Chemicals in House Dust: Potential Contributors to Metabolic Disorders

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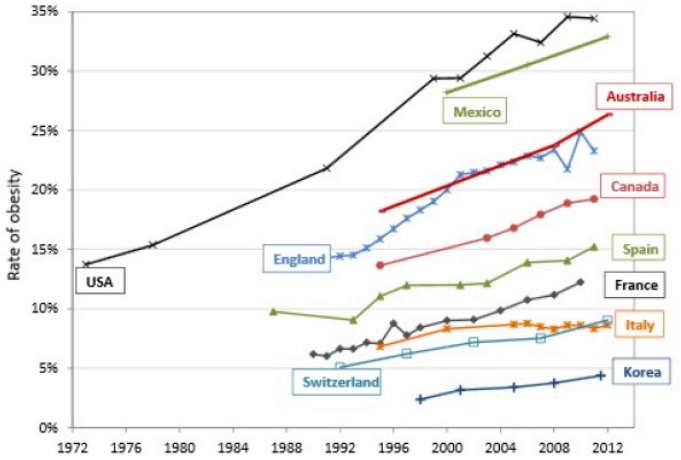
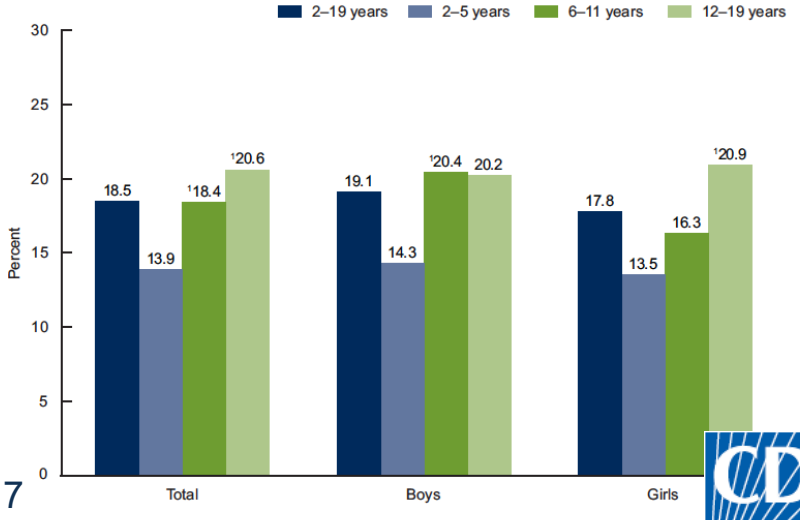
Outline

- Metabolic Syndrome & Molecular Mechanisms
  - Metabolic disruptors, adipogenesis
- Diverse Indoor SVOCs as Metabolic Disruptors
- Indoor House Dust Extracts and Metabolic Disruption

Prevalence of Obesity Epidemic in US, Globally

- Currently ~40% of US adult population is obese.
  - ~9% infants/toddlers
  - ~19% of 2-19 year-olds
- >$215B in US health care costs (2010)
- Increased comorbidities
  - T2D, CVD, hypertension
- Interventions have produced only modest effects

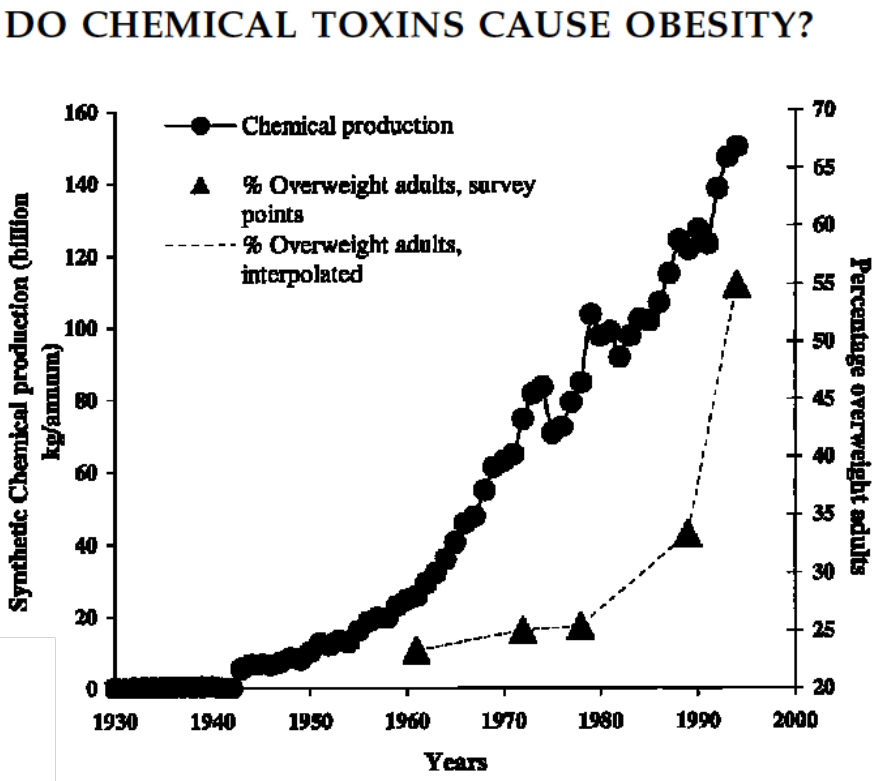




CDC Data Brief, Oct 2017

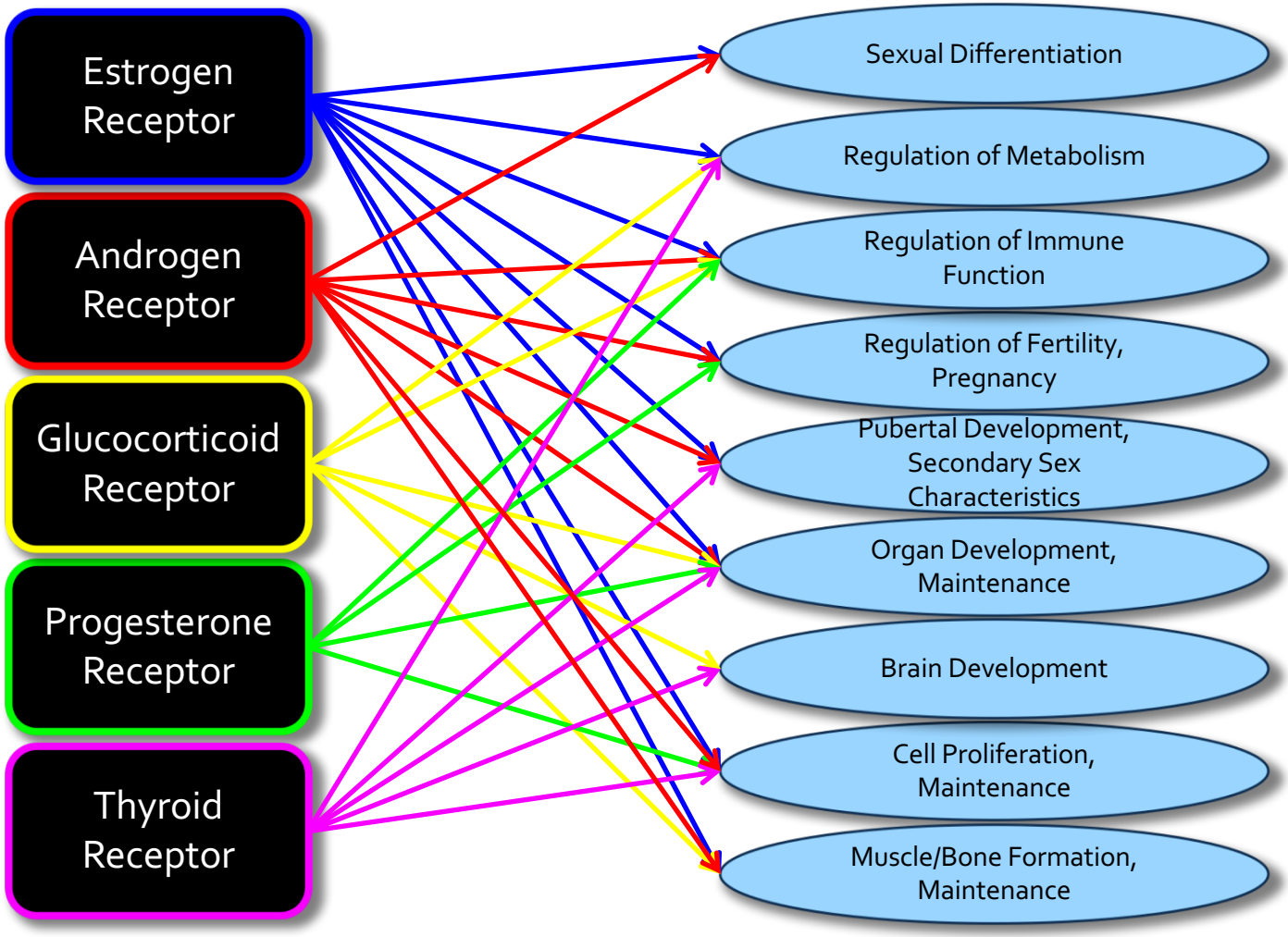
Potential Role of Chemicals in Increasing Obesity Rates in Humans

- 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*

Normal Hormonal Function



Adipocyte Differentiation Process

Adipocyte commitment

Adipocyte differentiation

Mesenchymal stem cell

Other pathways:
Myoblasts
Osteoblasts
Chondroblasts

Adipoblast

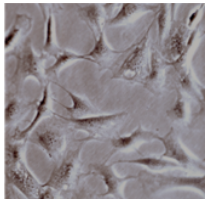
Pref-1

Preadipose cell

A2COL6/pOb24
LPLF A transport
PPARδ

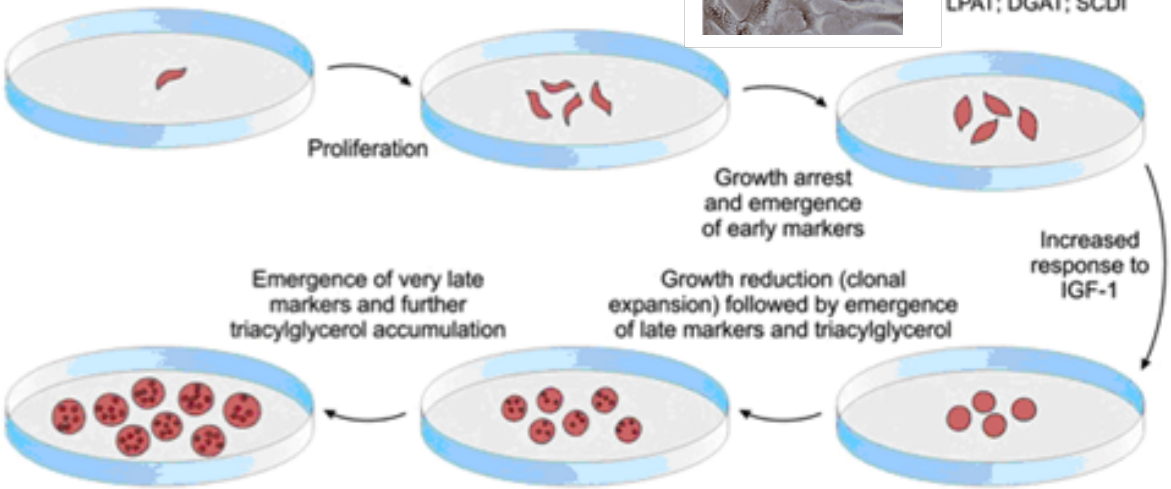
Preadipose cell

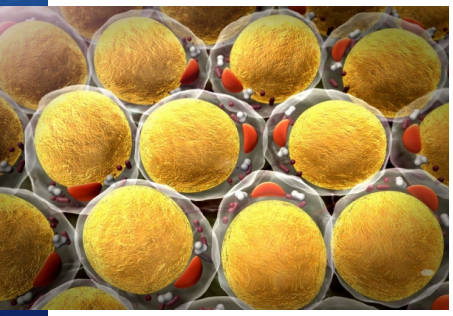
C/EBP β/δ
IGF-1
PRAR γ2



Immature adipose cell

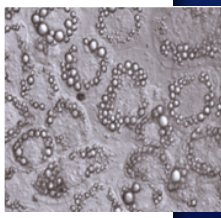
C/EBP γ; GLUT4; β2 AR; β3 AR; ACC FAS; ME; ATP-citrate lyase; GPDH; HSL; ALBP; perilipin; apoE; low Km PDE; GPAT; LPAT; DGAT; SCDI





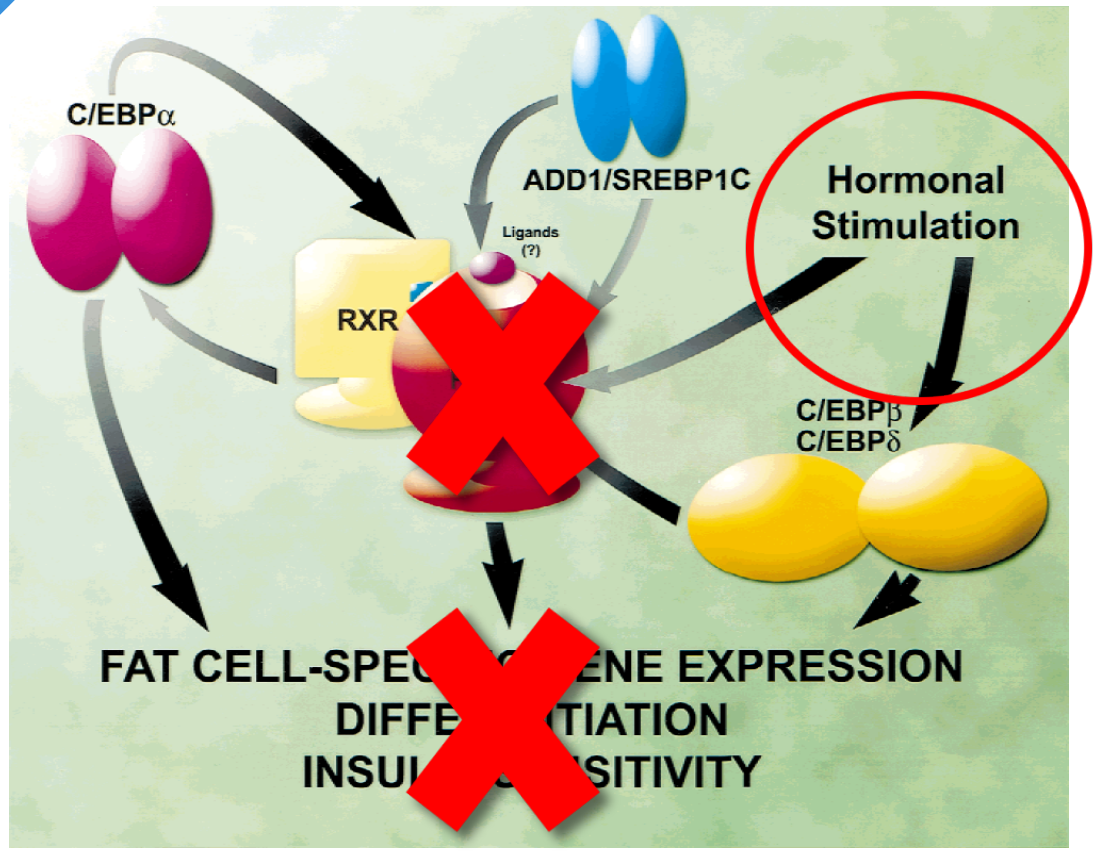
Resemble mature white adipose cell

Resemble brown/developing white adipose cell

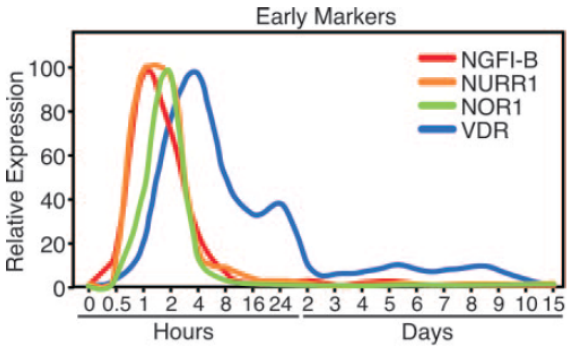


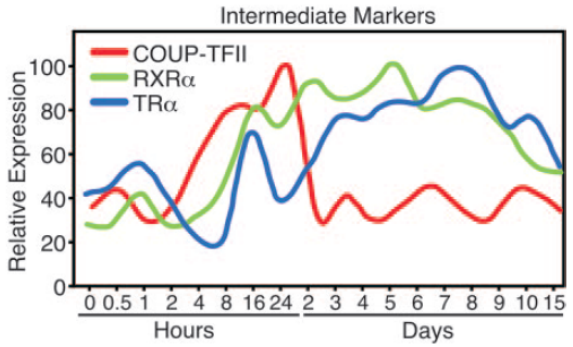
Nagy et al. 2011, *Mol Med*

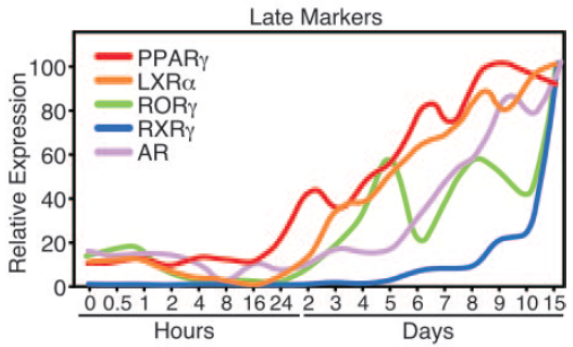
PPARγ-Dependence of Adipocyte Differentiation

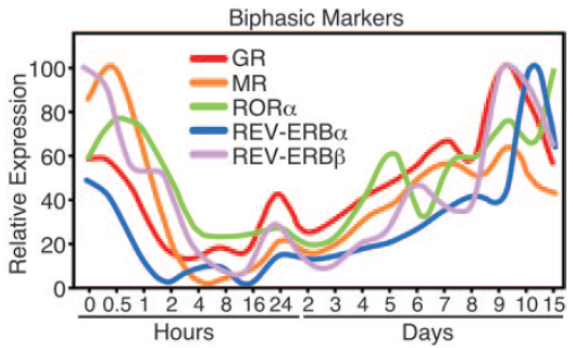


Rosen et al. 2000, *Genes & Development*



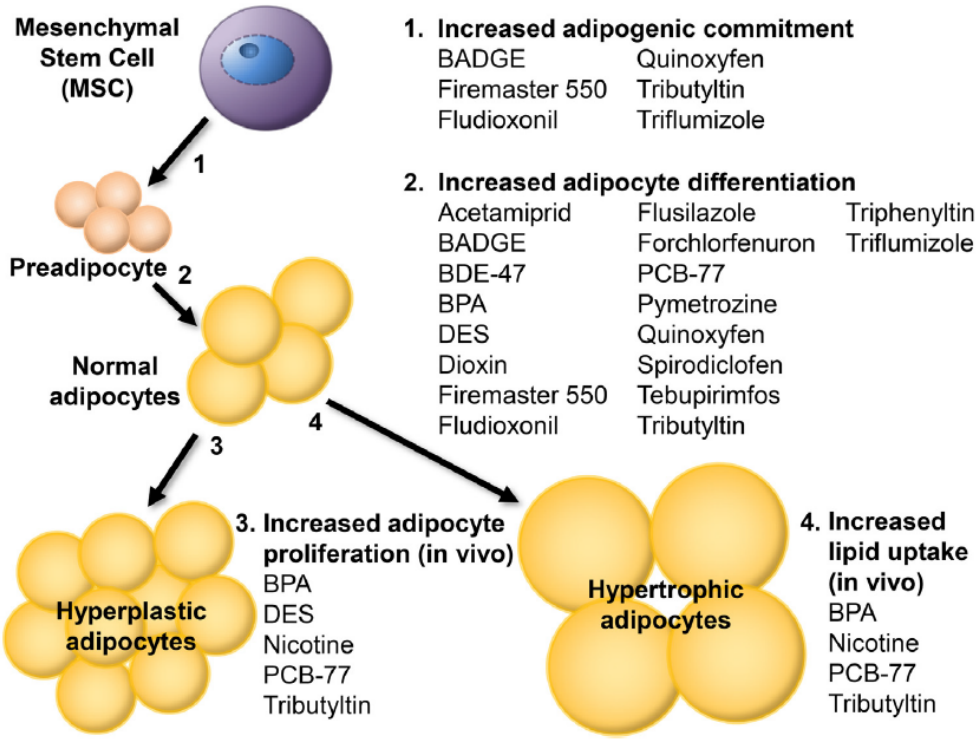






Fu et al. 2005, *Mol Endo*

Potential Mechanisms of Metabolic Dysfunction

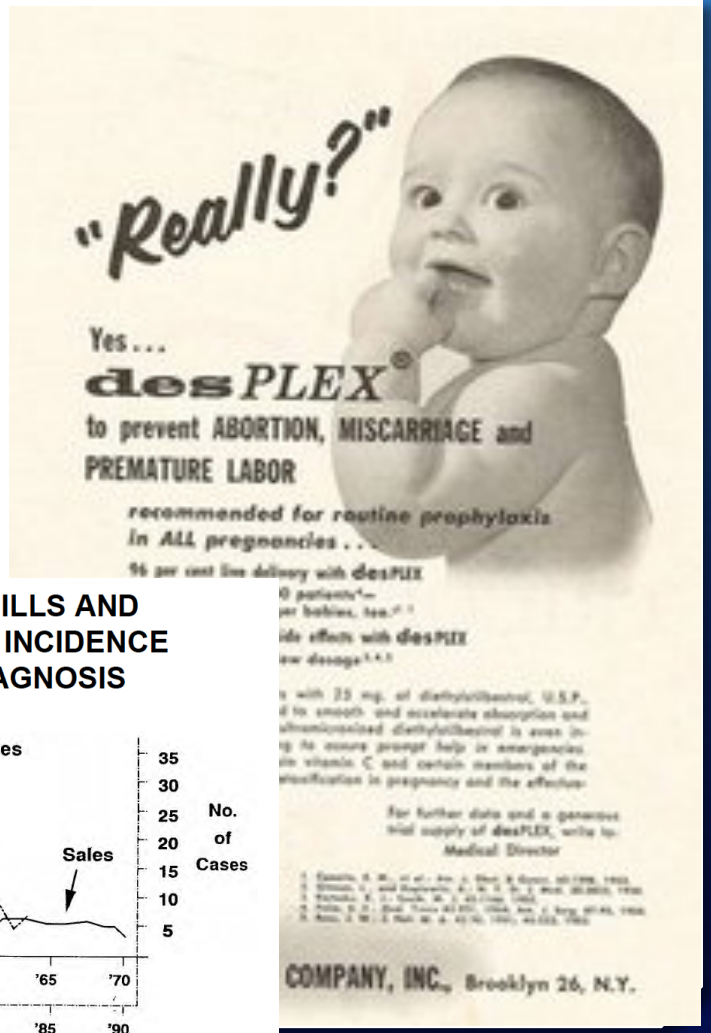


Heindel et al. 2017, *Repro Tox*

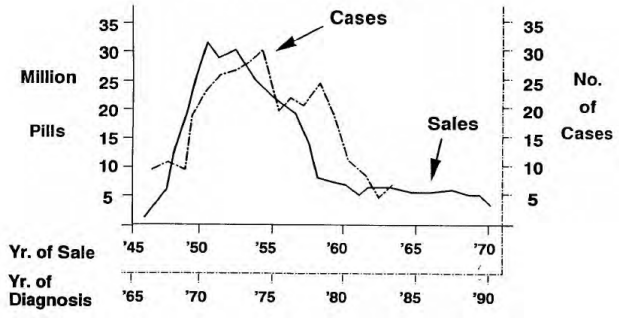
- Numerous potential mechanisms of metabolic disruption:
  - Adipocyte commitment from MSCs
  - Adipocyte differentiation from precursor cells
    - Increased pre-adipocyte proliferation
    - Increased lipid uptake
  - Shifting energy balance to favor calorie storage
  - Altering basal metabolic rate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

Diethylstilbestrol and Developmental Exposure

- Synthesized 1938
- Pregnant women, 1940's-1971
- Growth hormone (livestock), 60's-70's
- Adverse health outcomes (children):
  - Vaginal clear cell adenocarcinoma, reproductive tract malformations, infertility, testicular cancer

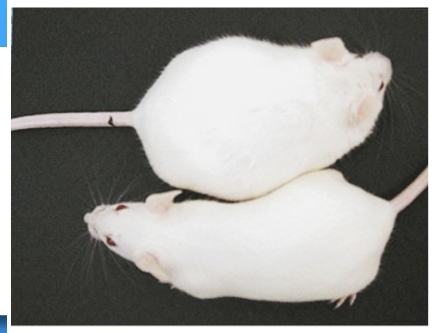


SALES OF DES PILLS AND VAGINAL CANCER INCIDENCE BY YEAR OF DIAGNOSIS

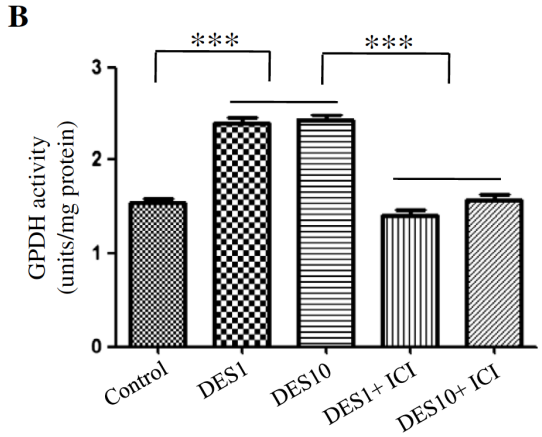


Source: Melnick et al, 1987 and Market Share Litigation Exhibit

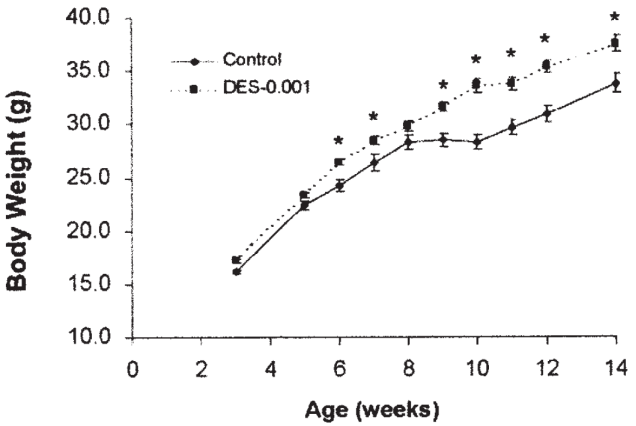
DES and Metabolic Disruption



- DES promotes triglyceride accumulation in *in vitro* models.
  - Appears to occur through an estrogenic mechanism
  - DES induces adipogenic regulators and markers *in vitro*.
- Gestational & perinatal DES exposure in mice increases body weight throughout life.
  - Increased body fat, altered serum profiles
- Some evidence for increased risk of obesity in adults exposed prenatally (Hatch et al., 2015, others).



Hao et al. 2012, *Tox App Pharm*

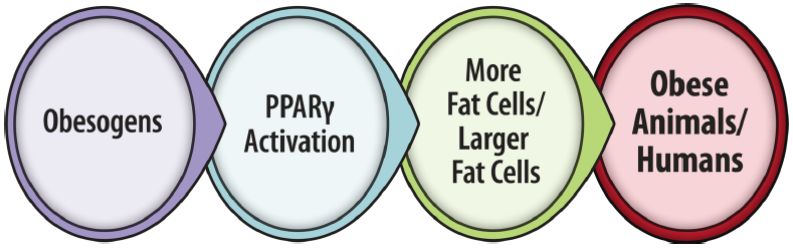


Newbold et al. 2007, *Repro Tox*

Indoor SVOCs as Metabolic Disruptors

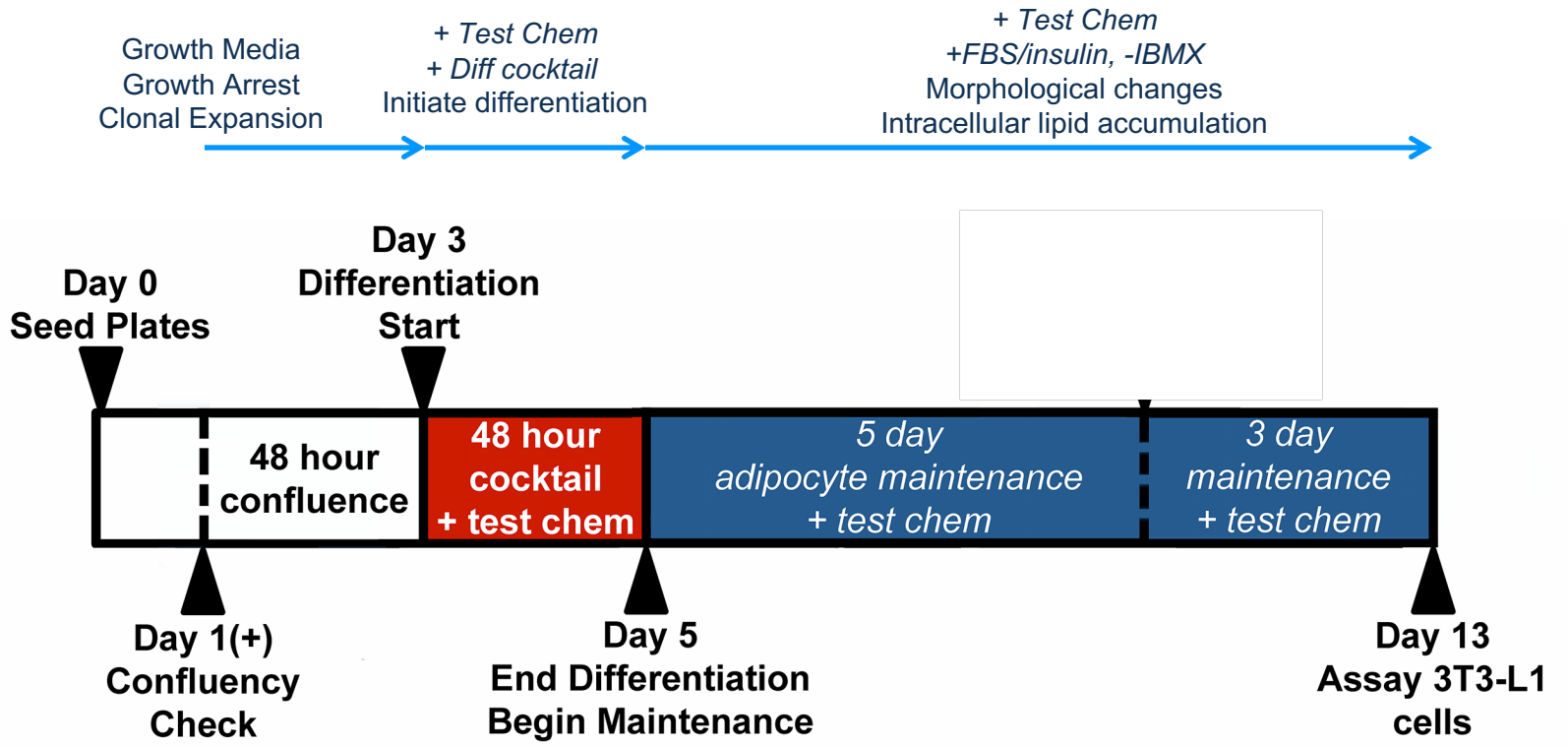
3T3-L1 Pre-adipocyte Adipogenesis Assay

- 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 reliable for predicting metabolic disruption *in vivo*.
- Limitations:
  - Length of differentiation window: ~2 weeks
  - Depends on contact inhibition to arrest cell cycle and differentiate
  - Fails to incorporate other mechanisms of metabolic disruption



Heindel and Schug 2014, *Curr Envir Health Rpt*

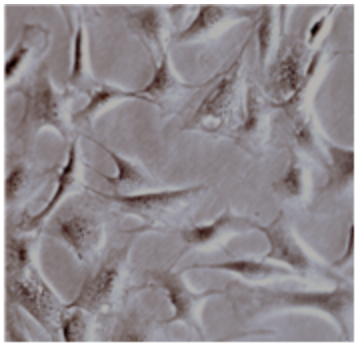
Adipogenesis Induction Timeline



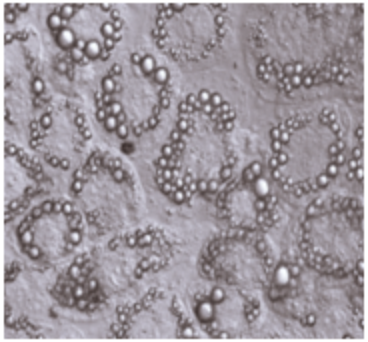
Differentiation cocktail:
5% NCS -> FBS,
1 µg/mL insulin,
800 mM IBMX

Adipogenesis Assay Measures

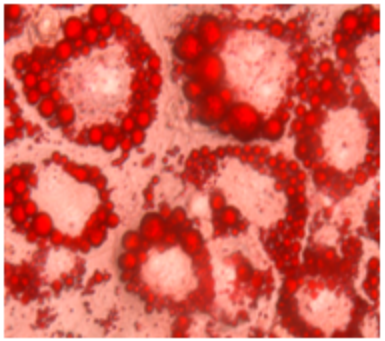
- Triglyceride (lipid) accumulation
  - AdipoRed - hydrophilic fluorescent dye (Nile Red)
    - Partitions into lipid droplets in the cells, fluoresces



(A)

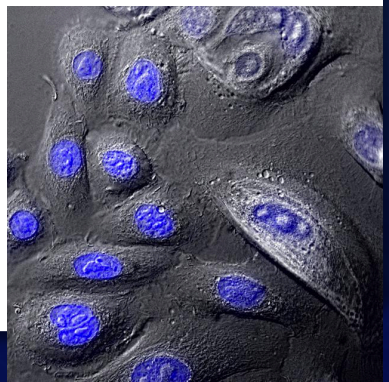


(B)

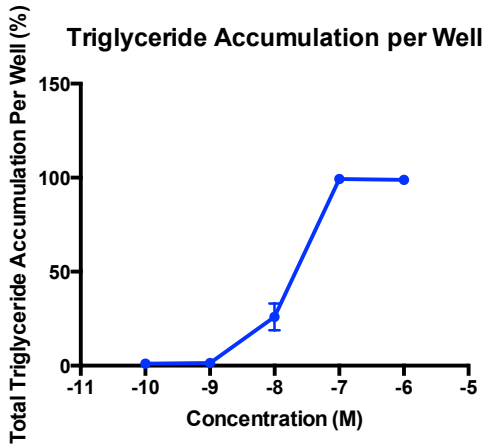


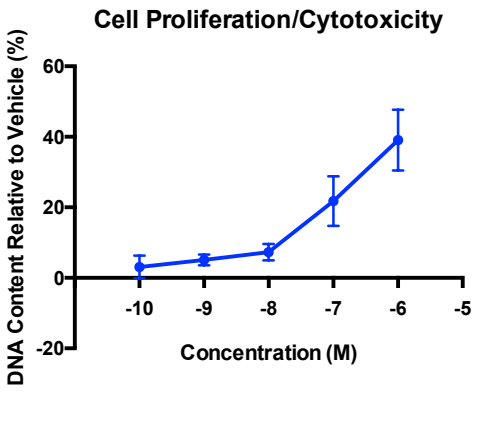
(C)

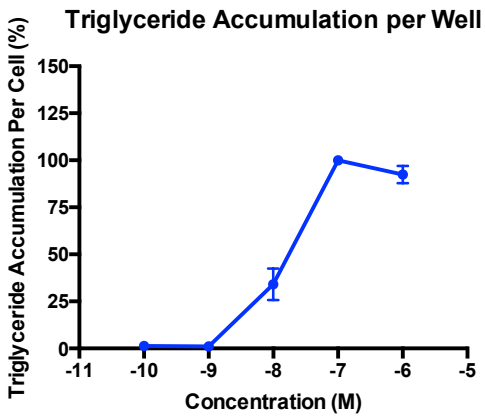
- Cell proliferation/cytotoxicity
  - NucBlue DNA dye (Hoechst 33342)
    - Partitions into nuclei and fluoresces upon binding DNA



Rosiglitazone Control Adipogenesis Assay Results



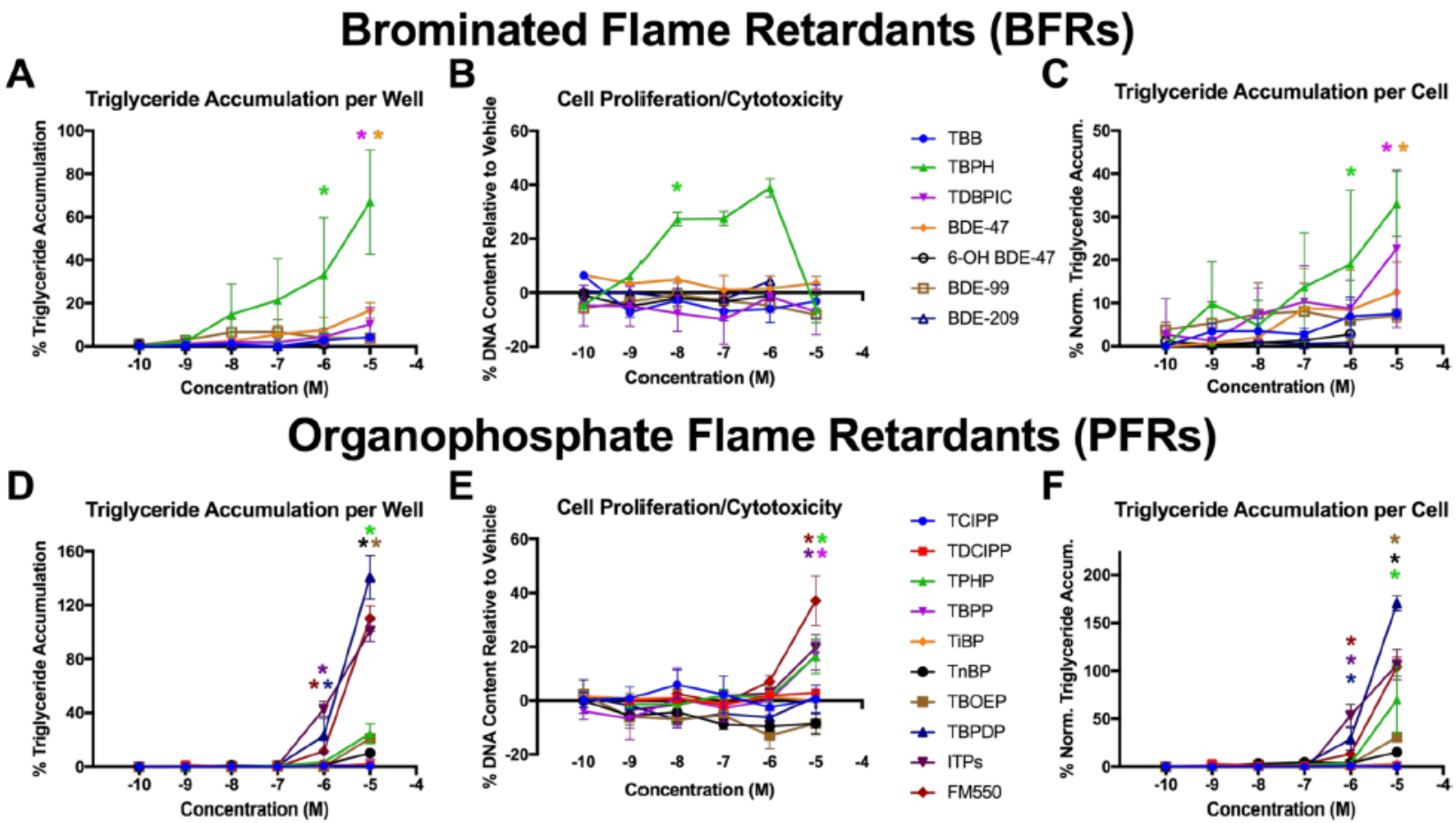




- Triglyceride accumulation per well – total response, normalized to maximal rosiglitazone-induced response.
  - Measure of whether chemical stimulates incorporation of lipids (fat cell size)
- Cell proliferation – DNA content relative to differentiated vehicle control (0.1% DMSO).
  - Measure of whether chemical stimulates increased number of fat cells
- Triglyceride accumulation per cell – response normalized to DNA content, normalized to maximal rosiglitazone-induced response.

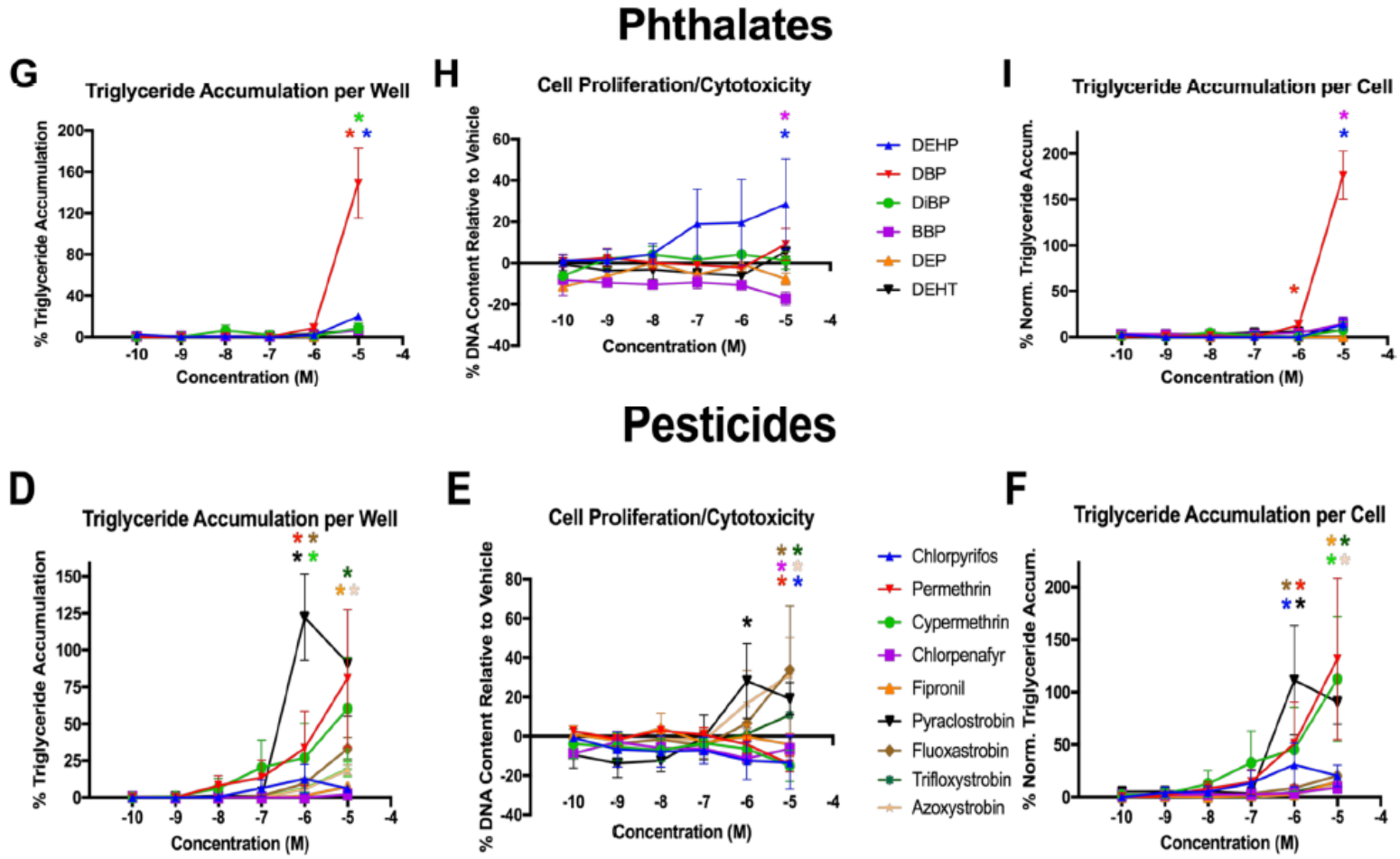
Kassotis et al. 2017, *ES&T*

Brominated and Organophosphate FRs Promote Substantial Fat Cell Development



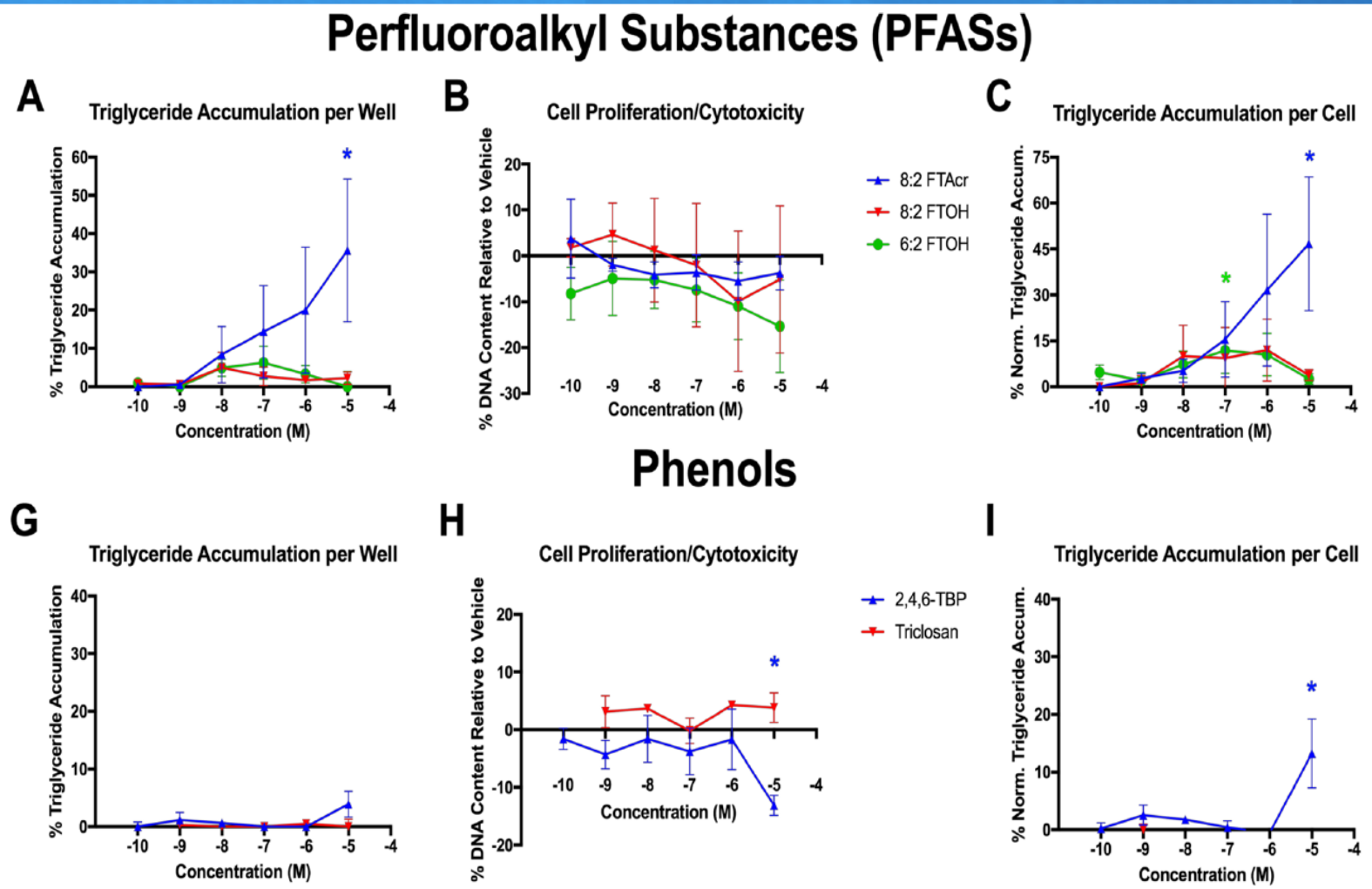
Kassotis et al. 2017, *ES&T*

Several Phthalates and Pesticides Promote Significant Fat Cell Development

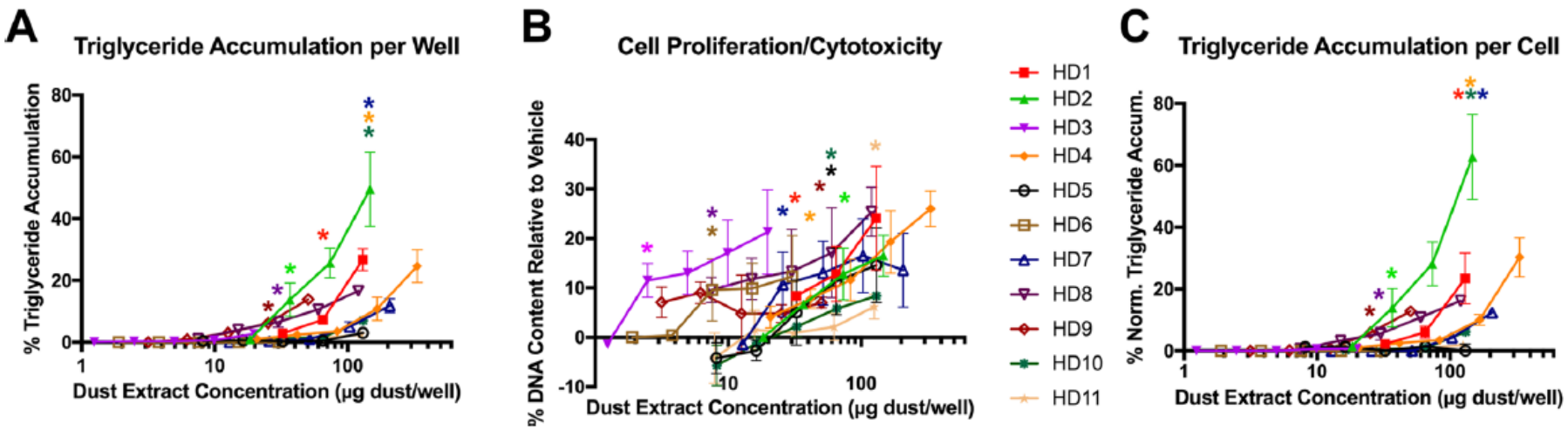


Kassotis et al. 2017, *ES&T*

Perfluorinated Compounds and Parabens Induce Limited Fat Cell Development



Indoor House Dust Extracts and Metabolic Disruption



- Seven of 11 extracts exhibited significant triglyceride accumulation.
- Nine of 11 extracts exhibited significant pre-adipocyte proliferation.
- One of 11 extracts exhibited no significant adipogenic activity.

Kassotis et al. 2017, *ES&T*

Summary of Findings

- >2/3 of the semi-volatile indoor contaminants were active in promoting triglyceride accumulation and/or pre-adipocyte proliferation.
  - PFRs exhibit much greater activity than the majority of the BFRs.
    - BFRs are generally weakly adipogenic, though novel BFRs (TBPH, TBBPA, TDBPIC) are more active than legacy PBDEs.
  - Pyraclostrobin, TBPDP, and DBP were the most active chemicals tested.
    - Several appear to exhibit effects through mechanisms other than PPARγ
  - PFASs, phenols, parabens exhibited minimal activity overall.
    - Previous work found moderate activity for bisphenols (TBBPA, BPAF, etc.)
- Many house dust extracts exhibited significant triglyceride accumulation and pre-adipocyte proliferation at < 10 µg.
  - Children estimated to consume ~50 mg of dust each day (EPA)

Future Directions, Next Steps

- Assessing activity of larger numbers of house dust samples.
  - CIE cohort (n=~150), TESIE cohort (n=~200)
- Assessing mechanisms through which house dust promotes fat cell development.
  - Causative constituent chemicals
  - Causative molecular pathways
- Assessing relationships between house dust driven fat cell development and metabolic health outcomes in residents.

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NIH National Institute of Environmental Health Sciences