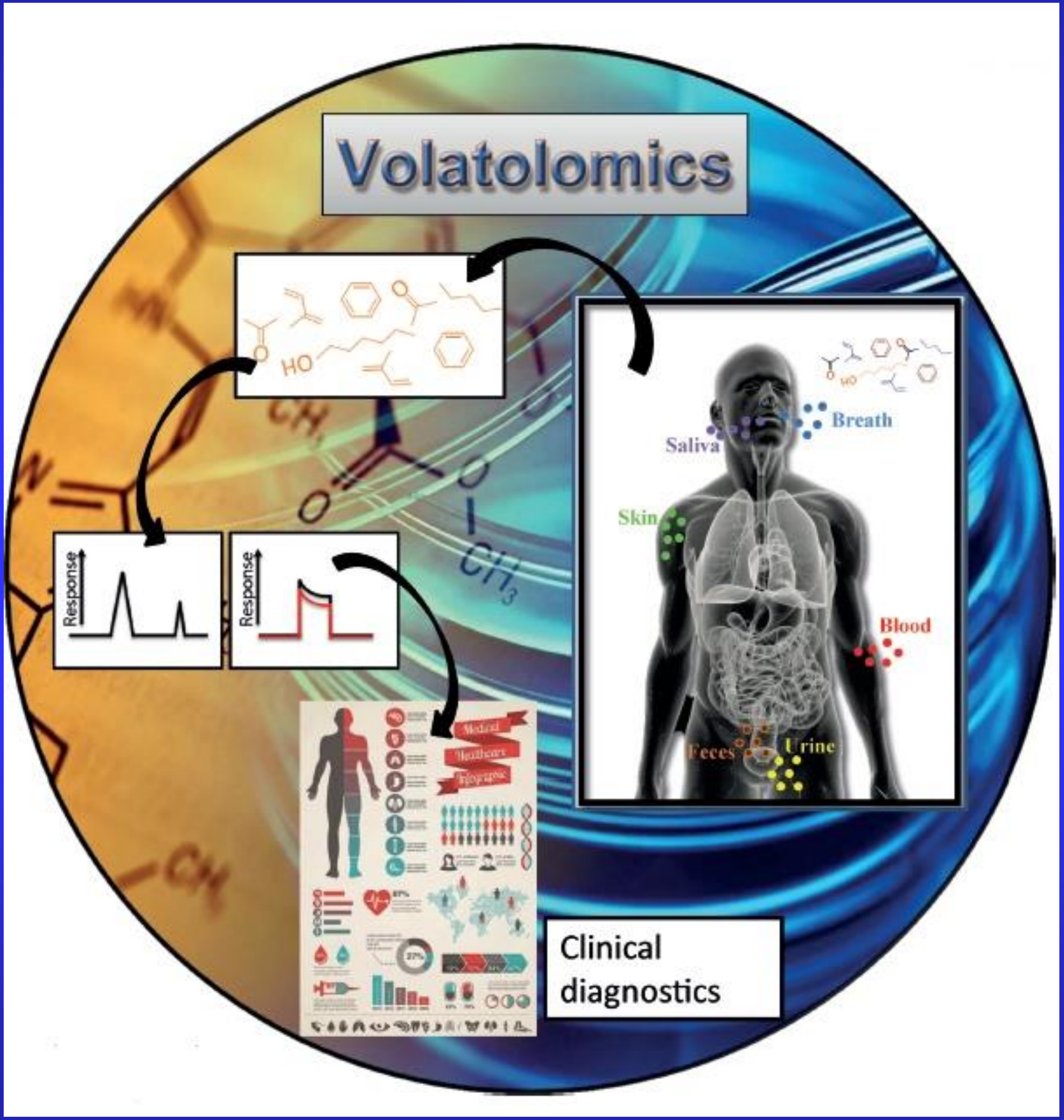
 # Blood
154 compounds

 # Feces
381 compounds

 # Saliva
359 compounds

 # Milk
256 compounds

1840 VOCs

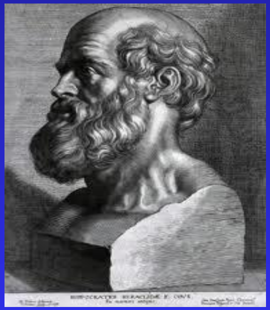


[De Lacy Costello et al., J. Breath Res. 8: 014001 (2014)]

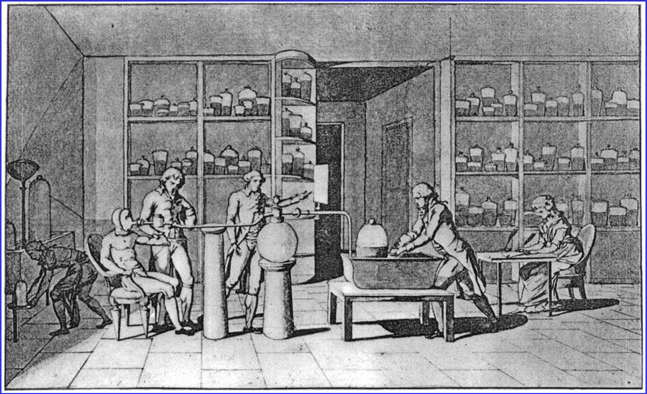
[Angew. Chem. Int. Ed. 2015, 54, 11036 – 11048]

Historical overview

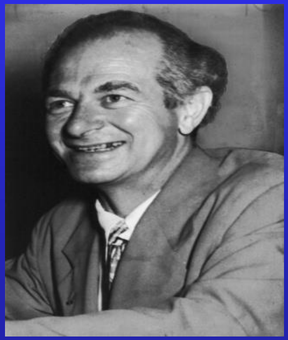
Milestone	Observation	Instrument
Hippocrates (460–370 BC, Kos, Greece)	Correlated smell of breath with illness	Smell of patient's breath; Hippocrates described *fetor oris and fetor hepaticus* in his treatise on breath aroma and disease
Antoine Lavoisier (1784)	Discovered CO_2 and its production in guinea pigs	An in-house apparatus
Nebelthau (1897)	Preliminary measurement of acetone in breath of diabetics	Colorimetric assay – diabetic's acetone change the colour of alkaline iodine solution
Francis E. Anstie (1874)	Isolated ethanol from breath (the first ethanol breath test)	Colorimetric assay - (breath alcohol turned the chromic acid solution from red-brown to green)
Linus Pauling (1971)	Human breath is a complex gas, containing over 200 different VOCs in picomolar concentrations	Gas–liquid partition chromatography analysis
> 1990s	Detection and identification of various medical diseases; early stage lung cancer, breast cancer, heart transplant rejection, tuberculosis, pseudomonas	Chromatographic and spectrometric methods with preconcentration enrichment step, optical techniques (e.g. laser absorption spectrometry, infrared spectroscopy), chemical sensors, sensors array (e-noses), etc.



Hippocrates deduced that bad breath could be indicative of diseases….



Antoine Lavoisier discovered carbon dioxide and its production in the body of guinea pigs with subsequent exhalation …



In 1971, Linus Pauling used gas chromatographic techniques to demonstrate that many different compounds (not yet identified at the time) appear in exhaled breath and therewith confirmed Hippocrates' early observations, boosting the research and interest around human breath…

[Amann & Agapiou, Mobihealth 2014, DOI 10.4108/icst.mobihealth.2014.257396]

Chemical synthesis of breath air

❖ Breath is a complex mixture of gases, vapors (evaporation) and aerosols (condensation)

❖ Breath gases & vapors, although being a small fraction, are hundreds in number and associated with **normal metabolism (endogenous VOCs)**.

❖ Others are entering the human body by exposure to environmental VOCs **(exogenous VOCs; food, drug, beverages, environmental pollutants).**

❖ The majority of breath volatiles is appearing in the **ppb_v to ppt_v** concentration range. The most abundant VOCs in human breath are **acetone** (median concentration approximately 400 ppbv), **isoprene** (~100 ppbv), methanol (~150 ppbv) and **ethanol** (~100 ppbv)

❖ However, their concentration is changing in pathological conditions enabling their use as novel **medical diagnostic tools for various types of cancers, oxidative stress, asthma, diabetes, kidney-, liver- failure and other medical disorders.**

Advantages of breath analysis

1. **New, safe non-invasive method**

2. Breath can be sampled **ANYWHERE BY EVERYBODY as often as it is desirable**, even in real time, or during sleep, exercise (e.g. during pedaling an ergometer); **during almost any human activity…**

3. **Even from animals…despite their size**

Small-size

Medium-size

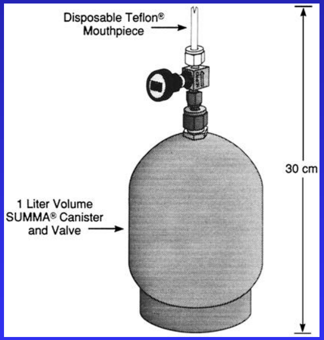
Large-size

[Anal. Chem., 2014, 86 10616–10624]

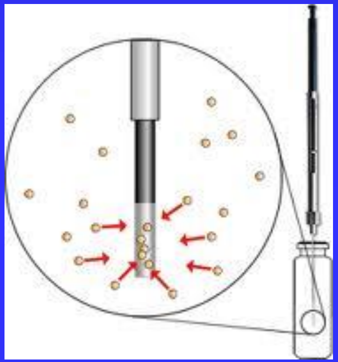
Human breath sampling

- ✓Tedlar bags or canisters
- ✓Sorbent tubes
- ✓SPME Fibers (Solid Phase Micro Extraction)
- ✓Commercial breath samplers
- ✓In-house made devices

Tedlar bags



Canister



SPME

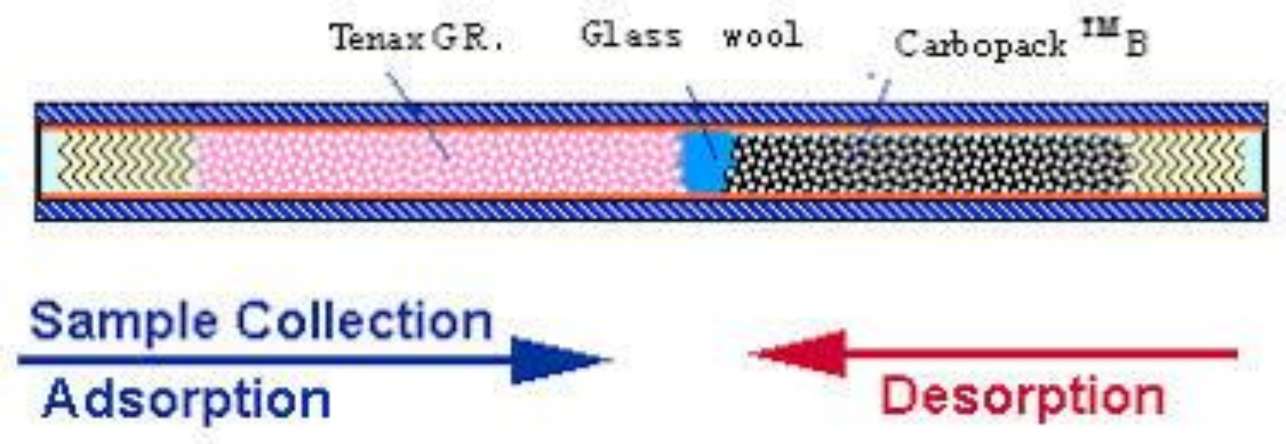
(Breath Research Institute)

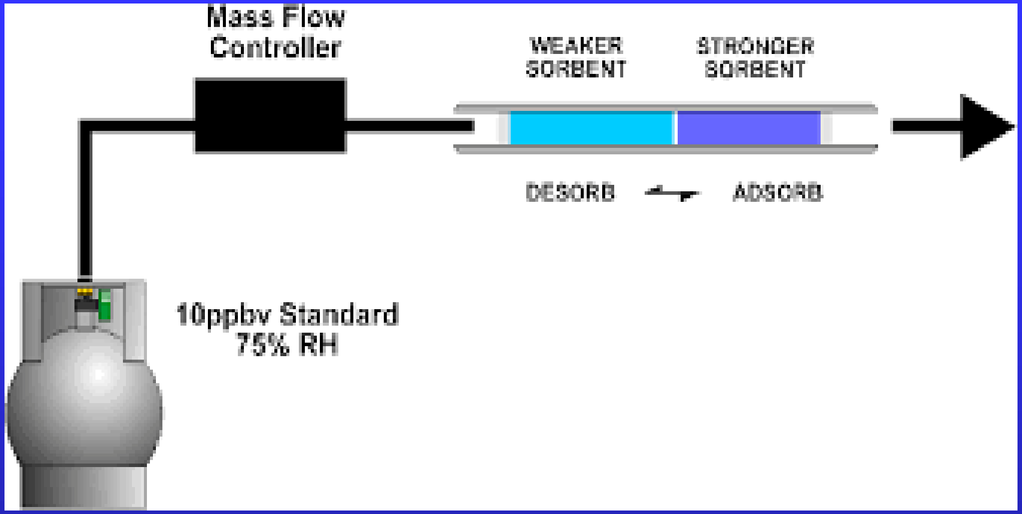
Sorbent tubes

Bio-VOC sampler

Human breath sampling

Multi-sorbent tubes





Human breath instrumentation

- ✓GC-MS (SPME) or TD-GC-MS
- ✓ PTR-MS (Proton transfer reaction - mass spectrometry)
- ✓SIFT-MS (Selected ion flow tube - mass spectrometry)
- ✓IMS, MCC-IMS (Ion mobility spectrometry)
- ✓Laser spectroscopy
- ✓Sensor, sensor arrays, E-sensors (e-nose)

TD-GC-TOF-MS

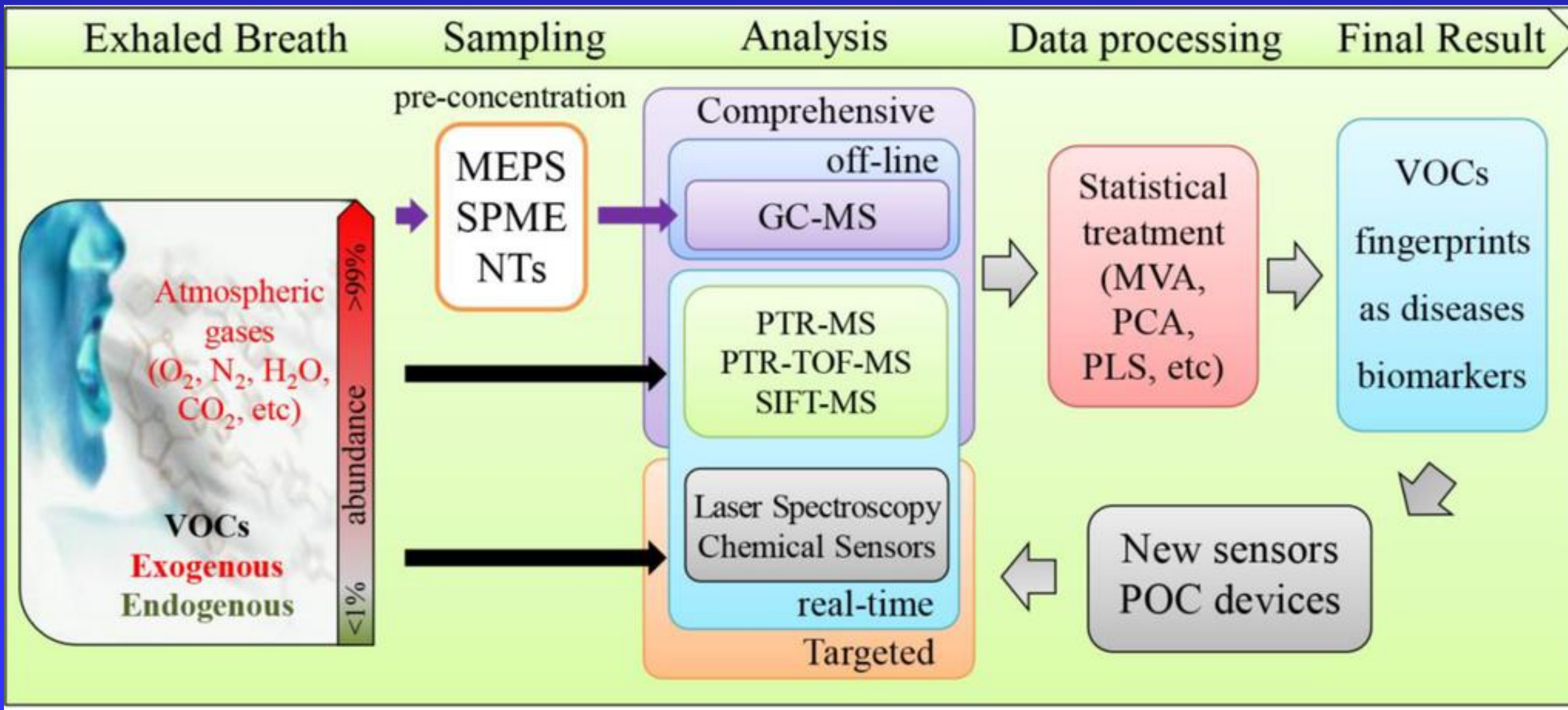
Cyranose e-nose

MCC-IMS

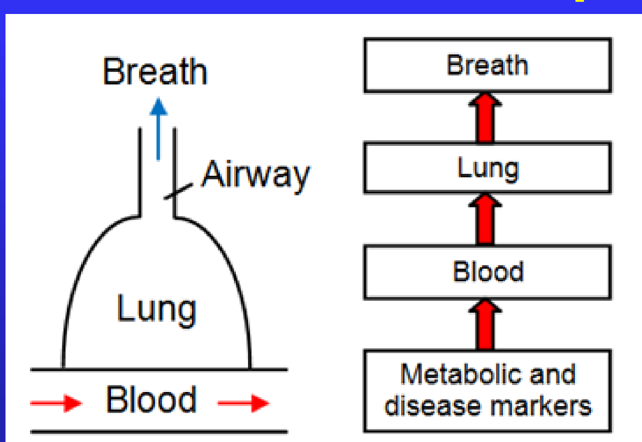
SIFT-MS

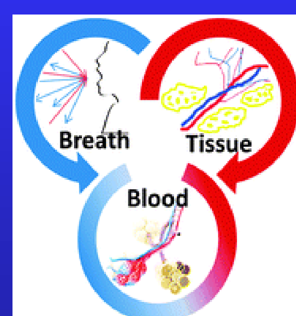
PTR-MS

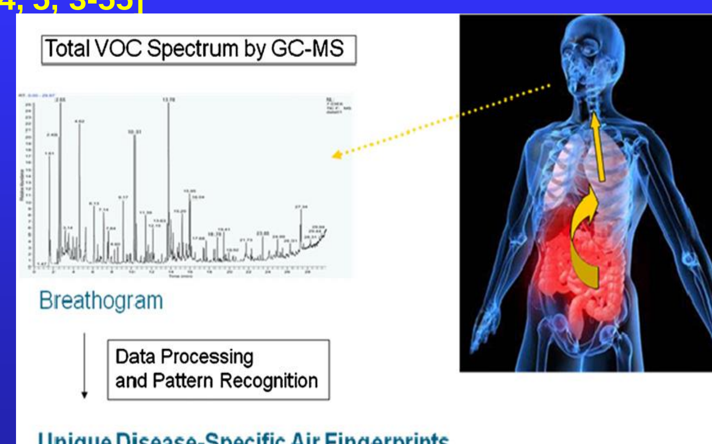
Generic layout for exhaled breath



[Pereira et al., Metabolites 2014, 5, 3-55]



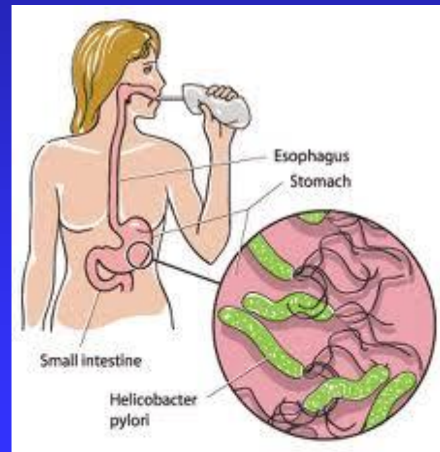




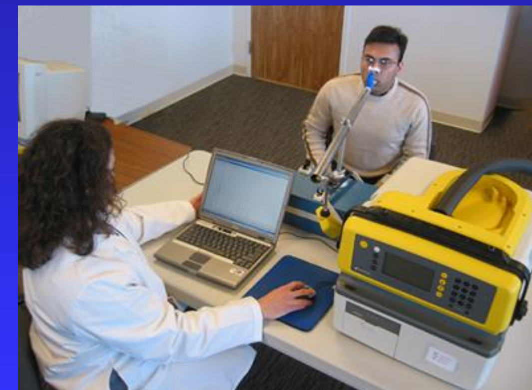
[J. Breath Res. 6 (2012) 027108]

Applications

Alcoholmeter



Helicobacter pylori



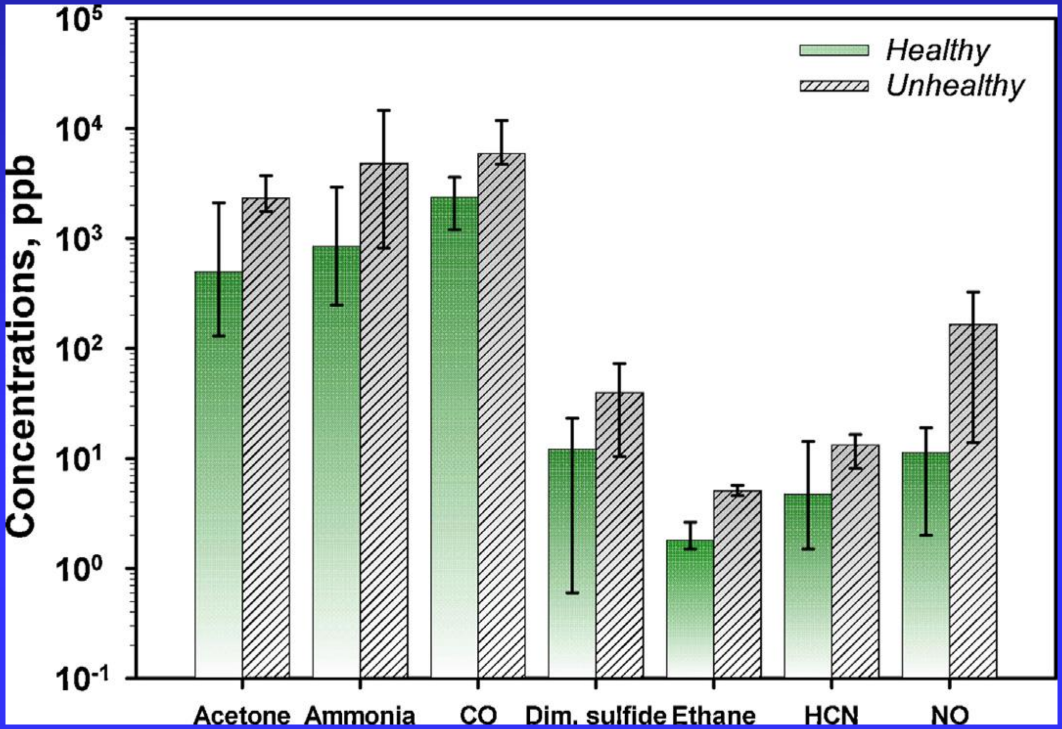
Hearts breath test"
(FDA approval, 2004)

Breath tests approved by US FDA
1. Breath CO_2 test or capnography
2. Breath CO test for neonatal jaundice
3. Breath H_2 test to detect disaccharidase deficiency, gastrointestinal transit time, bacterial overgrowth, intestinal status
4. Breath NO test for asthma therapy
5. Breath test for detection of heart transplant rejection
6. Urea breath test for detection of H. pylori infection
7. Breath ethanol test (law enforcement)

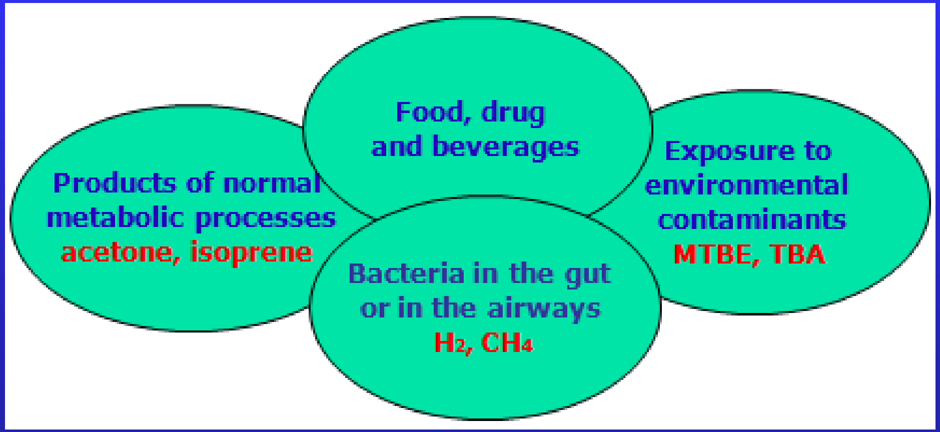
Volatile analysis fields
Human mouth hygiene (e.g. halitosis)
Human odor signature (analysis of breath or of skin emanations)
Physiology and medicine (e.g. cancer, diabetes, asthma, oxidative stress, liver kidney dialysis, ventilated ICU patients, uremia, etc.)
Headspace analysis of cells and bacterial cultures
Human daily activities (e.g. *real-time* analysis of exhaled breath during cycling, sleeping, etc.)
Safety and security applications (e.g. alcoholmeter, detection of entrapped victims under the debris of collapsed buildings after earthquakes, explosions and other catastrophes)
Forensic applications (e.g. drug abuse)
Exposure to xenobiotic volatiles (e.g. industrial/working/manufacturing exposure, contaminated water exposure, swimming in chlorinated water, post-anesthesia units, etc.)
Non-human applications (e.g. livestock, animal welfare monitoring)

[Amann & Agapiou, Chemistry in Australia, April 2014, 16-19]

Diseases and breath markers



[Righettoni et al., Materials today 18, 2015, 163-171]



Select breath markers with their average concentrations for healthy and unhealthy humans:

✓**acetone (diabetes)**
✓**ammonia (kidney disease)**
✓**carbon monoxide (lung inflammation)**
✓**dimethyl sulfide (liver disease)**
✓**ethane (schizophrenia)**
✓**hydrogen cyanide (bacterial infection)**
✓**nitric oxide (asthma)**

Aerocrine NIOX MINO breath analyzer for NO measurements in asthma

What Your Breath Reveals

EXHALED BREATH CONTAINS thousands of chemical compounds that can signal health issues. Scientists are developing tests to diagnose a growing list of diseases based on breath. Some diseases—and the clues that come out of your mouth:

ASTHMA: **Nitric oxide** levels rise when airways are inflamed.

STOMACH ULCERS: The gut bacteria H. Pylori, when mixed with a chemical tracer, emits a **carbon isotope** in breath.

LUNG CANCER: Tumors create dozens of unique **volatile organic compounds,** while sensory arrays identify telltale patterns.

DIABETES: Elevated levels of **acetone in breath** indicate ketosis, which reflects insufficient glucose.

KIDNEY DISEASE: 'Electronic nose' test recognizes **ammonia-like odor** linked to renal failure.

LIVER DISEASE: Patients whose livers can't metabolize a tracer solution containing methacetin show changes in **carbon dioxide levels.**

IRRITABLE BOWEL SYNDROME: **Elevated hydrogen** in breath can indicate bacterial overgrowth in small intestine.

LACTOSE MALABSORPTION: Undigested lactose in the colon is fermented by bacteria, **raising hydrogen breath levels.**

HEART TRANSPLANT REJECTION: Rejection creates 'oxidative stress' that produces **alkanes and methylakanes** in breath.

Med Info on metabolic/pathophysiological state

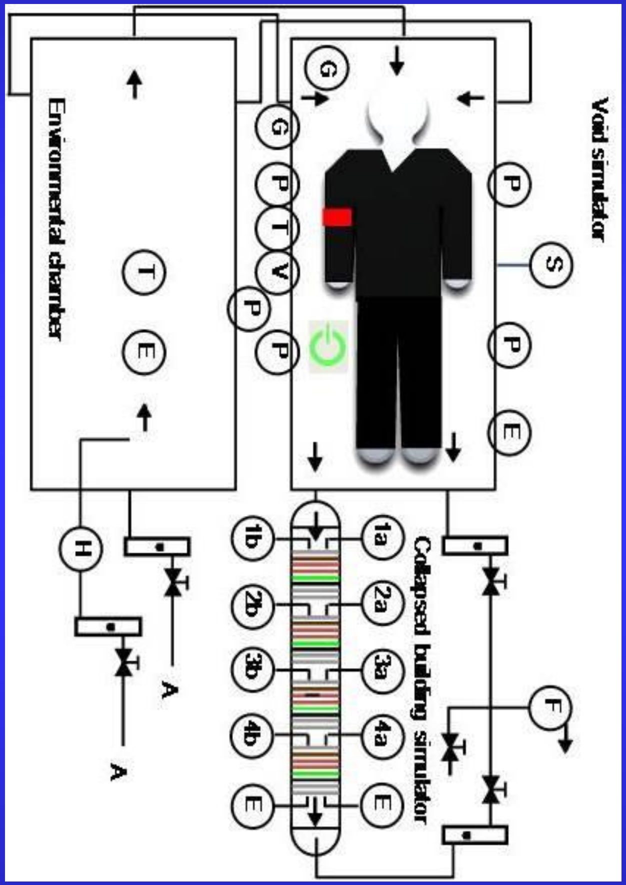
Source: WSJ reporting

Novel Applications: SaR Safety and Security

Second Generation Locator for Urban Search and Rescue Operations
SGL for USaR
Technologies for Heroes

Picture from Angela Eaton Snovak, Guide to search and rescue dogs, Barron's 2004

FP 7 EC Security Project
USaR applications
www.sgl-eu.org/

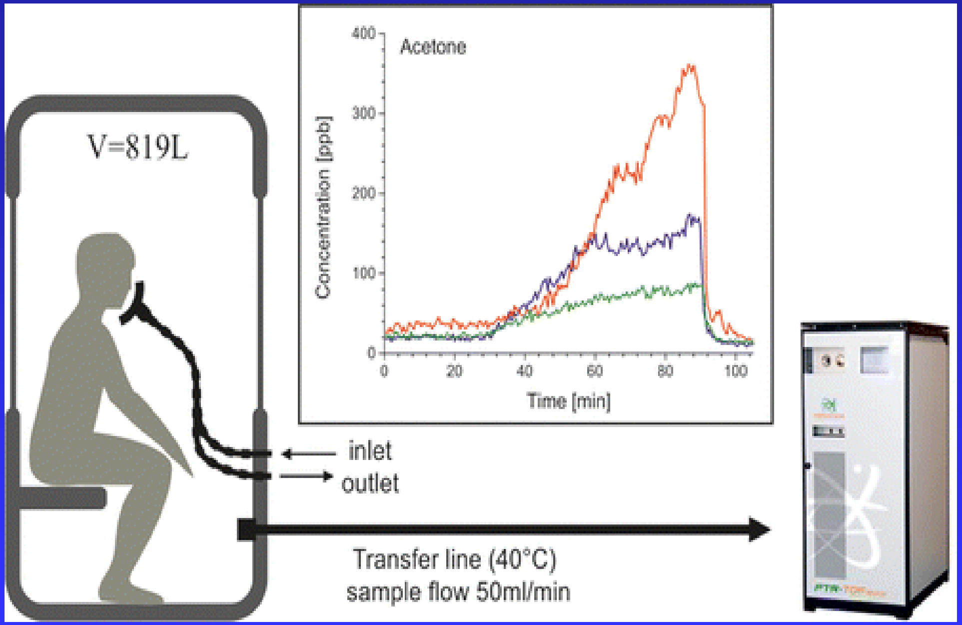


J. Breath Res. 5 (2011) 046006

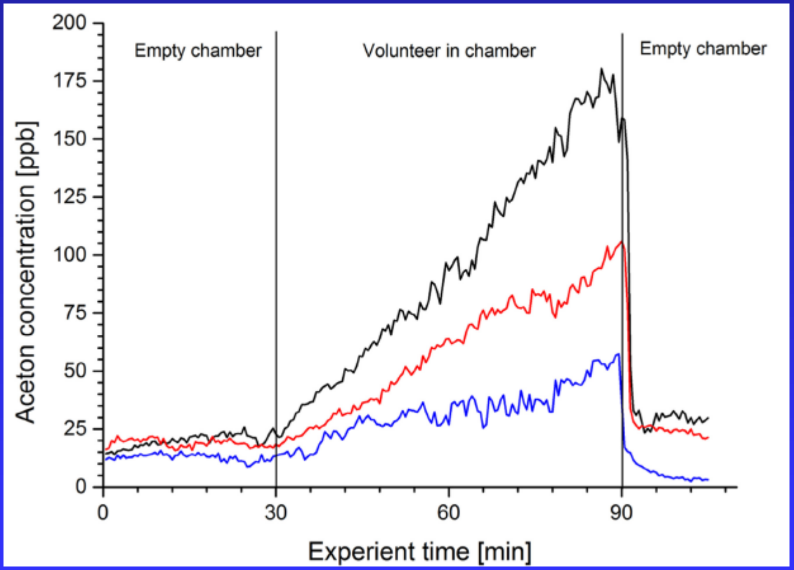
Confined space experiments

ISBN: 978-0-444-62613-4
Volatile biomarkes,
(Elsevier, 2013)

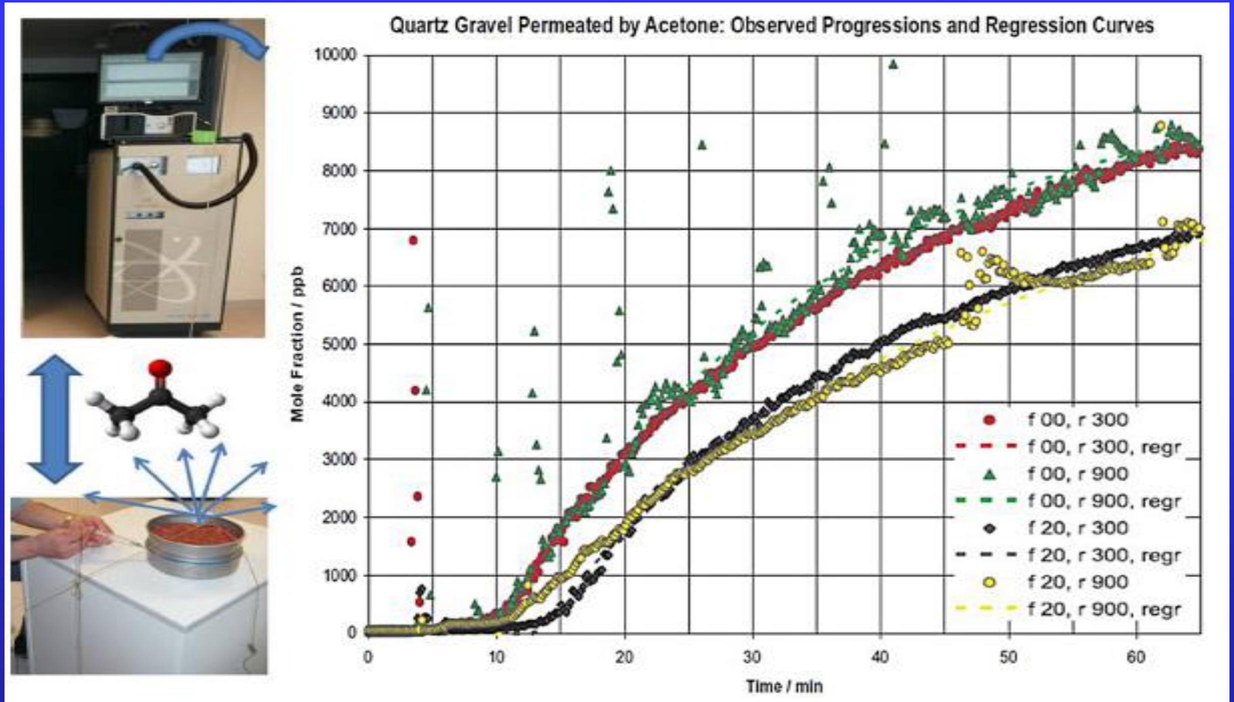
Body-plethysmography chamber



[Anal. Chem., 2014, 86 (8), pp 3915–3923]



Confined space experiments

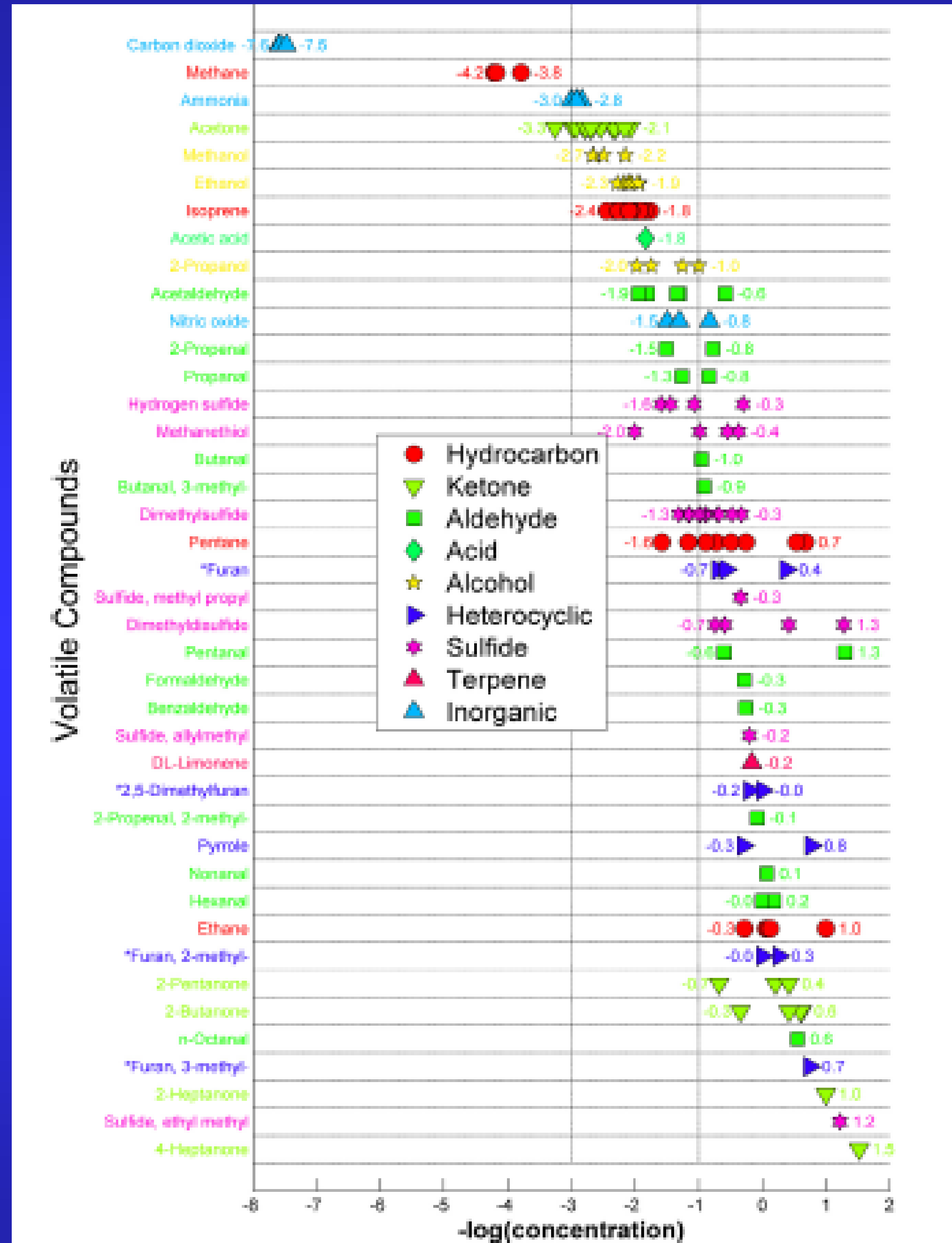


Spatiotemporal measurements of acetone standards over quartz gravels using proton-transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS) (plume detection and monitoring)

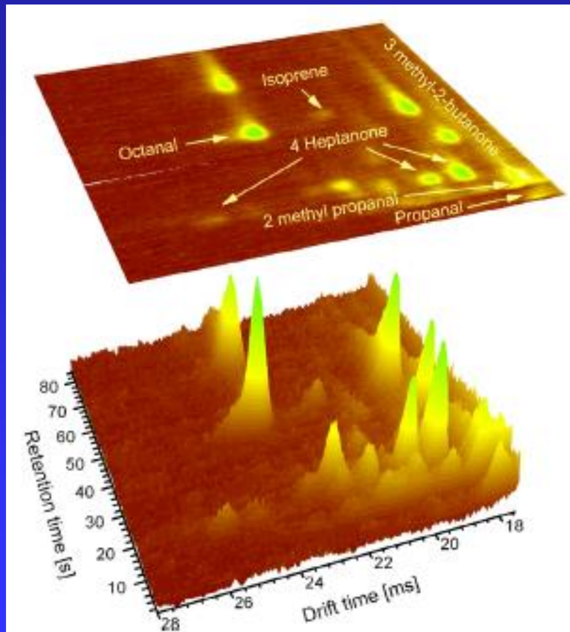
[A. Agapiou et al., Trends in Analytical Chemistry 66 (2015) 158–175]

Typical mean/median concentrations of selected breath volatile marker compounds in logarithmic scale.

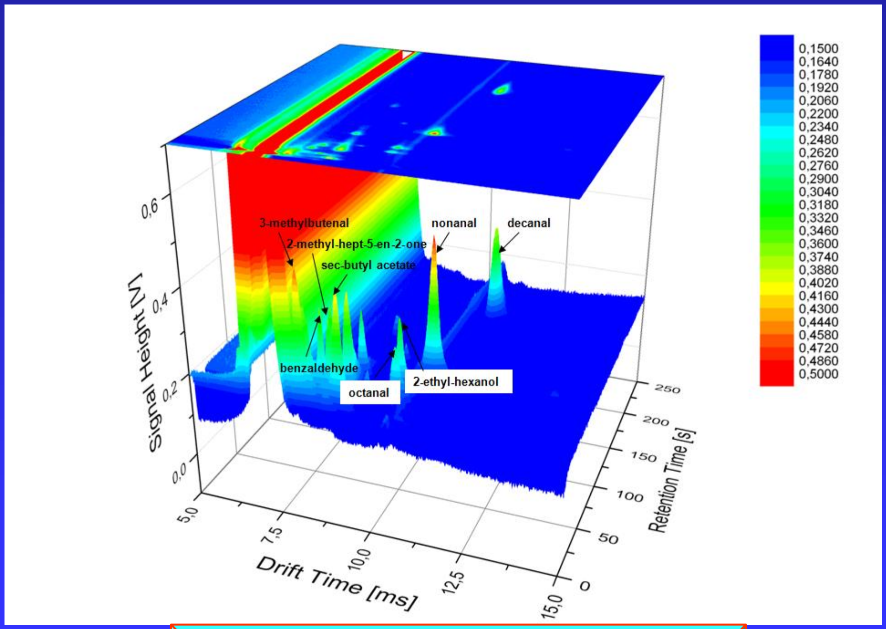
Compounds with an asterisk are considered smoking related compounds (e.g., furan and its methyl derivatives).



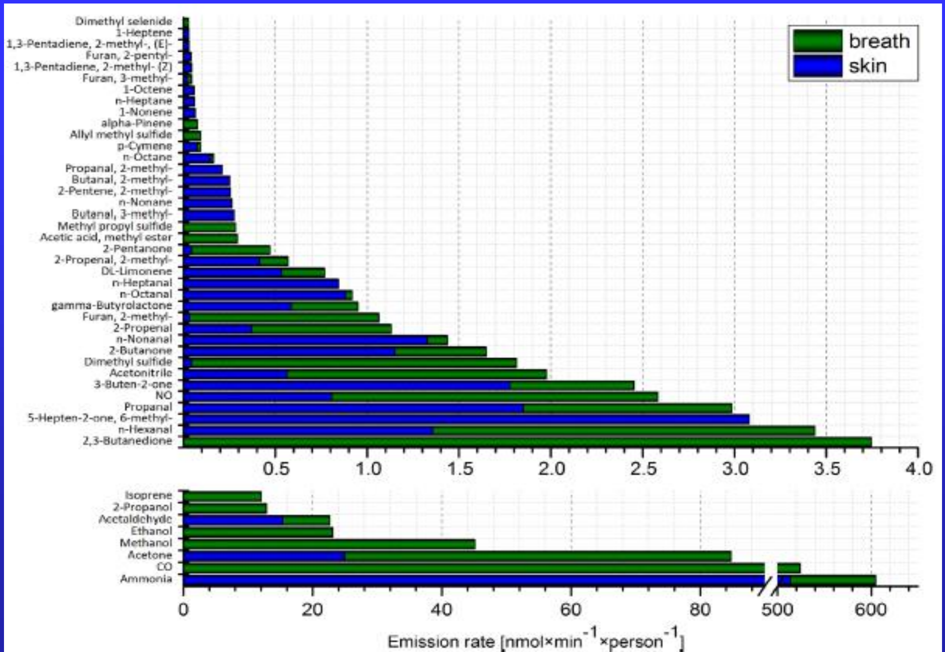
[Agapiou et al., Trends in Analytical Chemistry 66 (2015) 158–175]



Urine VOCs

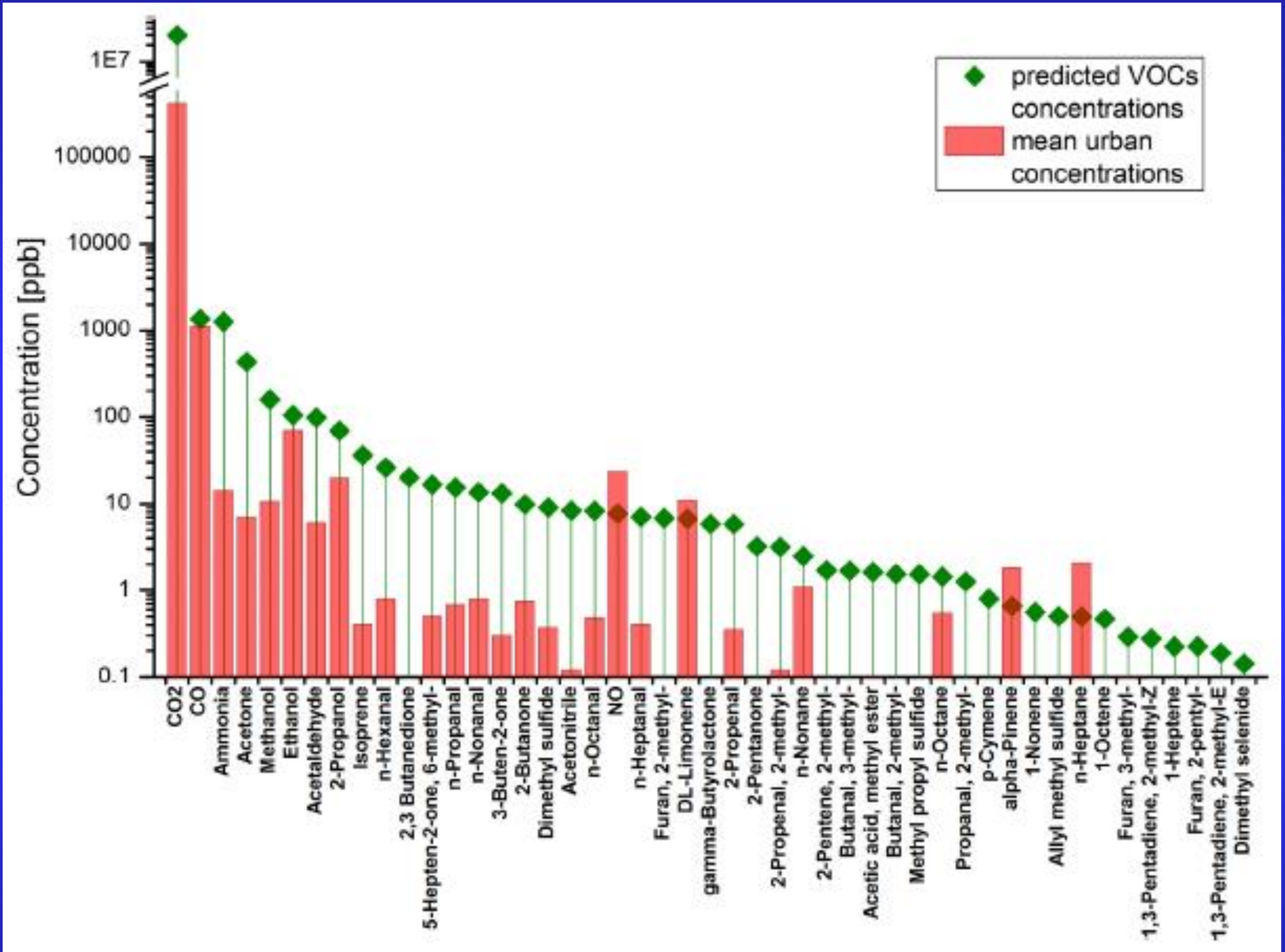


Skin emanations



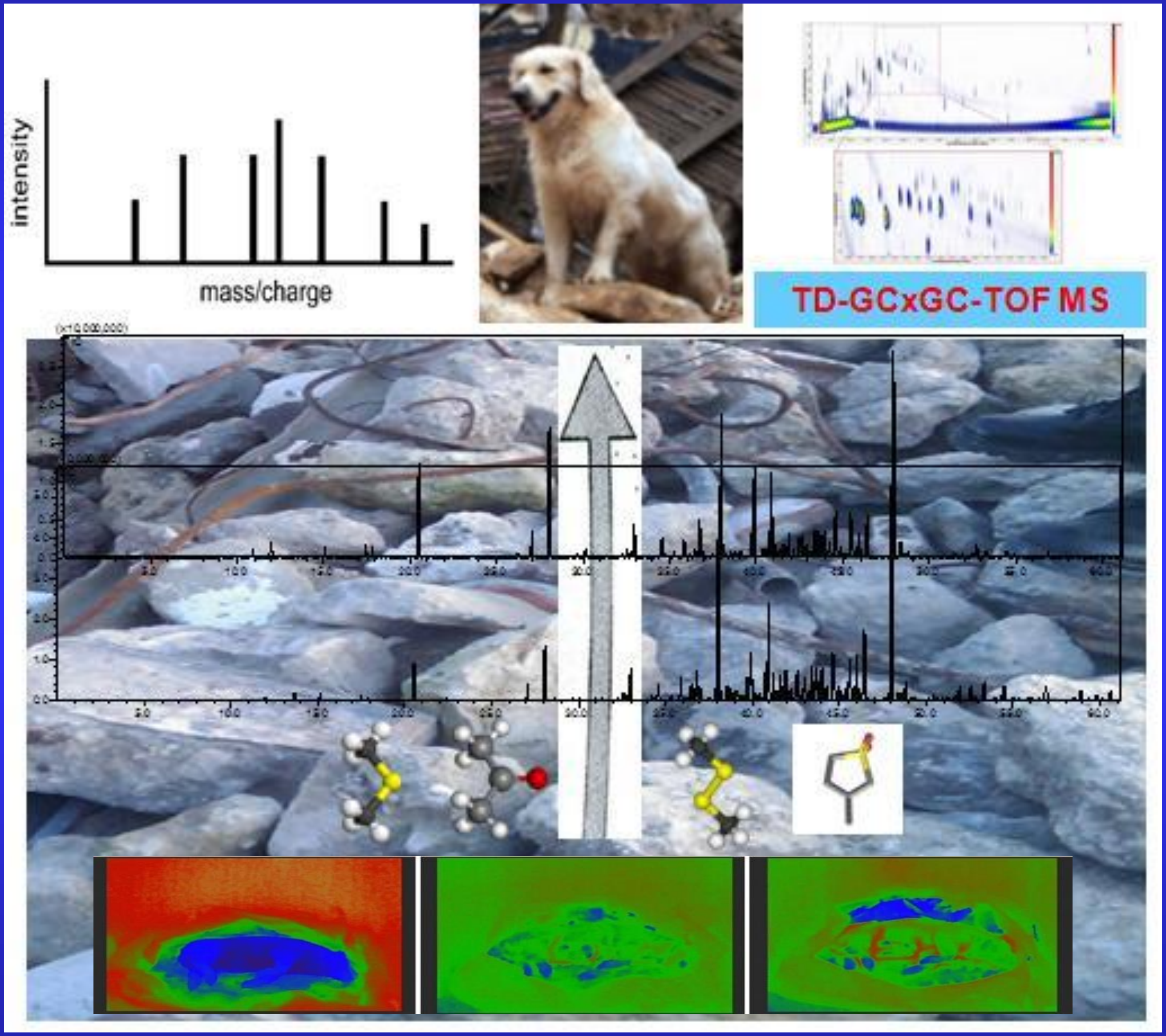
Total emission rates (considering means) of potential volatile markers of human presence from the human body. CO2 was excluded for reasons of clarity

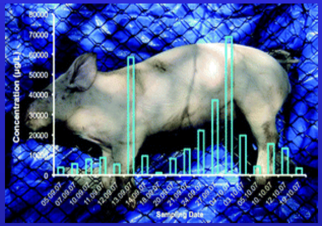
[P. Mochalski et al., Trends in Analytical Chemistry 68 (2015) 88–106]



Exemplary chemical signature of entrapped person predicted for a point located 3 m from a survivor and debris-to-air ratio 3:1. Redbars indicate mean urban air levels of compounds of interest
[P. Mochalski et al., Trends in Analytical Chemistry 68 (2015) 88–106]

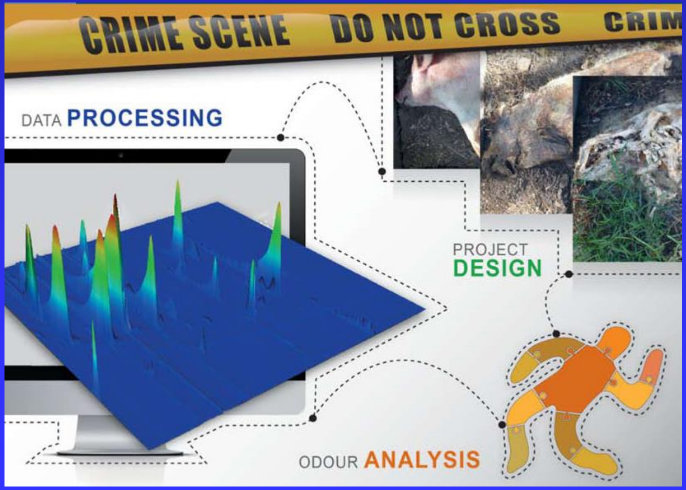
Forensic applications

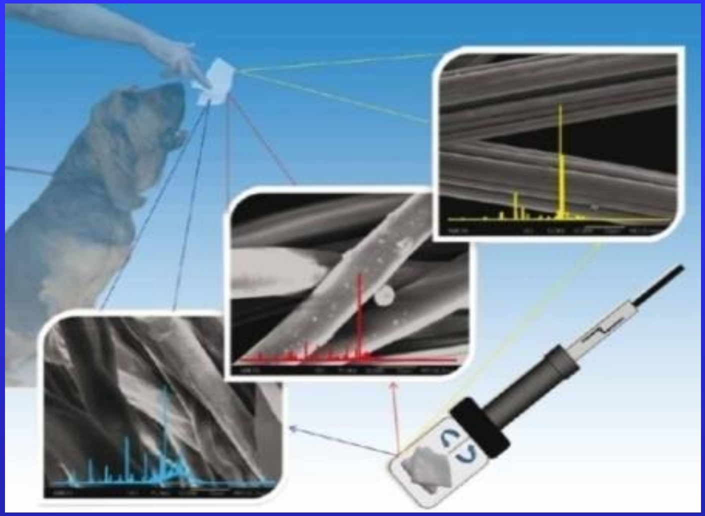




[Agapiou et al., Analytica Chimica Acta 883 (2015) 99–108]

Importance of cadaveric VOCs





The knowledge acquired from cadaveric studies is important for use by:

1. Forensic entomologists
2. Law enforcement police forces for training human remains detection (HRD) dogs
3. Medical experts for revealing the etiology of death and identifying the postmortem interval (PMI)
4. Forensic experts for the location of clandestine graves
5. SaR teams for the location of dead bodies in collapsed buildings after natural and man-made disasters

The ultimate goal:
development of portable cadaveric detection devices

Synopsis:
The future is personalized medicine…

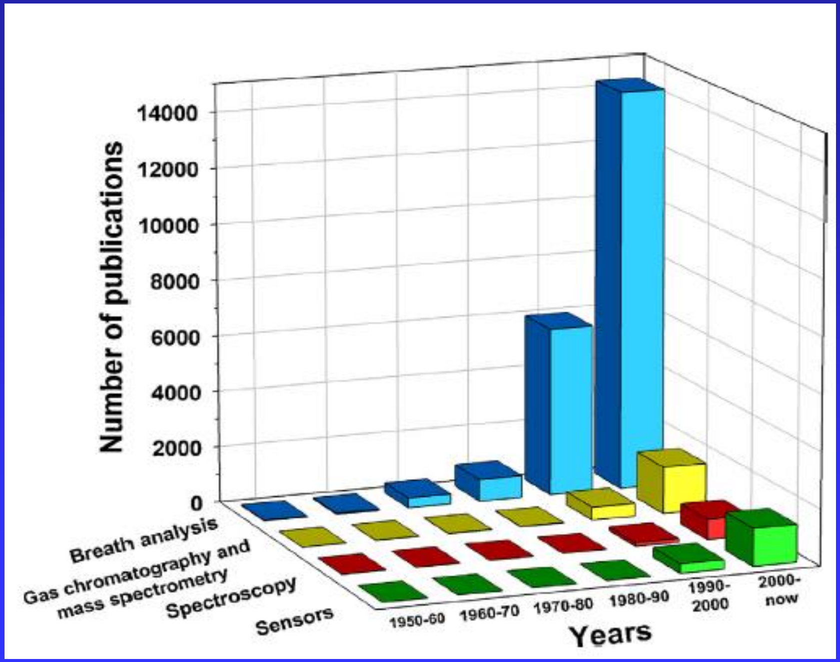
✓Nowadays, VOCs' profiling has become an attractive diagnostic method for clinicians and researchers. This medical knowledge should be integrated into **personal care hand-held monitoring devices**

✓Time has come for **breath αanalysis** to be transformed to novel **hand-held portable diagnostic devices**

✓ Emerging technologies: **Microfabrication** (e.g. development of micro GCs) and silicon **micromachine** technologies (e.g. pre-concentrators, separation columns, detectors); **Nanoscale sensor technology**; semiconducting DNA-carbon nanotubes and coated gold nanoparticles

✓A quite promising trend is the use of chemical sensors in **smart phones**

BACtrack for iPhones and Breathometer for iPhones and Android: measuring blood alcohol levels aiming to prevent drunk driving



H2020
Smart Phone for Disease Detection from Exhaled Breath

Thank you for your attention!

Do not waste your breath….