



LITTLE THINGS DO MATTER

Assessing Ambient Air Quality for Human Health Risks: A Novel Screen

AIR POLLUTION AS A SOURCE OF CHRONIC DISEASE

- The potentially long induction time between environmental exposures and the onset of health effects adds to the difficulty of establishing causation .
- Air pollution can contribute to angina, myocardial infarction, arrhythmias, and congestive heart failure causation.
- The pathophysiology linking particulate exposure to death includes pulmonary inflammation, accelerated atherosclerosis, and cardiac dysfunction causation.



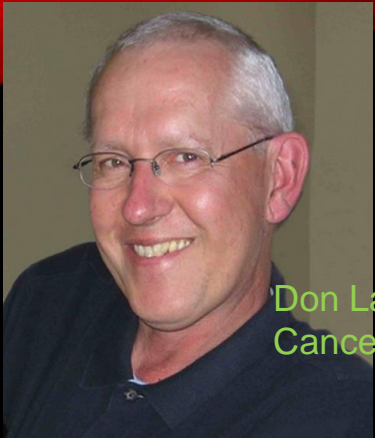
Reality: Conventional practitioners in Canada have no resources and little training to be able to diagnose or recognize environmental determinants of chronic disease.

CHRONIC DISEASE IN CANADA

- Rates of diseases with potential links to chemical exposures have been increasing nationwide.
- Asthma in children under age five has increased by 160%
- Autism has increased by 1000% since the mid-1980s
- Congenital misplacement of the urinary opening in the penis, has increased by 100% and now affects one of 125 male babies born
- Cancer in children has increased by 26%
- Acute lymphocytic leukemia (62%)
- Brain and nervous system cancers (50%)
- Testicular cancer in young men has increased by 85% and is now the most common cancer in men ages 15–35

As per the American Cancer Society, only 5–10% of all cancers can be attributed to inherited factors; the rest occur from environmental exposures and other damage throughout our own lifetimes.

LABORATORY MANAGEMENT WITH CANCER AND OFFSPRING BORN 2004- 2007



Don LaBerge, President of ETL – 2005, 57 – Brain
Cancer

Type of Cancer	Status
Brain Cancer - 2	Both Deceased <60
Paranasal Sinus Cancer - 2	Remission
Prostate Cancer - 1	Remission
Melanoma - 1	Remission
Lung Cancer - 2	Both Deceased <60
Breast Cancer – 3	1 Deceased <60
Cervical Cancer - 1	Remission

Rob Deverall, ALS– 2014, 59 – Lung Cancer



Type of Issue	Number
Autism	3
ADHD	2
Other neurodevelopmental disorders	2
Seizure Disorders	1
Asthma	5
Down Syndrome	2

**WE WILL ADAPT, RESISTANCE
IS FUTILE! YOUR LIFE, AS IT
HAS BEEN - IS OVER. FROM
THIS TIME FORWARD, YOU
WILL SERVICE US.**

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LIFE SCIENCE FORENSICS



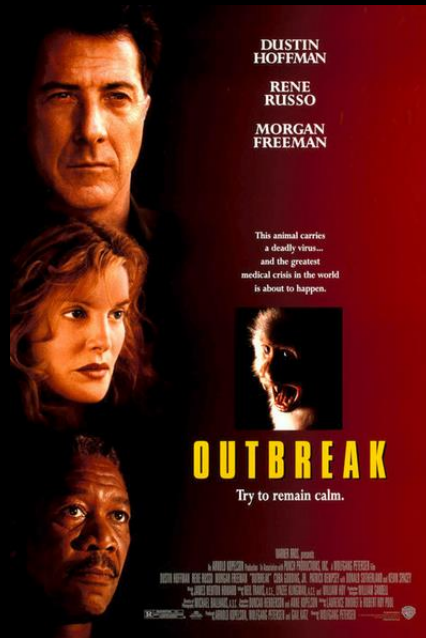
EPIDEMIOLOGY

The branch of medicine that deals with the incidence, distribution, and possible control of diseases and other factors relating to health.

THE GENOME OF A POPULATION DOES NOT CHANGE IN 100 YEARS

We deal with environmental determinants of chronic disease in addition to dealing with infectious disease.

OUR GOAL IS TO MARRY TOGETHER THE FIELDS OF ENVIRONMENTAL CONSULTING AND ENVIRONMENTAL MEDICINE/TOXICOLOGY



ADVERSE HEALTH EFFECTS FROM AIR POLLUTION



- One of the most common requests that we get is to evaluate the potential for adverse health effects due to odors generated in the field due to oil and gas operations.
- Ambient air monitoring equipment is adequate for routine monitoring purposes, as are passive filters, but cannot address novel or high-end parameters in terms of detection limits and the limitations to small amounts of potential analytes.



ADVERSE HEALTH EFFECTS FROM AIR POLLUTION

- Point source testing using summa canisters, bags, or charcoal tubes is much more accurate, but still requires pre-knowledge of the contaminants of concern in order to test for them.

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SO WHAT DID WE DO ABOUT IT?

- Summa canisters and charcoal tubes require a technician to sample regularly in field if extended periods of time are required to be monitored.
- An alpha/beta Continuous Air Monitor (iCAM) was used to collect a month's worth of air samples, with no changing of filters required.
- Traditionally, this instrument measures airborne alpha and beta particulate activity with radon/thoron alpha and beta background compensation.
- This has now been used in real world applications as well.



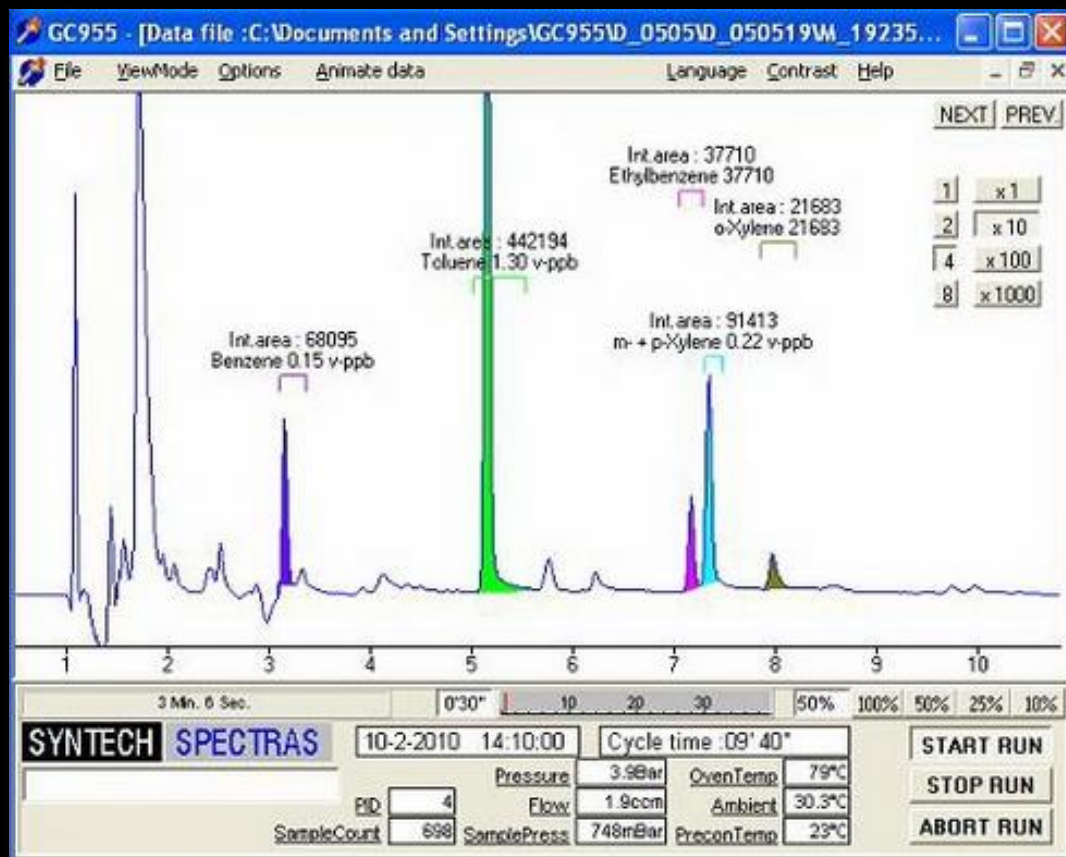
SAMPLING PROTOCOL

- Samples were placed in a ditch of a median on Stoney Trail for a period of 1 month, from mid-September to mid-October, 2014. Note that pesticide levels still persist, despite being the end of the lawn and garden season.
- 4 test monitors were placed on site, to ensure that enough sample was garnered for the extensive list of analytical.
- Samples were checked and collected every week to ensure that they maintained integrity, although this was not strictly required.
- **Air sampling and screening can provide evidence that a problem exists, whereupon conventional testing can be done to pinpoint and address the issue.**



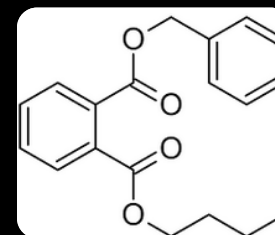
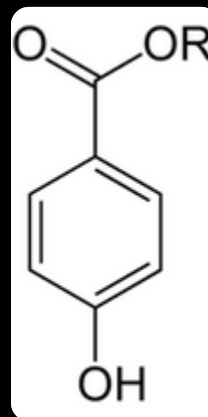
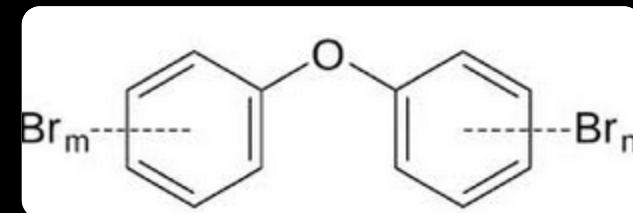
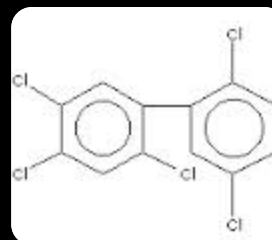
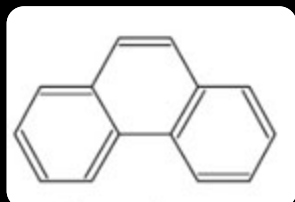
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STUDY AREA



WHAT WE TESTED FOR...

- In place of the fixed, card mounted filter, which must be changed manually at regular intervals, the mechanism passes a continuous belt of filter material under the detector. The filters were collected and run for a number of parameters, including:
- PAHs
- PCBs
- Phthalates
- VOCs
- BTEX F1-F4
- SVOCs
- Metals
- Particle Size
- OC and OP Pesticides



POINT SOURCE TESTING RESULTS

- Elements and compounds of note are listed on the next slide.
- Compounds with values below detection limit are not included.
- Overall, 238 parameters were analyzed.
- Results are summarized to show a yearly intake, based on a conservative proposed one hour of outside exposure a day.



Analyte (ug/L)	YEARLY SUM
Metals	
Aluminum	9453.36
Barium	13293.60
Boron	15317.80
Calcium	16379.59
Iron	1068.47
Magnesium	2020.47
Potassium	11352.35
Sodium	28910.38
Zinc	10164.40
PHCs	
F2 PHCs (C10-C16)	1902.0
F3 PHCs (C16-C34)	1409.4
VOCs	
1,3-Dichlorobenzene	1500.6
1,4-Dichlorobenzene	1502.2
1,2-Dichloroethane	3122.8
SVOCs	
4-Chloro-3-methylphenol	1419.4
Azobenzene	6367.2
Benzo [b] fluoranthene	730.00
Bis(2-chloroethoxy)methane	1054.44
Bis(2-chloroisopropyl)ether	932.78
Bis(2-ethylhexyl)phthalate	1987.2
Dibenzo [a,h] anthracene	202.78
Diethylphthalate	358714
Di-n-butylphthalate	40231
Isophorone	648.89
Nitrobenzene	3001.1
Pesticides	
Atrazine	1404.5
2,4-D	1210.9

This is all well and fine...very scientific...and expensive!
 ~ \$60,000 worth of testing, and so what is it telling me? Will I get sick breathing this air based on these results?



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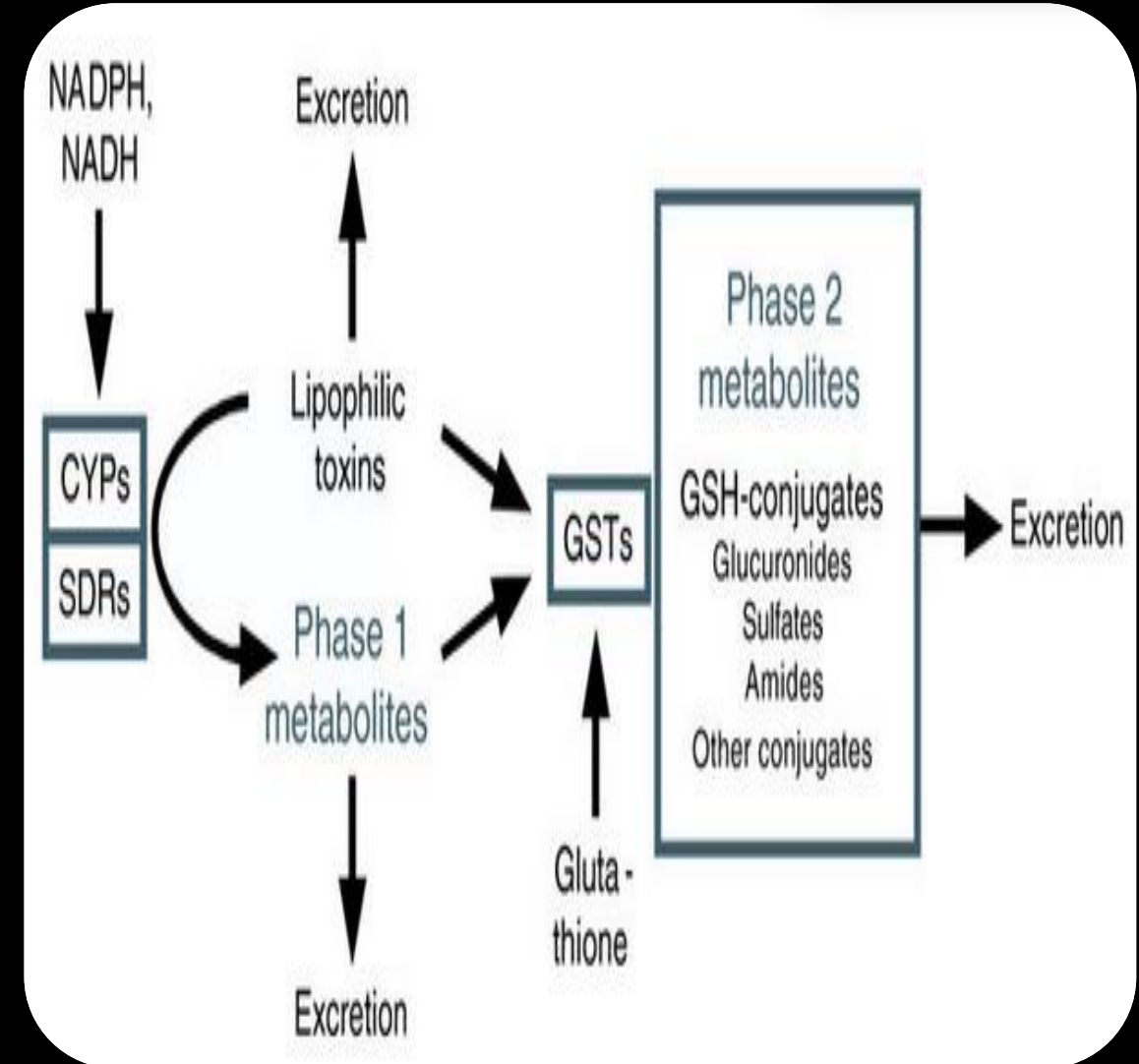
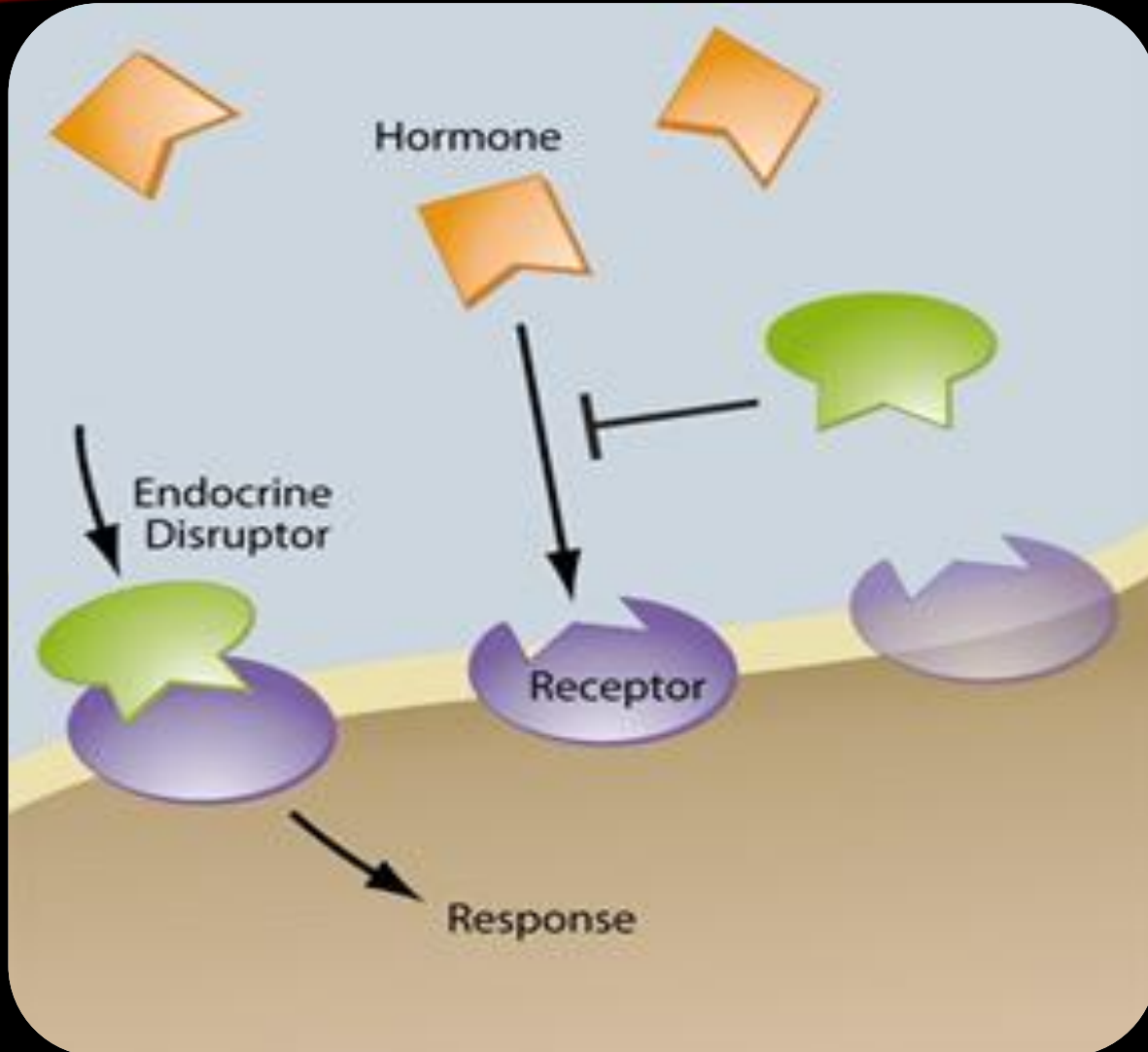
YEAST ESTROGEN ASSAY

Because point source testing has yielded some results, the question becomes “What of them?” and “Will they cause adverse health effects?”

In this case: from doing yeast estrogen assays, the answer is, likely yes – there was a strong estrogenic response to this novel screen and extraction technique that shows that...

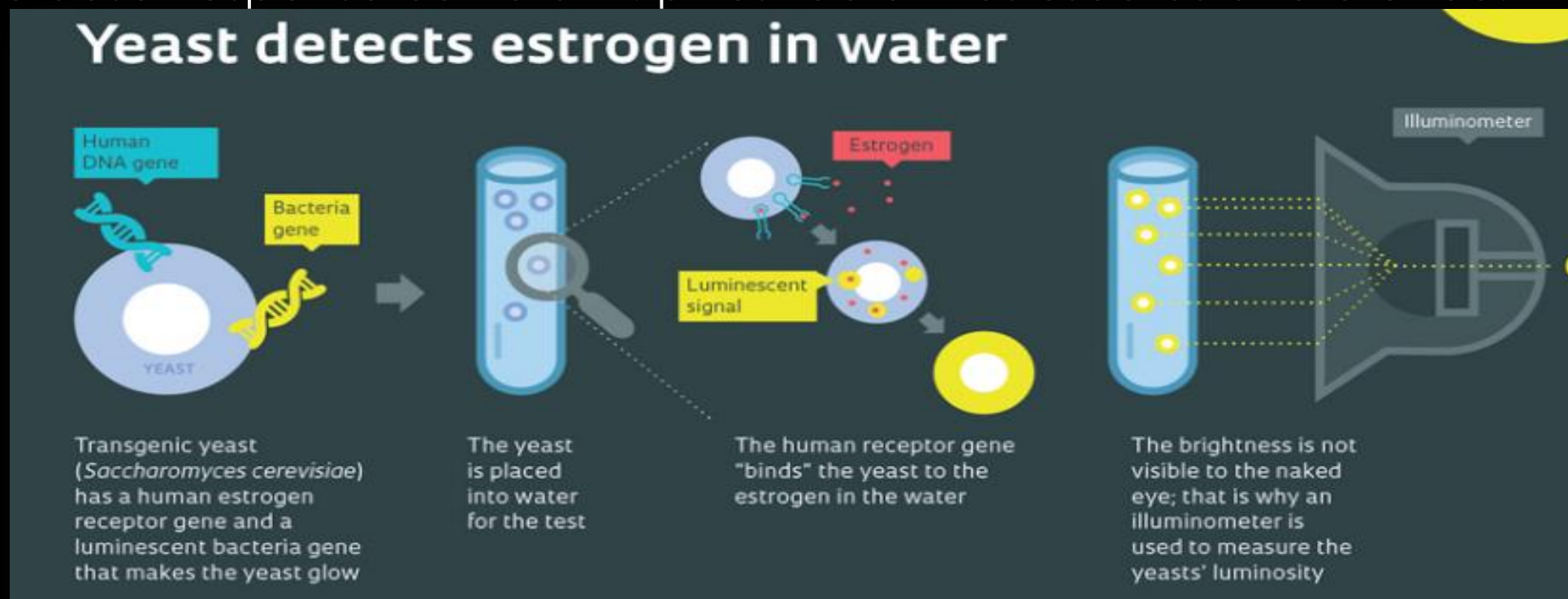
There are chemicals of concern that have the capacity to cause adverse health effects in samples that **PASS ambient air quality criteria.**

MECHANISMS OF ACTION

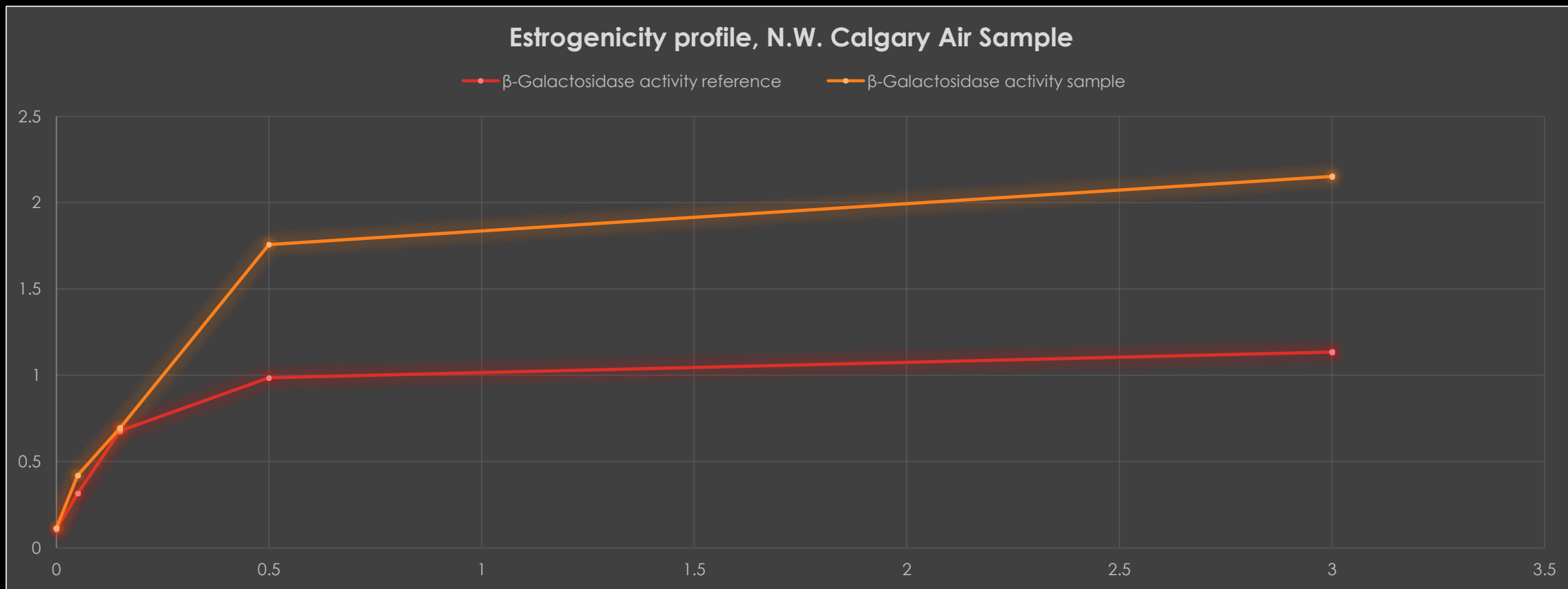


ESTROGENIC RESPONSE

- Yeast that has been transfected with the human estrogen receptor (hER) gene as well as expression plasmids that carry an ERE and the reporter gene LacZ that encodes the enzyme X-galactosidase was used to test for estrogenic response potency.
- A positive dose-response curve of 17β -estradiol was used as a reference.

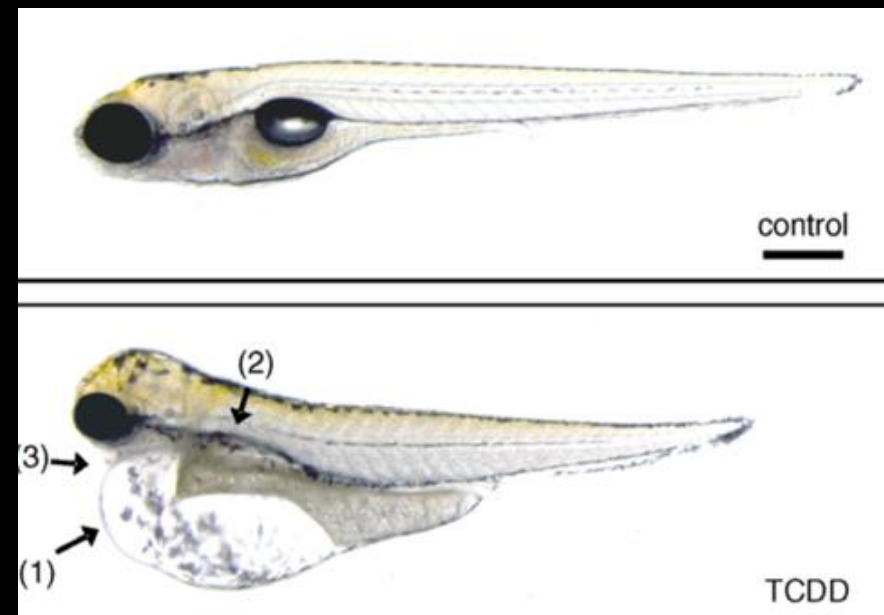
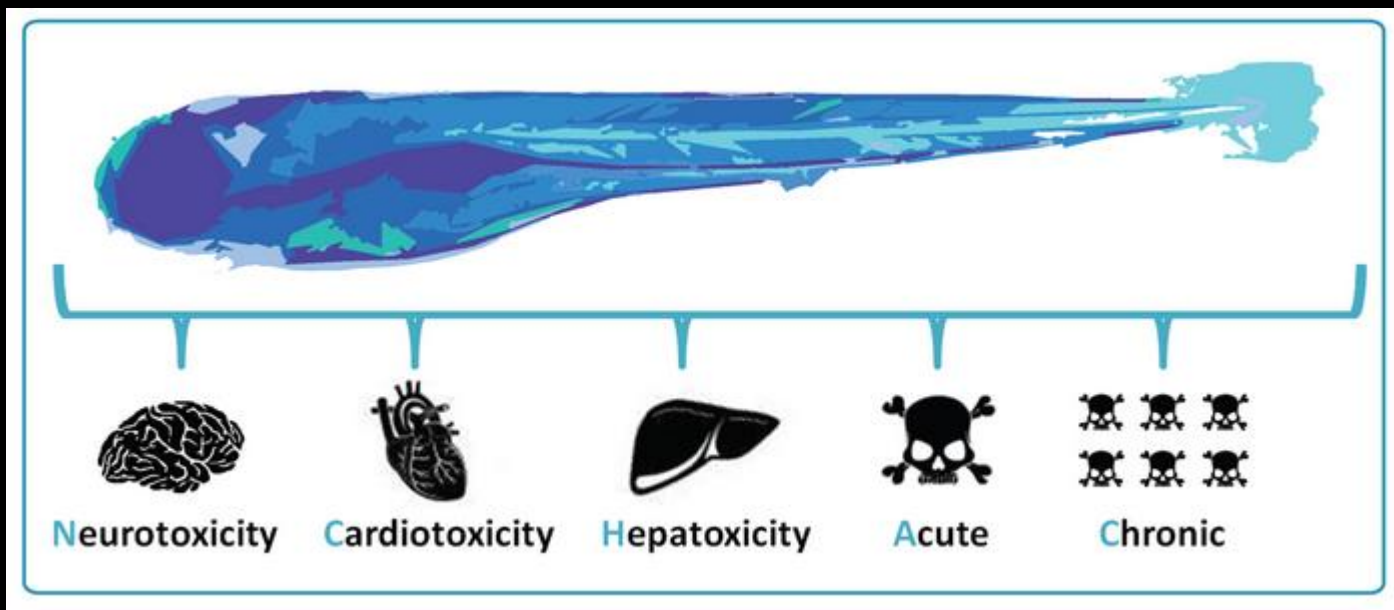


ESTROGENICITY PROFILE



ZEBRA FISH NEUROTOXICITY AND MUTAGENICITY

Further potential steps also exist...



2,3,7,8-Tetrachlorodibenzodioxin

WHAT DID WE FIND OVERALL?

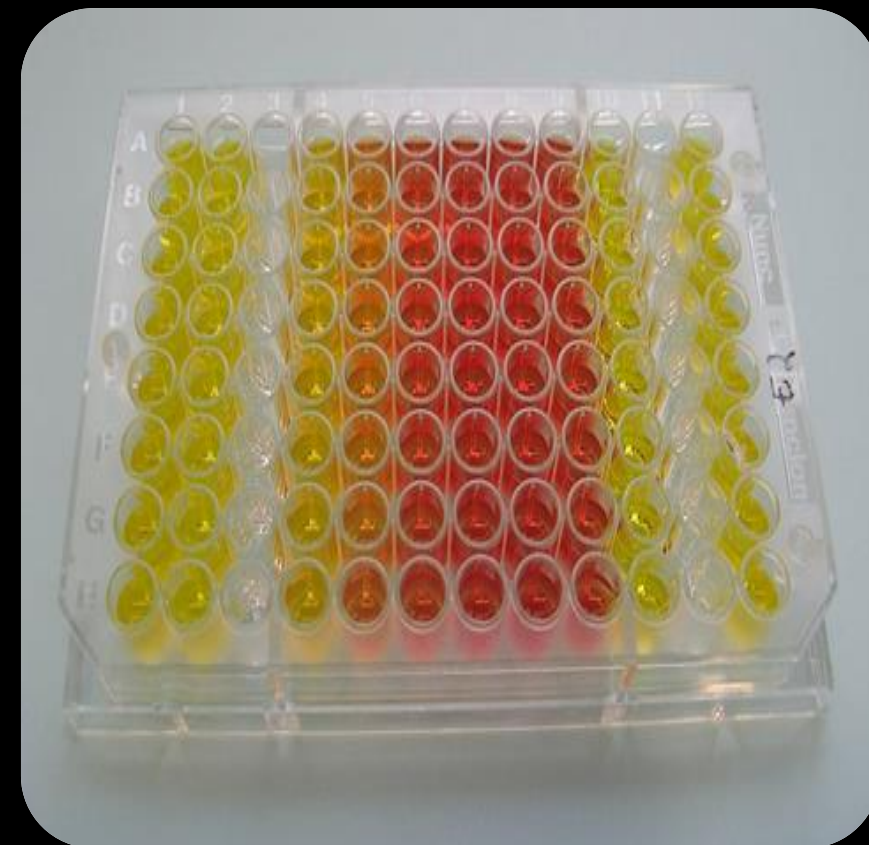
It is likely that these exhaust fumes as well as those from gasoline engines have been captured in this study.

Further, the site is less than 5 km from the Spy Hill landfill.

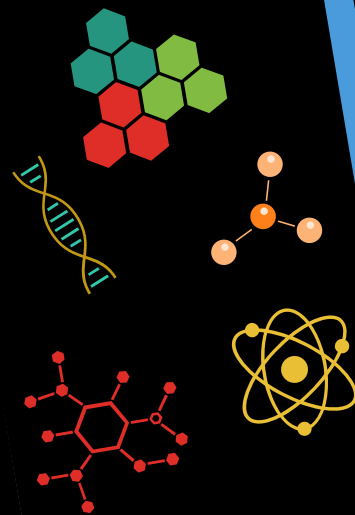
It was not known what had caused the results of this test to show estrogenic effects; however, it is clear that these results cannot purely be just the result of what has been captured in the discreet analyses.

In other words, part of the picture is missing and there have to be some chemicals that have yet to be accounted for in these samples.

SO THEN WE LOOKED A LITTLE FURTHER



Doing High-throughput screening in conjunction with other testing significantly cuts down on the kind of testing that is needed.



- ✓ Several Different Types Possible
- ✓ Endocrine Disruption
- ✓ Neurotoxicity
- ✓ DNA Damage and Oxidative Stress
- ✓ Mutagenicity

GOING EVEN FURTHER

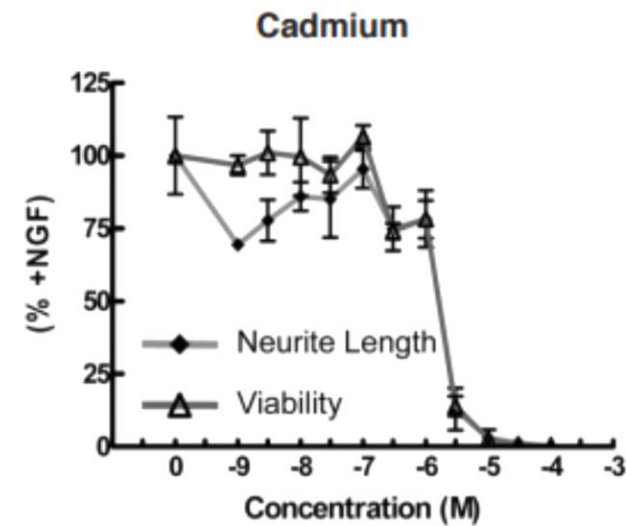
High-Throughput Screening

USEPA Approved *in-vitro* Methods Through ToxCast

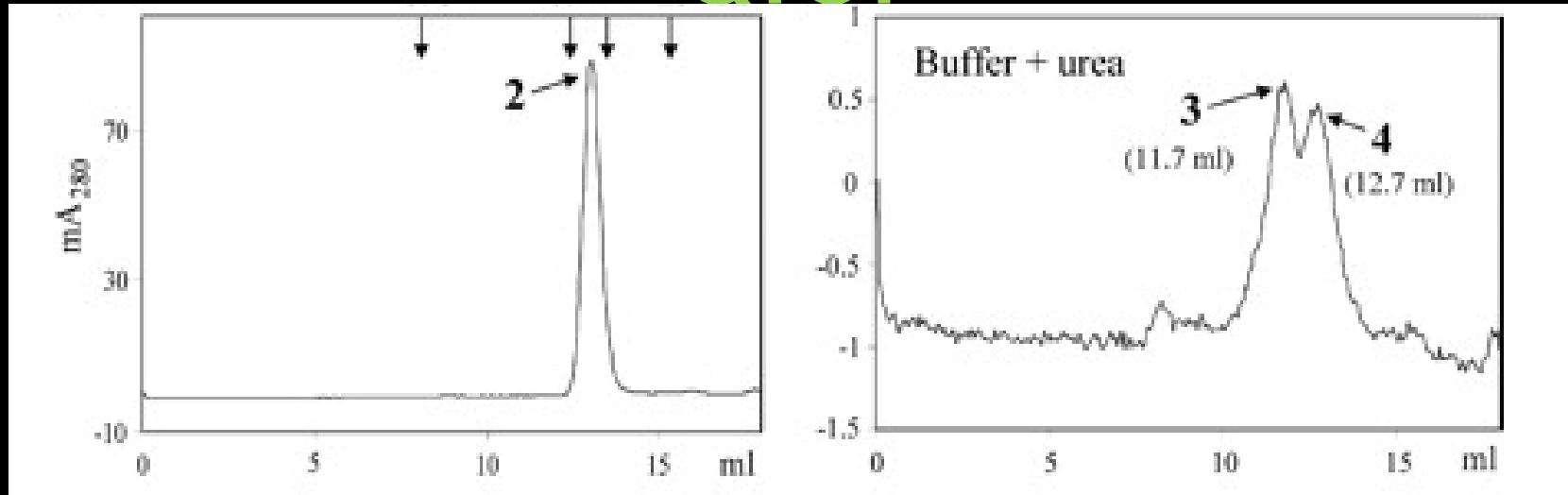
Toxicological Specificity of Neurite Outgrowth

High-Throughput Screening

- 1- Neurotoxicity
- 2- Endocrine Disruption
- 3- DNA Damage, Oxidative Stress
- 4- Mutagenicity



AND THEN AFTER THAT WE LOOKED EVEN FURTHER - NEXT STEPS: LC/MS QTOF



Quantum Time of Flight LCMS

Conventional Methods

- A GC-APCI source can be added on to connect the QTOF to a GC so we can do both GCMS and LCMS on one instrument.



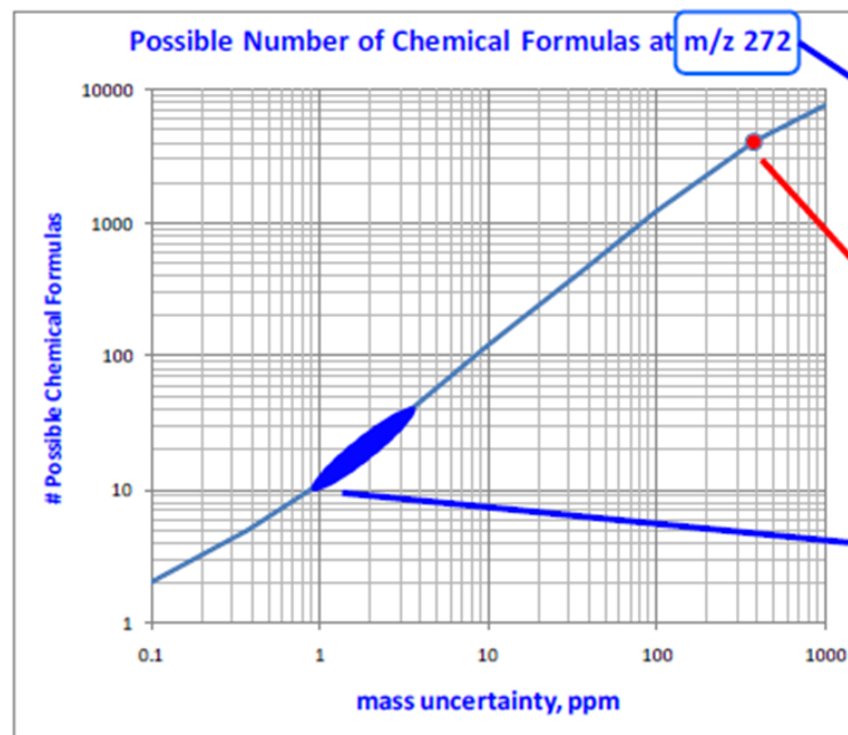
IN A NUTSHELL...



LC/MS INSTRUMENTS

6560 Ion Mobility Q-TOF
LC/MS

Accurate Mass Reduces the Number of Possible Chemical Formula



Octafluoronaphthalene (CAS 313-72-4)
 $C_{10}F_8 = 271.98667$

mass uncertainty		# of Possible Formulas
ppm	amu	
1000	0.3	7657
368	0.1	4050
100	0.03	1223
37	0.01	466
10	0.003	120
4	0.001	43
1	0.0003	11
0.4	0.0001	5
0.1	0.00003	2

Formulas made of:
C, H, N, O, F, & Cl

Accurate mass reduces risk of investing effort on the wrong molecule

WHAT DID WE FIND?

- **Glyphosate – not a conventional parameter – a powerful Endocrine Disruptor**
- 2,682,656 kg are sold in the province of Alberta, per year.
- This is 3 times more than the next most used herbicide, MCPA, and 3.5 times more than 2,4-D, the third most common pesticide.
- Glyphosate can induce excessive consumption and wasting of glutathione and ascorbate.
- These two antioxidants are at the center of the antioxidant recycling network that protects delicate cell components from free radical oxidative damage during physiologic homeostasis and immune tolerance.
- Once antioxidants are selectively depleted, free radical damage can “run rampant” in oxidizing and making dysfunctional important cell systems

CHEMICALS WIDELY “KNOWN” TO BE SAFE...OR ARE THEY?



(Samsel & Seneff, 2013)

Mice exposed at low-levels to theoretically “non-toxic” RoundUp (glyphosate) – a chemical that exists on almost all vegetables grown in existence today.

AIR PARTICULATES

- What has been captured in the analytical results is likely the result of absorbance to fine particulates. Particulate matter (PM_{2.5}) values were measured at 18 µg m³, less than the 30 µg/m³ as a 24-hour average concentration guideline within Alberta.

Relevance

- Over 104,000 chemicals have been introduced with few studied for toxicities and only a handful for their interactions.
- The human cost is a reduction of 8.8 years of life for the average person due to the effects of these toxicants which represents 10% of most people's lives.
- **As shown in this study, it is possible for risks to exist even if intensive analytical testing is performed – for what does it all mean? What actually constitutes too much? AND IT SHOWS HOW EASY IT IS TO BE LED TO THE WRONG CONCLUSION BASED ON POINT SOURCE TESTING ALONE**

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