Impacts of Chemical Mixtures Isolated from Household Dust on Metabolic Health

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UNIVERSITY

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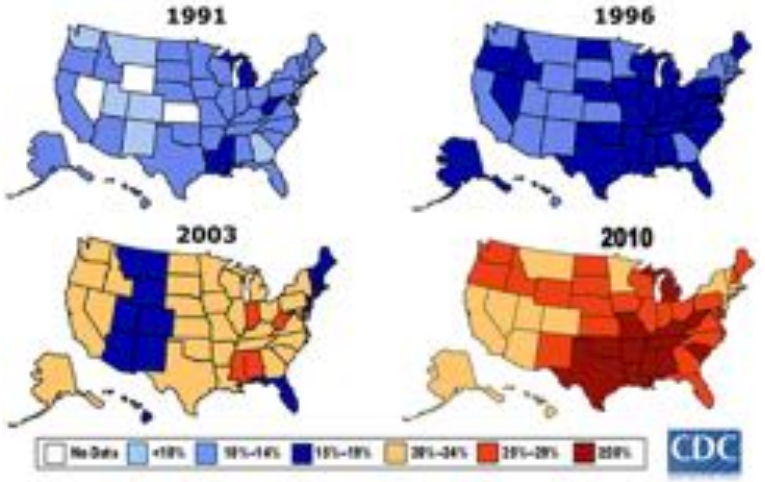
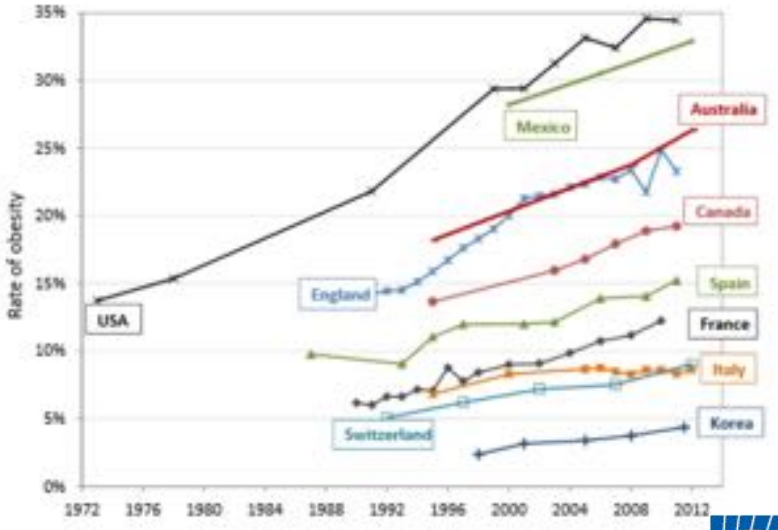
Prevalence and Consequences of Obesity Epidemic in US, Globally

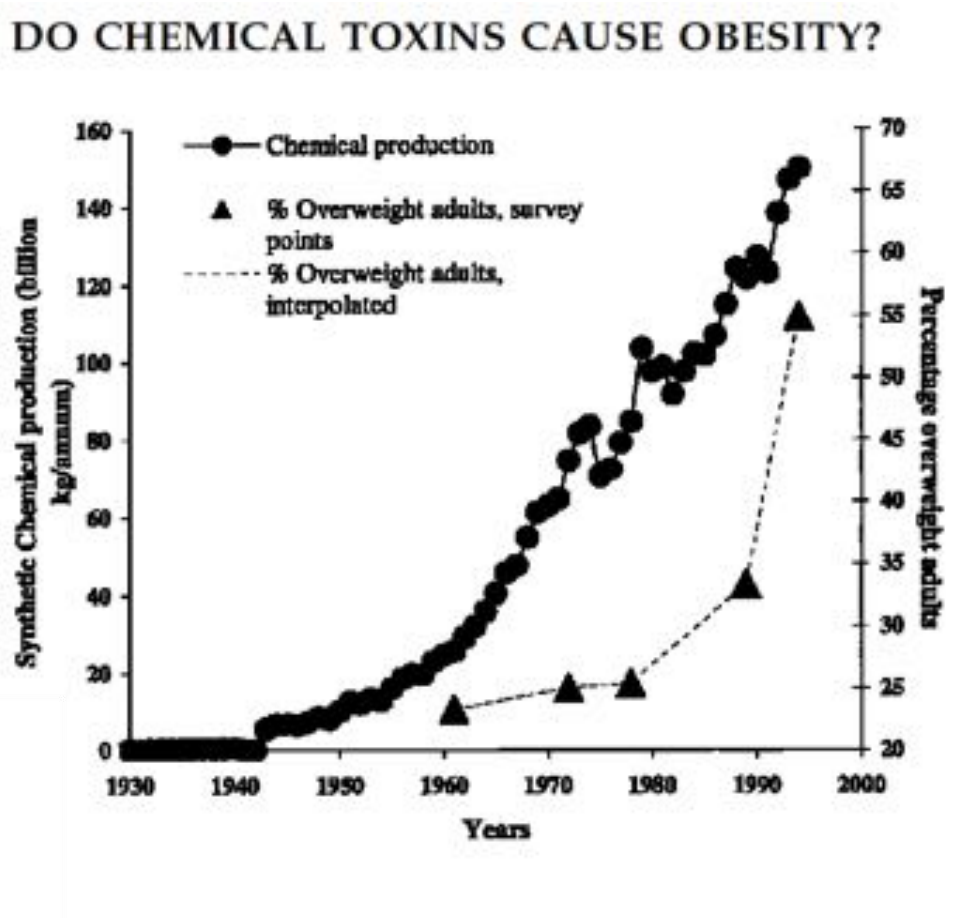
- Currently ~40% of US adult population is obese.
  - ~9% infants/toddlers
  - ~19% of 2-19 year-olds
- >$265B in US health care costs on obesity related illnesses (2015)
  - ~8% of total US health care costs (>12% in NC, OH, WI; 2018)
- Increased comorbidities
  - T2D, CVD, hypertension
- Interventions have produced only modest effects





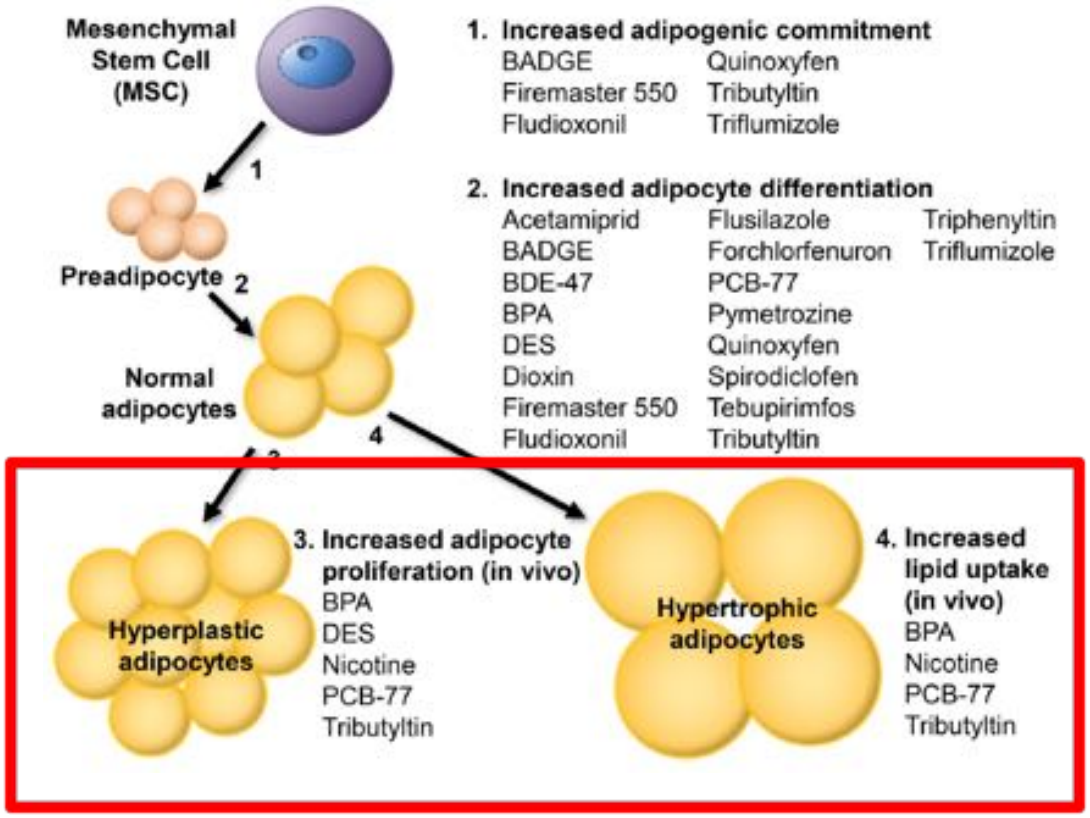
Potential Role of Chemicals in Increasing Obesity Rates in Humans

- First posited in 2002, despite decades of experimental evidence.
- Challenges caloric intake, activity, genetics as sufficient factors to explain magnitude/speed of observed trend.
- Summarizes wealth of animal evidence on antibiotics, PCBs, plastics, pharmaceuticals, pesticides, organophosphates, heavy metals, etc.



Baillie-Hamilton et al. 2002, *J Alt Comp Med*

Potential Mechanisms of Metabolic Dysfunction



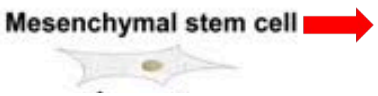
Heindel et al. 2017, *Repro Tox*

- Numerous potential mechanisms of metabolic disruption:
  - Adipocyte commitment from MSCs
  - Adipocyte differentiation from precursor cells
    - Increased pre-adipocyte proliferation
    - Increased lipid uptake
  - Shifting energy balance to favor calorie storage
  - Altering basal metabolic rate
  - Altering hormonal control of appetite and satiety
  - Altering brain circuitry that controls food intake, energy expenditure

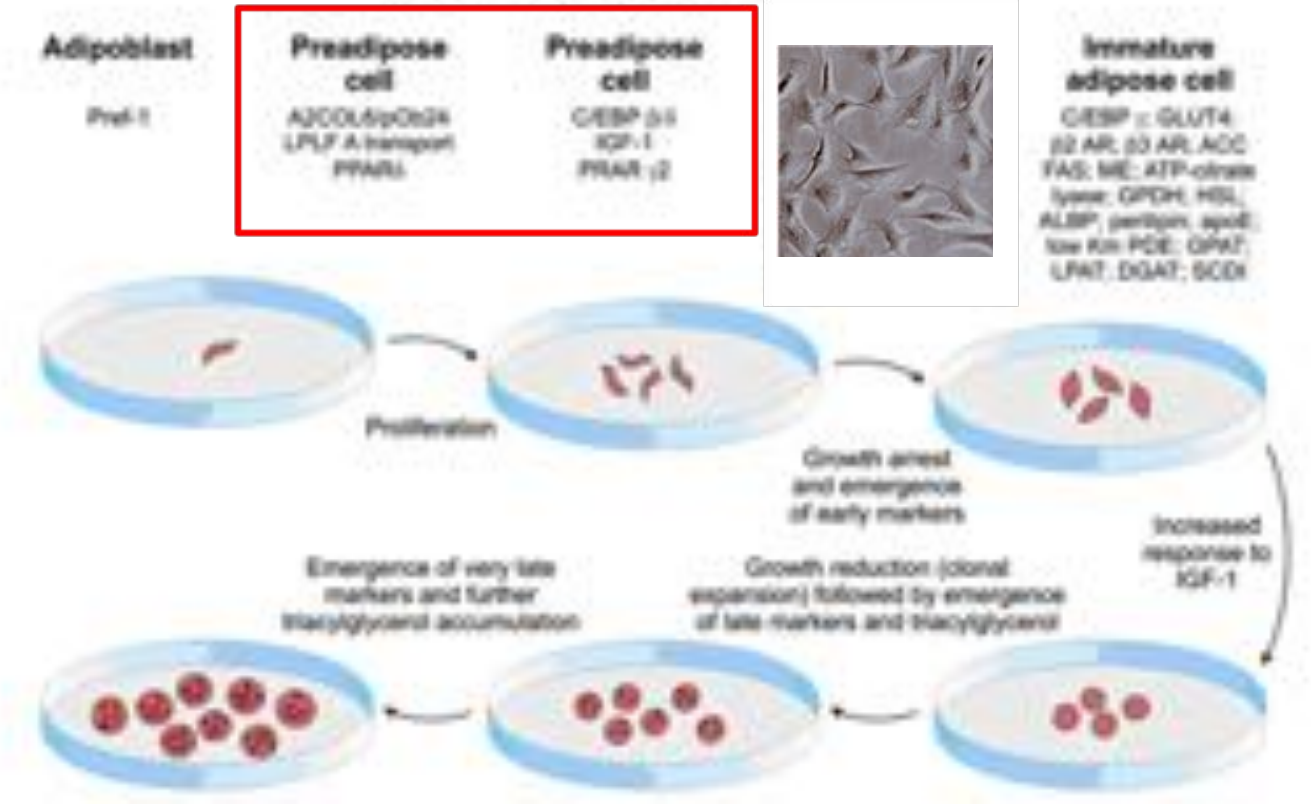
Adipocyte Differentiation Process

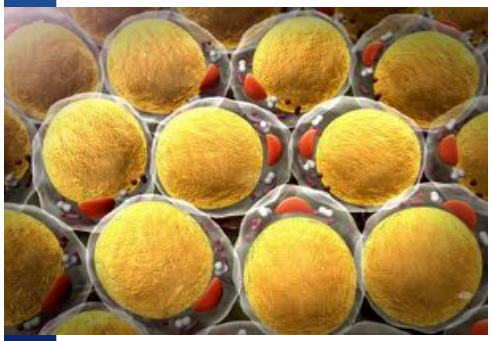
Adipocyte commitment

Adipocyte differentiation



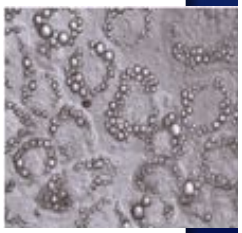
Other pathways:
Myoblasts
Osteoblasts
Chondroblasts





Resemble mature white adipose cell

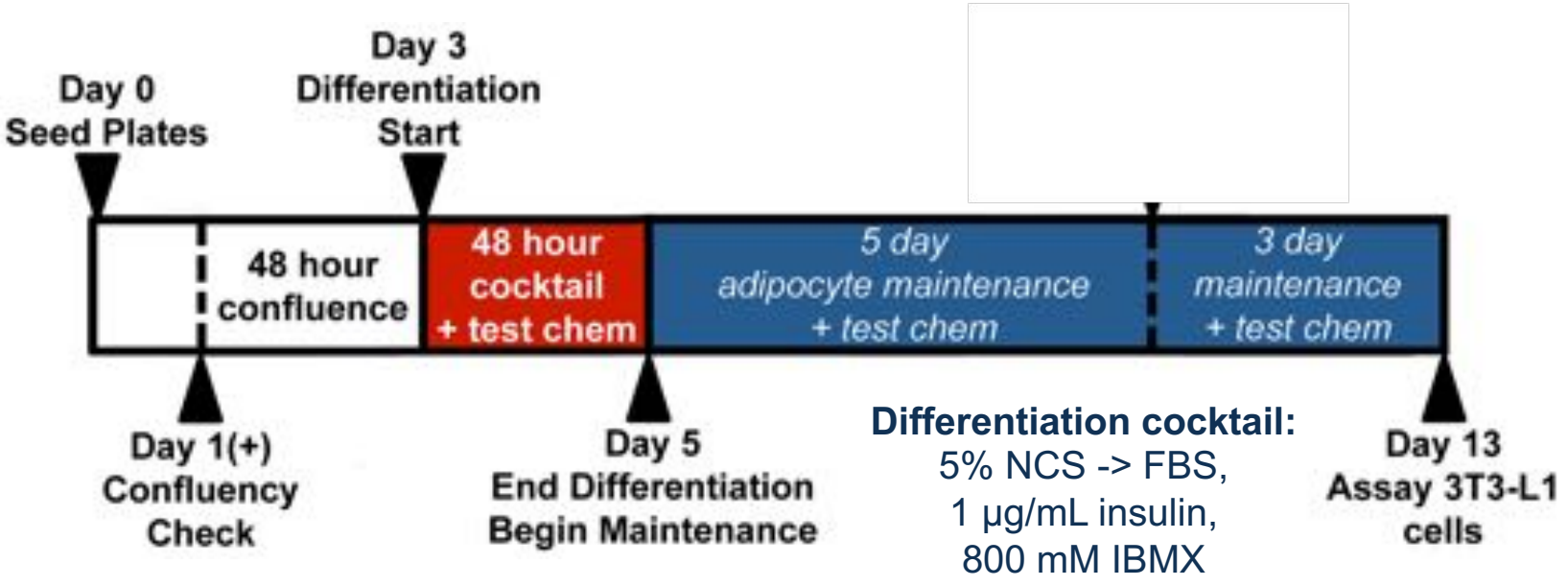
Resemble brown/developing white adipose cell



Nagy et al. 2011, *Mol Med*

3T3-L1 Pre-adipocyte Adipogenesis Assay

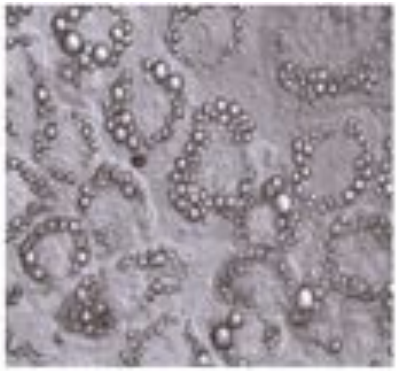
- Swiss albino mouse embryonic fibroblast cell line – committed pre-adipocytes
- Extensively used over decades to evaluate adipogenesis
  - Mechanisms of adipocyte differentiation well understood
  - This assay, particularly coupled with PPARγ reporter gene assays, has proven a reliable *in vitro* model for metabolic disruption *in vivo*.



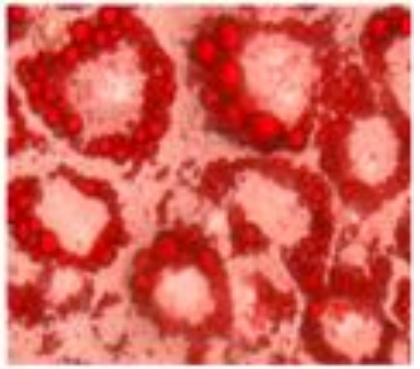
Adipogenesis Assay Measures

- Triglyceride accumulation
  - AdipoRed - hydrophilic fluorescent dye (Nile Red)
    - Partitions into lipid droplets in the cells, fluoresces

(A)

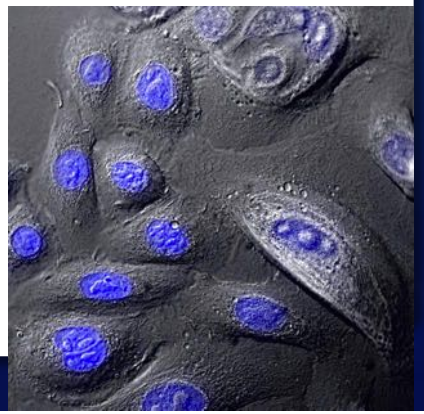


(B)



(C)

- Cell proliferation/cytotoxicity
  - NucBlue DNA dye (Hoechst 33342)
    - Partitions into nuclei and fluoresces upon binding DNA



Cancer in the Environment (CIE) Cohort

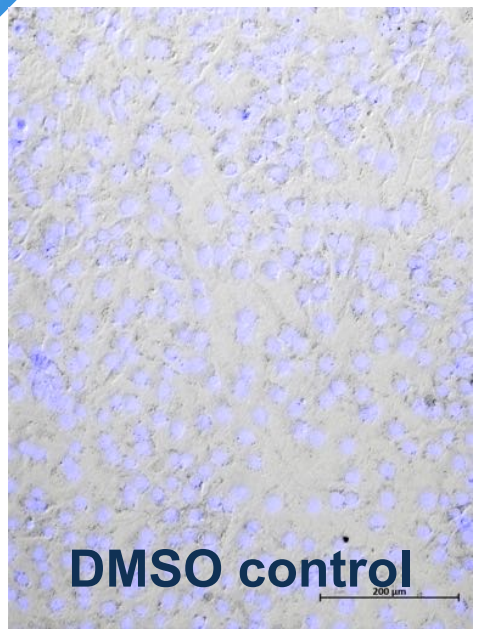
- N=137 adult participants recruited from central NC.
- Demographic, lifestyle, and environment information collected via questionnaire.
- Clinical data abstracted from medical records.
- Visited participants' homes and collected dust samples as a measure of long-term exposure.
  - ~200 mg dust sieved to <500 µm, solvent extracted in 50:50 DCM:hexane, concentrated under N2 gas.
  - Half of extract evaporated and reconstituted in DMSO for bioassays, half purified further for mass spec analysis.

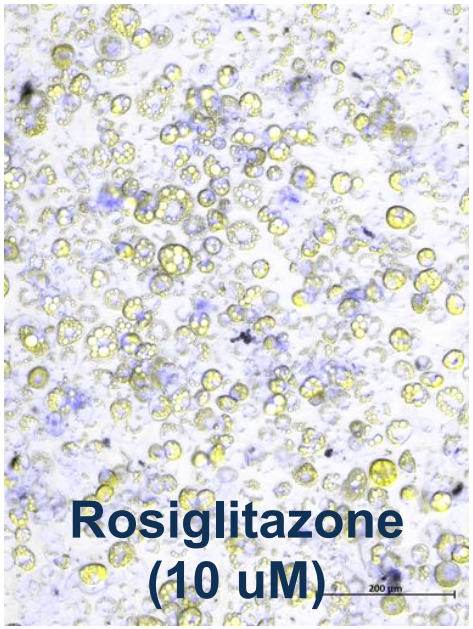
photo credit: Jared Lazarus
Duke Photography

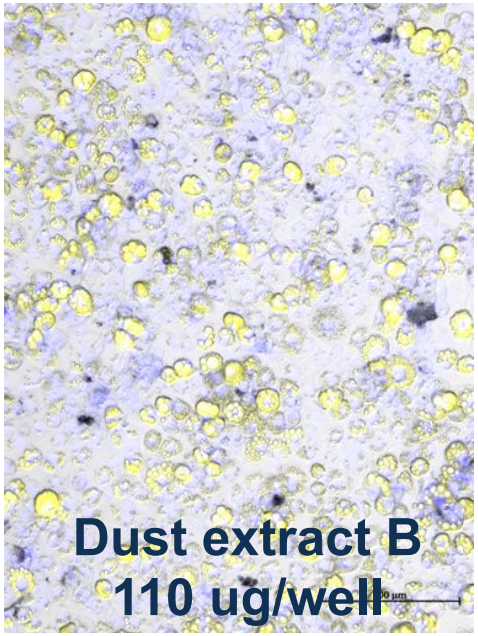
Chemical Exposure Markers: Indoor House Dust

- Household dust is a well-described reservoir for chemicals leaching from consumer products and materials in home.
  - Hundreds of contaminants have been measured in dust globally – a complex environmental mixture
  - Previous research has measured endocrine bioactivities for various receptors by household dust extracts
- Residents chronically exposed to chemicals present in dust via oral, dermal, and inhalation exposure routes.
- Research has demonstrated strong positive correlations between chemicals in dust and internal chemical/metabolite concentrations in serum/urine.

Majority of Dust Extracts Promote Adipocyte Development at Low Concentrations (<1 mg)



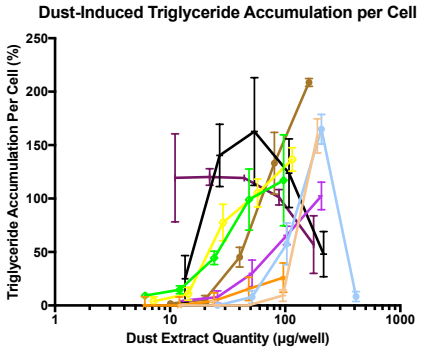


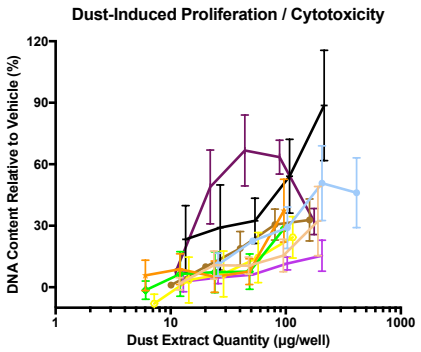


- Majority of dust extracts promoted significant adipogenic activity (~90%).
  - >60% exhibited significant triglyceride accumulation
  - >70% exhibited significant pre-adipocyte proliferation

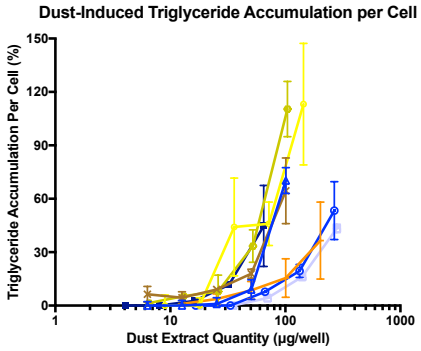
Kassotis et al. 2019, *STOTEN*

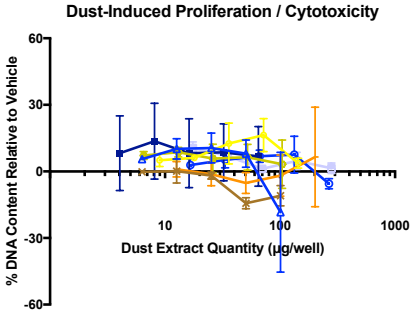
Adipogenesis Endpoints Shared and Distinct Across Dust Extracts



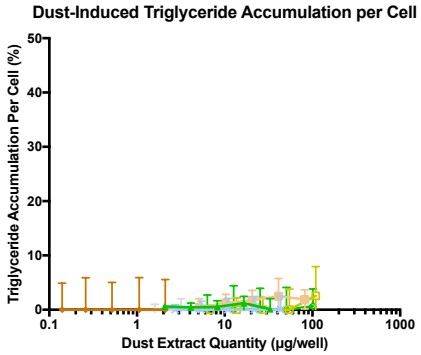


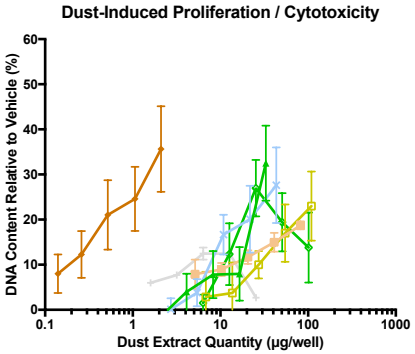
High triglyceride accumulation
High pre-adipocyte proliferation





High triglyceride accumulation
Minimal pre-adipocyte proliferation





Minimal triglyceride accumulation
High pre-adipocyte proliferation

Kassotis et al. 2019, *STOTEN*

BFR and PFR Flame Retardants Associated with Increased Triglyceride Accumulation

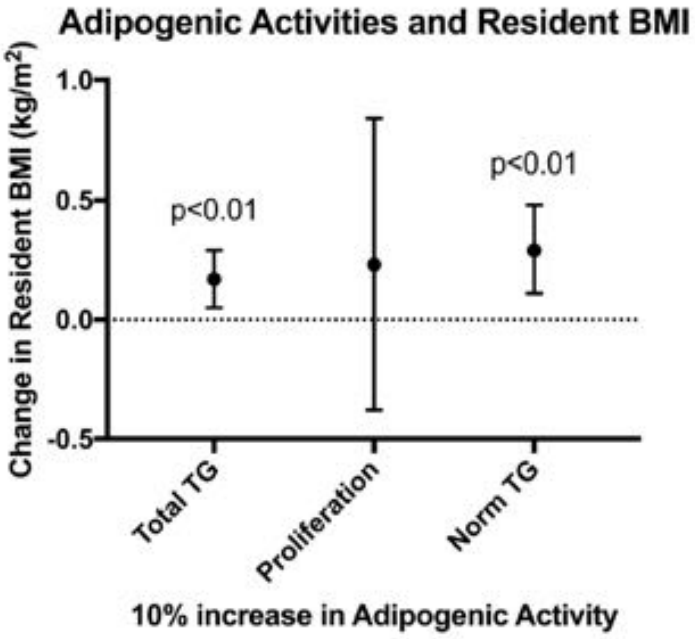
	Correlation Coefficients	
BFRs/PFRs	**Triglyceride Accumulation**	**Pre-adipocyte Proliferation**
BDE-47	**0.244****	*-0.096*
BDE-99	**0.294****	*-0.124*
BDE-100	**0.339****	*-0.043*
BDE-153	**0.385****	*-0.049*
BDE-154	**0.394****	*-0.073*
BDE-209	**0.462****	*0.060*
TBB	**0.324****	*0.006*
TBPH	**0.341****	*0.025*
TCEP	**0.343****	*-0.013*
TDCIPP	**0.397****	*-0.099*
TCIPP	**0.290****	*-0.041*
TPHP	**0.199***	*-0.011*

Spearman's correlations: * $p<0.05$; ** $p<0.01$

Kassotis et al. 2019, *STOTEN*

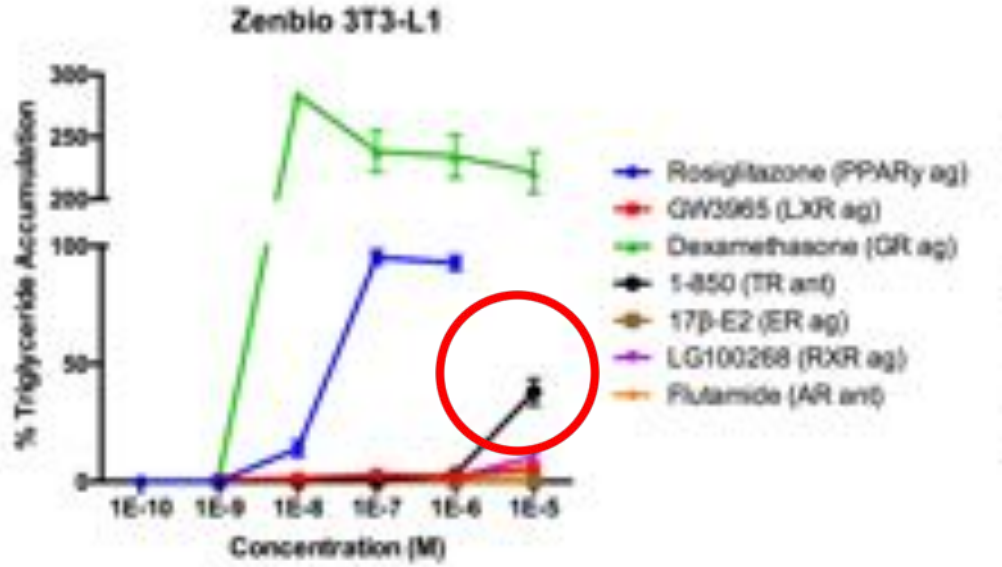
Regression Analyses of Health Outcomes and House Dust Extract Bioactivities

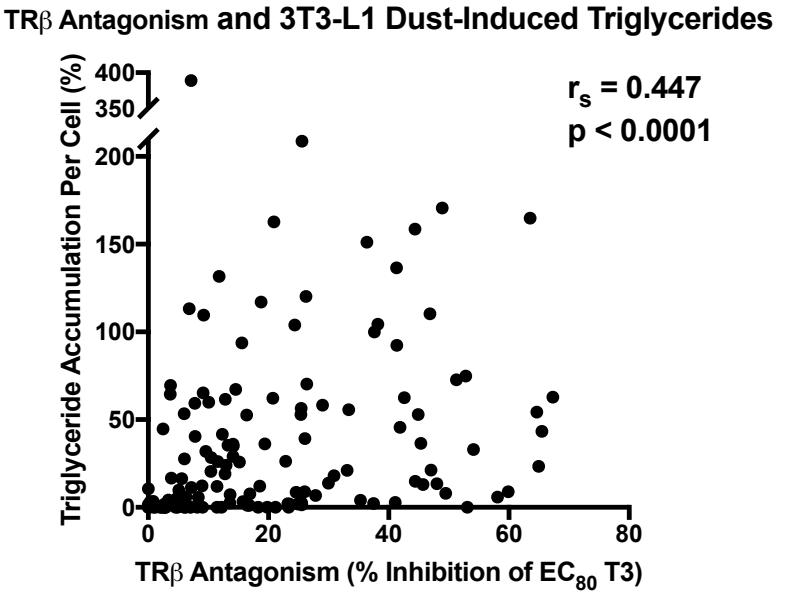
- Thyroid stimulating hormone in adult residents positively correlated with adipogenic activity of their house dust (normalized by concentration); free triiodothyronine (T_3) and thyroxine (T_4) negatively correlated.
  - TRβ antagonism promoting adipogenesis a likely factor in the TH suppression
- Performed regressions controlling for sex, age, race, and education as potential confounders.
  - Triglyceride accumulation efficacy was significantly associated with resident BMI.



Kassotis et al. 2019, *STOTEN*

Putative Role of Thyroid Receptor β Antagonism in Adipogenic Activity





- GR (dexamethasone) and PPARγ (rosiglitazone) are potent and efficacious regulators of adipogenesis.
- 1-850 (non-specific TRβ isoform antagonist) also significantly promotes adipocyte differentiation.

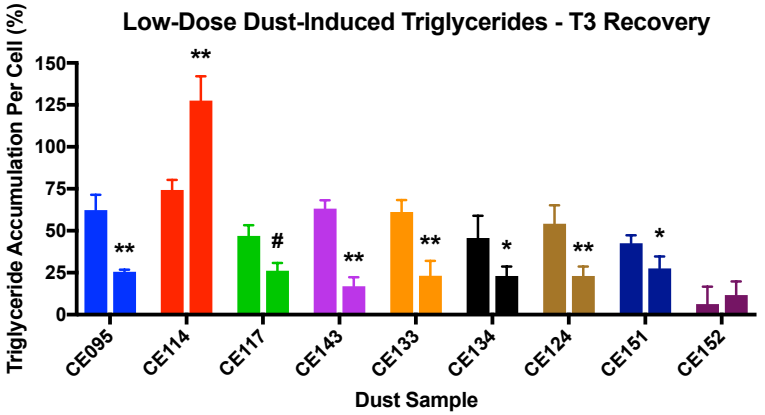
Kassotis et al. 2017, *Sci Rep*

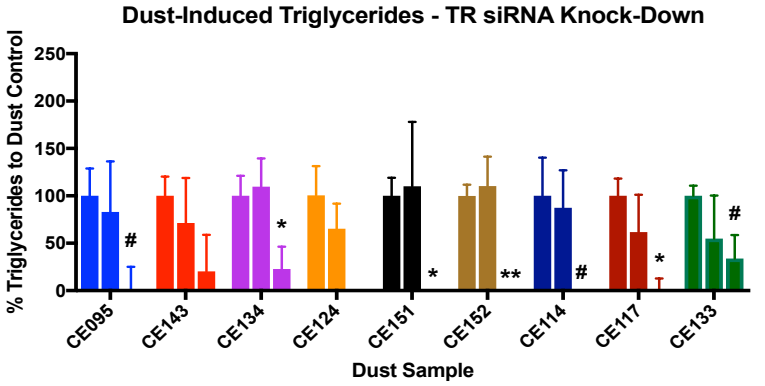
- Triglyceride accumulation (3T3-L1 cells) significantly correlated with TRβ antagonism in dust extracts.
  - Not correlated with pre-adipocyte proliferation

Kassotis et al. 2019, *STOTEN*

Contributory Role of TRβ Antagonism in Adipogenic Activity

- Two experiments bolster causative link between TRβ and triglyceride accumulation in 3T3-L1 cells:
  - Ligand recovery experiment. Dust + T3 (TR agonist):
    - Addition of T3 inhibited dust-induced triglyceride accumulation for 7 of 9 samples.
  - siRNA knock-down of TRα/β:
    - TR knock-down inhibited dust-induced triglyceride accumulation for 7 of 9 samples (two trending).





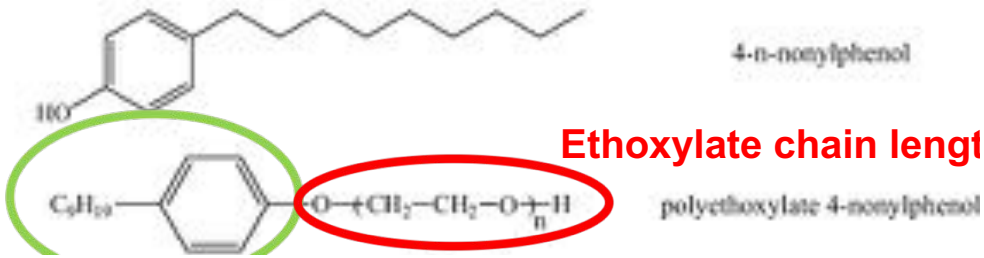
Each grouping: Dust alone, Dust+Negative Control siRNA, Dust+TRα/β siRNA

Kassotis et al. 2019, *STOTEN*

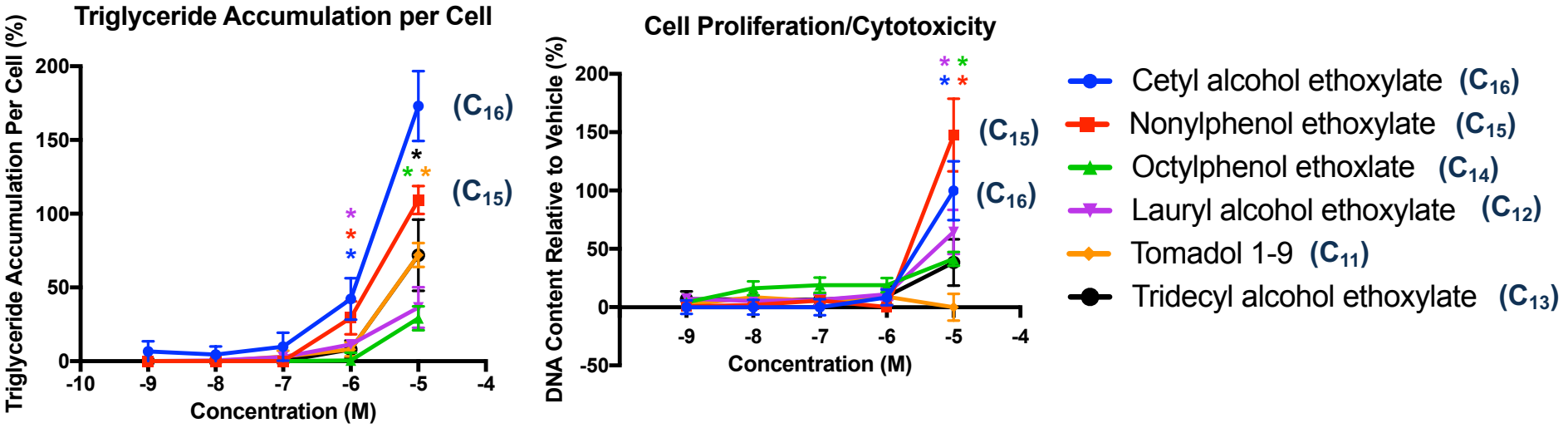
Ethoxylated Surfactants are Common Environmental Contaminants

- High-production volume chemicals
  - >13 million metric tons, 2008
  - >$33 billion global revenues, 2014
- Used widely in laundry detergents, hard-surface cleaners, paints, cosmetics, agriculture.
- Common environmental contaminants
  - Widely reported at µg/L conc. in water column (wastewater)
  - Detected with high frequency in indoor house dust samples
- Tested the ability of various ethoxylated surfactants to promote adipogenesis
  - 6 APEO/AEO surfactants with varying alkyl chain lengths (carbon backbones C11-16)
  - Select NPEOs with varying average ethoxylate chain lengths (2, 4, 6, 10, 20)

Alkyl chain length



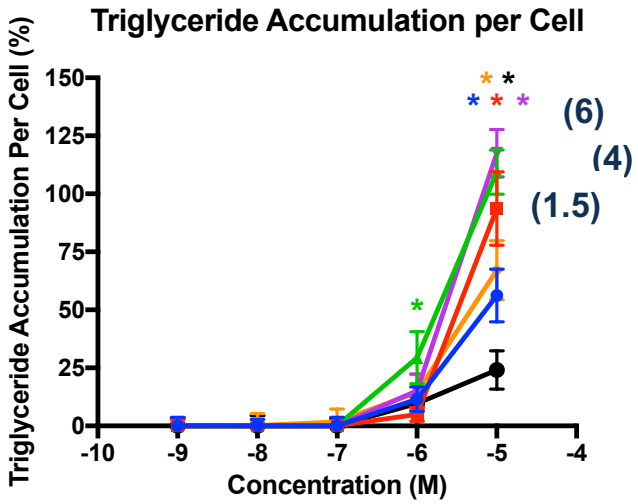
Various Alkyl Chain Length Surfactants Induce Adipogenesis to Varying Degrees

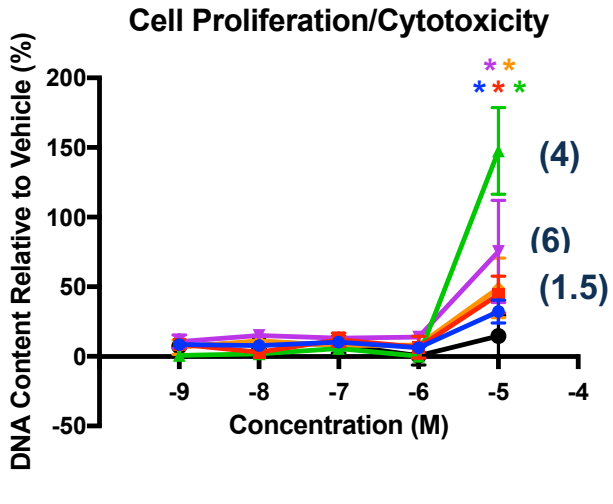


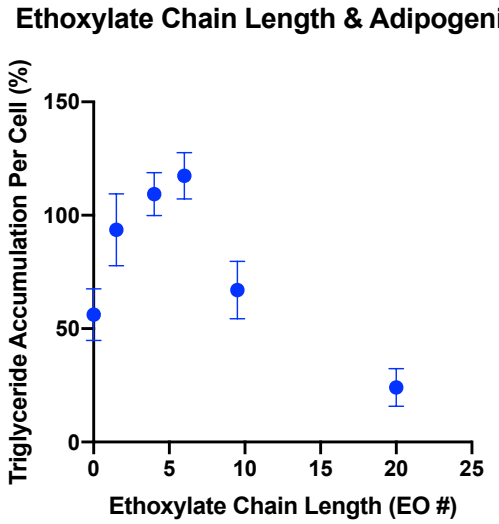
- Six ethoxylated surfactants (alkyl lengths 11-16) all induced triglyceride accumulation in 3T3-L1 cells.
  - Cetyl alcohol and NPEO induced greater maximal accumulation than the rosiglitazone control.
- 4/6 surfactants induced pre-adipocyte proliferation.

Kassotis et al. 2019, *Tox Sci*

Nonylphenol Ethoxylates Induce Chain-Length Dependent Adipogenic Effects







- NPEOs induced varied adipogenic responses.
  - Maximal response for medium-length (4/6) ethoxylate chains; decreasing activity with decreasing or increasing chain number.
    - Activity for NPEO(20) indistinguishable from base (0).

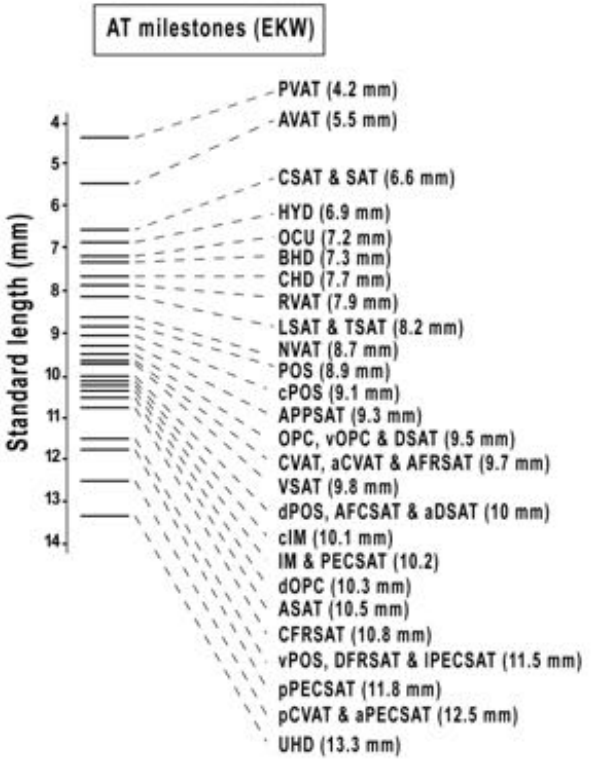
Kassotis et al. 2019, *Tox Sci*

Next Steps: K99/Roo Research Aims

- Utilization of the zebrafish model to assess whether select polyethoxylated surfactants (alcohol and alkylphenol) induce metabolic health effects following developmental exposure.
  - Weight gain (gross), adipose depot development (adipocyte staining and depot-specific quantification)
- Identification of molecular mechanisms driving the adipogenic effects of polyethoxylated surfactants across species.
  - Human and zebrafish *in vitro* models, cell-based and cell-free
- Utilize affinity-directed analysis and HRMS to identify causative adipogenic ligands in environmental samples.
  - Confirmation in pre-adipocyte models; role of APEOs/AEOs

The Zebrafish Model (*Danio rerio*) for Metabolic Health Research

- High genetic fidelity to humans – endocrine system is highly conserved, as is metabolic system
  - 84% of genes known to be associated with human disease have zebrafish counterpart
- Molecular mechanisms underlying adipocyte and lipid depot development are highly conserved
  - Energy storage functions and morphology of adipose tissue
  - Genes associated with adipocyte differentiation, lipolysis, and endocrine function
  - Control of adipose distribution into anatomically/ physiologically/molecularly distinct depots
- Fish adipose tissue also contains a heterogeneous cell population, including adipocyte progenitor cells – similar to mammals
- Imaging of whole-animal adipose imaging in mammals is limited, technically challenging, and generally low resolution



Minchin and Rawls 2017, *Disease Mod Mech*

Summary: Environmental Contaminants as Metabolic Disruptors

- Numerous common environmental contaminants and complex environmental mixtures can disrupt metabolic health *in vitro* at environmentally-relevant concentrations.
  - Evidence that some environmental mixtures might promote adipogenesis through mechanisms other than PPARγ
- In many mixtures, the causative chemicals promoting the activity have yet to be determined.
  - Need for new analytical tools to isolate and identify
  - Need for better application of molecular databases to ease translation of *in vitro* data to potential *in vivo* health effects
- Seems to be an association between the adipogenic activity exhibited by house dust and the metabolic health of residents living in those homes.
  - This is not necessarily causative; could be a measure of altered behavior in individuals who are already overweight, contributing to different chemical burdens in the indoor environment

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NIH National Institute of Environmental Health Sciences

I'm Recruiting!

- Incoming Assistant Professor in Institute of Environmental Health Sciences and Department of Pharmacology at Wayne State University in Detroit, starting September 1!
  - Recruiting grad students, technician(s), postdoc, etc. Email me for more information!