U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program
Office of Chemistry and Toxicology

Assessment of the effects of cannabidiol (CBD) and a CBD-rich hemp extract in *Caenorhabditis elegans*

Piper Reid Hunt, PhD

FDA/HFP/OLOAS/OCT/DT

C. elegans

1.5-fold

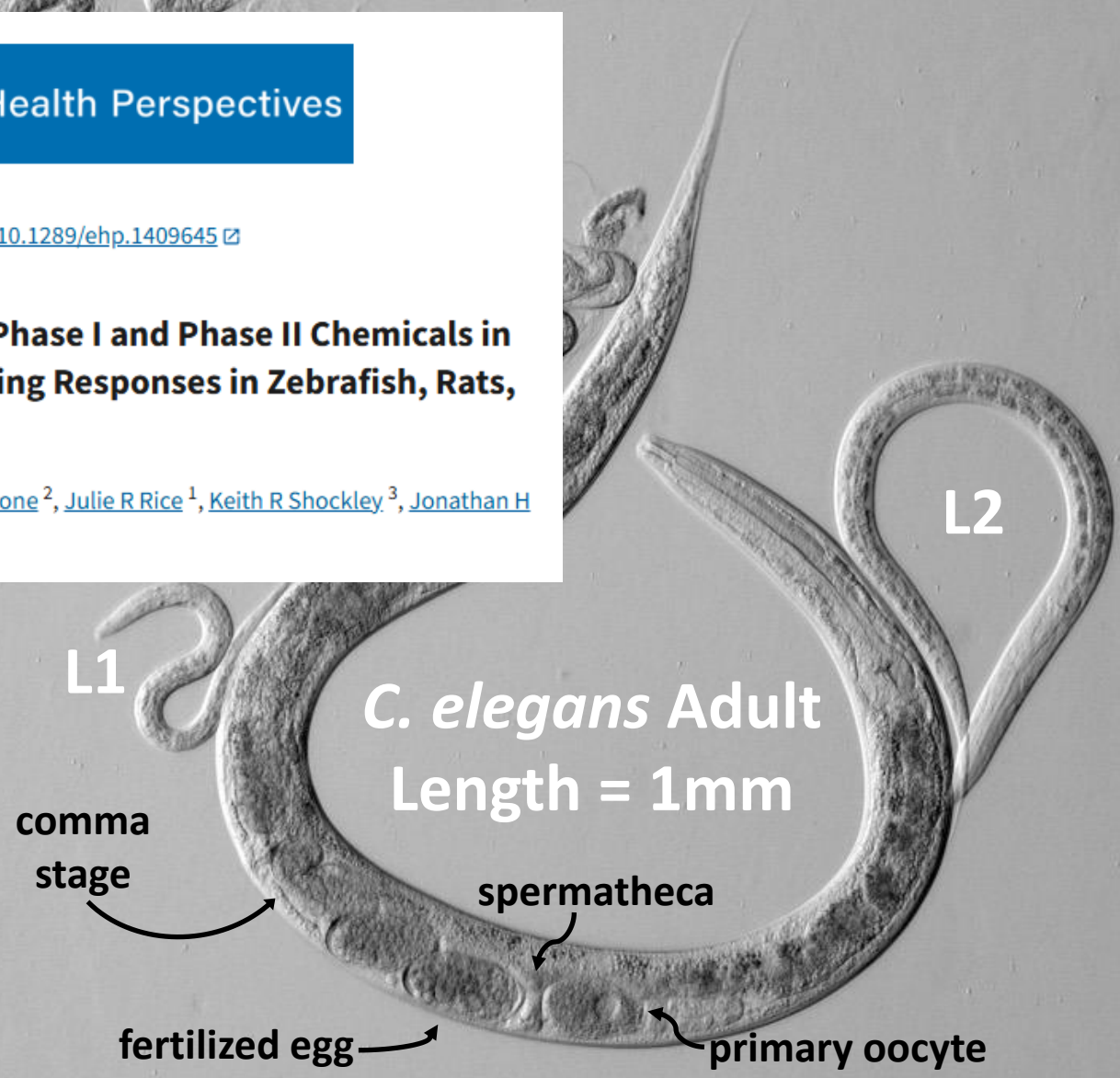
ehp Environmental Health Perspectives

Environ Health Perspect. 2015 Oct 23;124(5):586–593. doi: 10.1289/ehp.1409645

Developmental Effects of the ToxCast™ Phase I and Phase II Chemicals in *Caenorhabditis elegans* and Corresponding Responses in Zebrafish, Rats, and Rabbits

Windy A Boyd [1], Marjolein V Smith [2], Caroll A Co [2], Jason R Pirone [2], Julie R Rice [1], Keith R Shockley [3], Jonathan H Freedman [1,4,*]

For over 200 chemicals in the Phase I ToxCast™ chemical library with rat and rabbit data, *C. elegans* growth predicted rat and rabbit developmental effects nearly as well as these two mammalian species predicted each other.



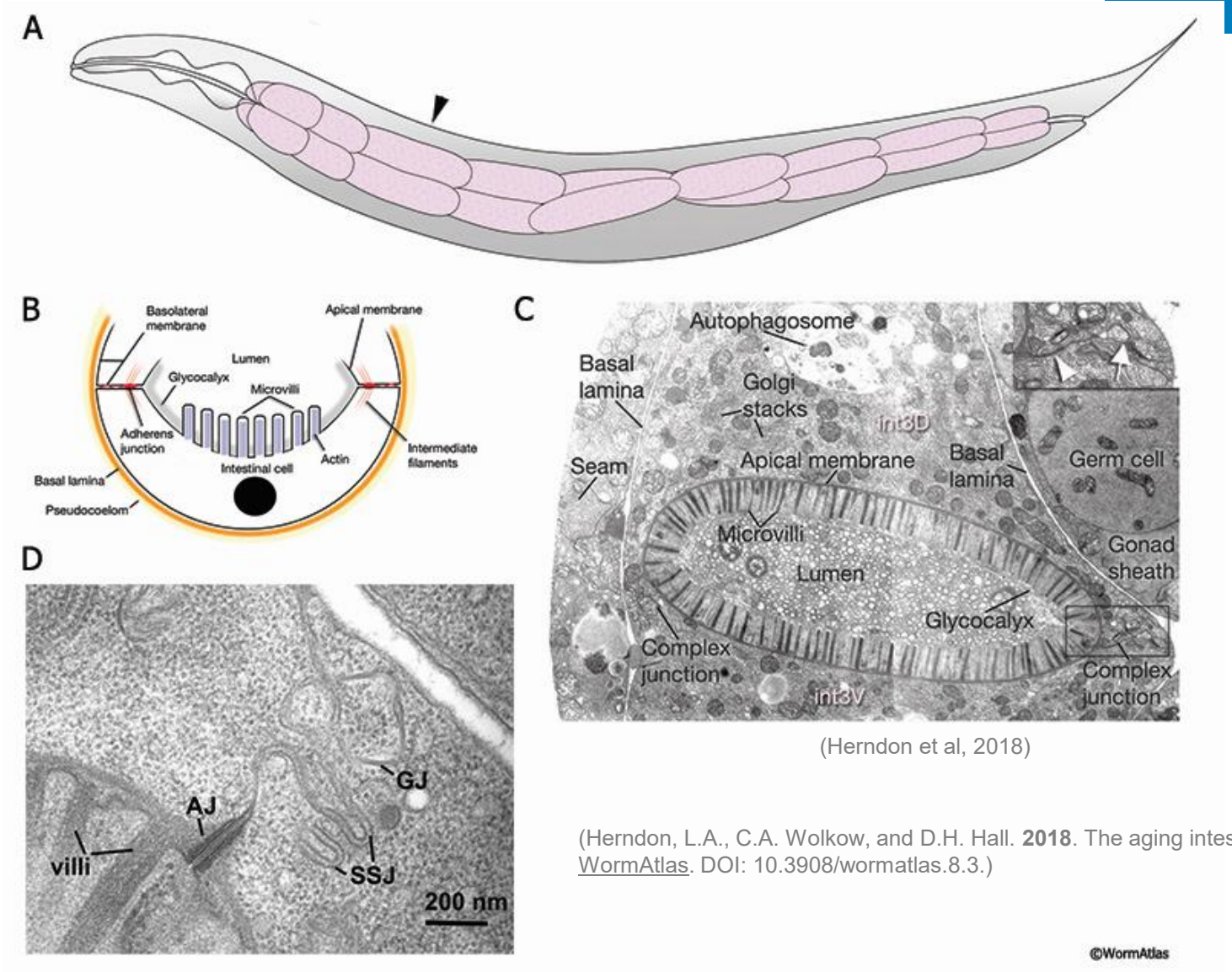
Microscopic
Nonpathogenic
3-week lifespan
3-day life cycle

Conserved Developmental Signal Transduction Pathways

- Apoptosis Pathway
- Bone Morphogenetic Protein
- Folate signaling
- Gap Junction Channels
- HOX gene regulation
- IGF1 pathway
- Integrin pathway
- Notch-Delta Pathway
- TGF-β signaling
- Wnt pathway via β-catenin

C. elegans is an *in vivo* oral toxicity test model

- microvilli
- peristalsis
- acidic and more neutral regions of the digestive tract
- conserved nutrient transport & endocytosis machinery
- conserved digestive & metabolic enzymes



(Herndon et al, 2018)

(Herndon, L.A., C.A. Wolkow, and D.H. Hall. **2018**. The aging intestine. WormAtlas. DOI: 10.3908/wormatlas.8.3.)

Some *C. elegans* Model Strengths & Limitations

- **Short lifespan = rapid testing**
  - ✓ acute vs. chronic, different life stages
- **Oral toxicity model**
  - ✓ lipid soluble phytochemicals can be emulsified and consumed
  - ✓ vs. zebrafish embryos = an absorption model
- **Genetic Conservation**
  - ✓ Pathways controlling morphogenesis, organismal development, and neuromuscular function are highly conserved
- **Active metabolism, digestion, endocrine signaling, sensory-motor responses**

- **Simple body plan & biology**
  - ✓ *C. elegans* lack eyes, a skeleton, a heart, and a liver (present in zebrafish)
  - ✓ No placenta or maternal transfer after fertilization, no adaptive immune system (also true of zebrafish embryos)
- **Chemical domain limited by solubility, volatility, adsorption to plastic, precipitation**
- **~70% of *C. elegans* genes have human homologs = ~30% do not**

Conservation of Endocannabinoid Signaling

- **Many aspects of endocannabinoid signaling are conserved from nematodes to humans**
  - ✓ 2-arachidonoylglycerol (2-AG) and anandamide (AEA) produced in all animals
  - ✓ 2-AG and AEA have indirect effects on monoaminergic neurotransmitter signaling in rodents and *C. elegans*
  - ✓ In *C. elegans* and mammals, AEA has analogous effects on appetitive and consummatory feeding behaviors
  - ✓ Transgenic human CB1 receptor gene *CNR1* can restore behavioral responses in *C. elegans npr-19* mutants
  - ✓ *C. elegans* encode members of each of the seven transient receptor potential ionotropic cannabinoid receptor subfamilies
  - ✓ ↑ endocannabinoids → ↑ latency to antinociceptive behaviors in mice and *C. elegans*

Conservation of Endocannabinoid Signaling

- **Many aspects of endocannabinoid sign nematodes to humans**
  - ✓ 2-arachidonoylglycerol (2-AG) and a animals
  - ✓ 2-AG and AEA have indirect effects c signaling in rodents and *C. elegans*
  - ✓ In *C. elegans* and mammals, AEA ha consummatory feeding behaviors
  - ✓ Transgenic human CB1 receptor ger responses in *C. elegans npr-19* muta
  - ✓ *C. elegans* encode members of each potential ionotropic cannabinoid rece
  - ✓ ↑ endocannabinoids → ↑ latency to a *C. elegans*

Drugs give biology's favourite worms the munchies too

Experiments with *C. elegans* suggest that the mechanism by which cannabis affects appetite evolved 500 million years ago.

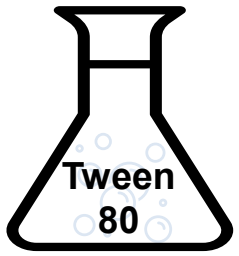
By Elissa Welle

Levichev, A., S. Faumont, R.Z. Berner, Z. Purcell, A.M. White, K. Chicas-Cruz, and S.R. Lockery. **2023**. The conserved endocannabinoid anandamide modulates olfactory sensitivity to induce hedonic feeding in *C. elegans*. Curr Biol. DOI: 10.1016/j.cub.2023.03.013.

CBD Emulsions: sesame oil in milk

emulsifier

+

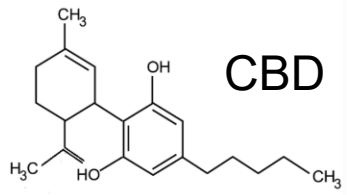


surfactant

+

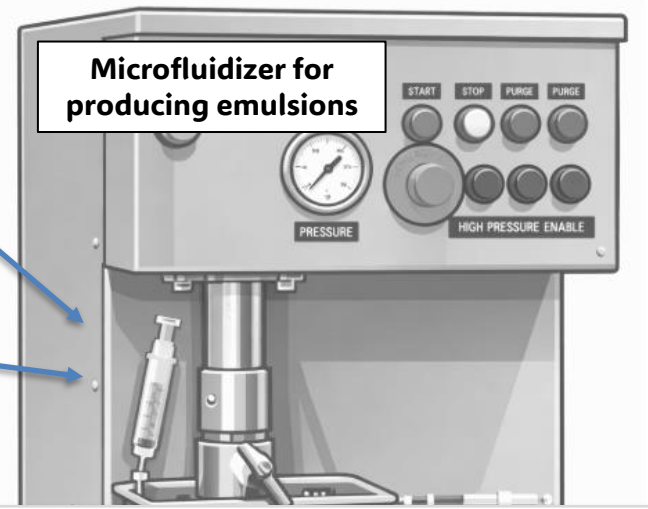
vehicle

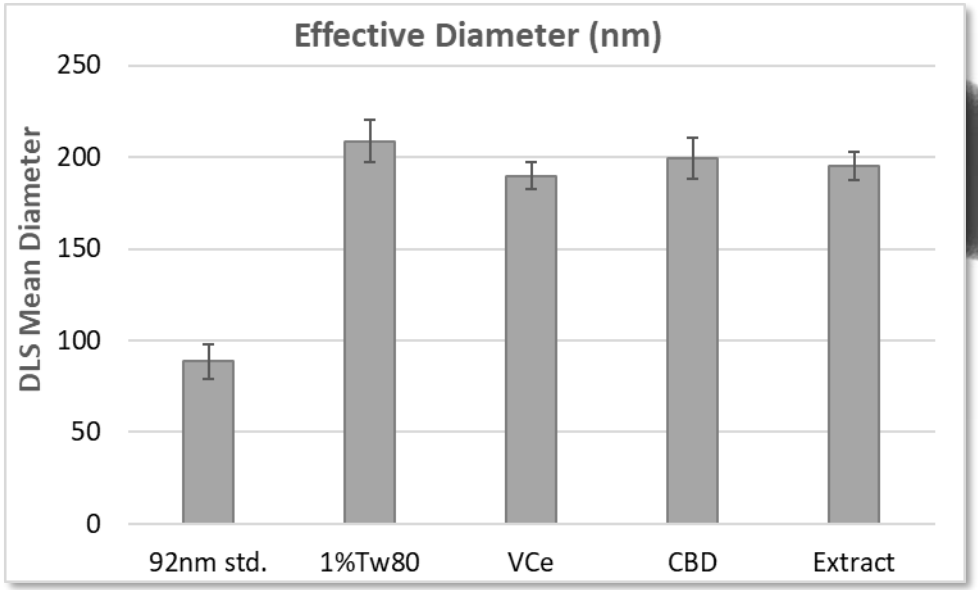
±



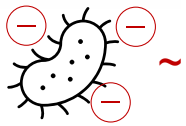
CBD

hemp extract



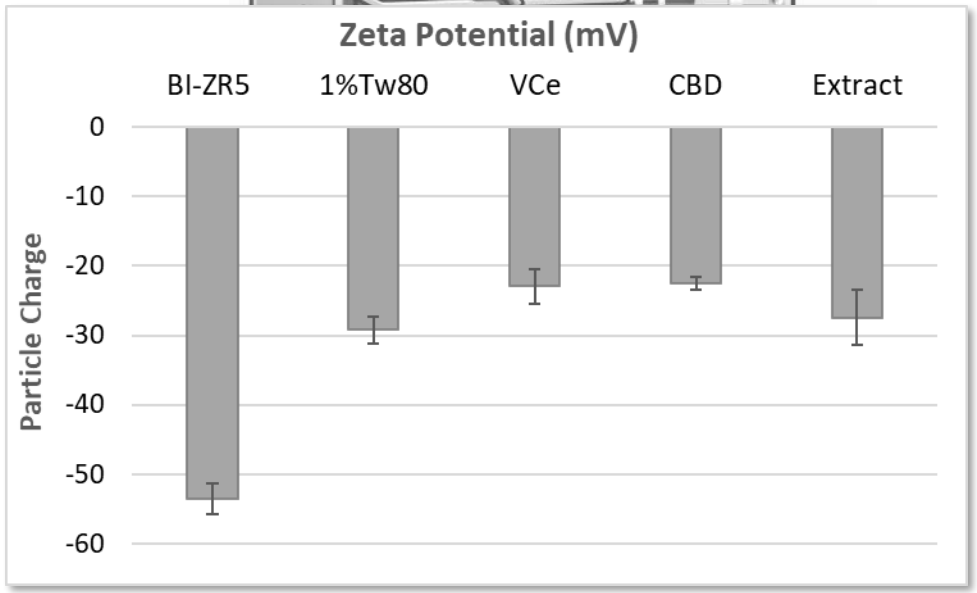


(Gubert et al. 2023)



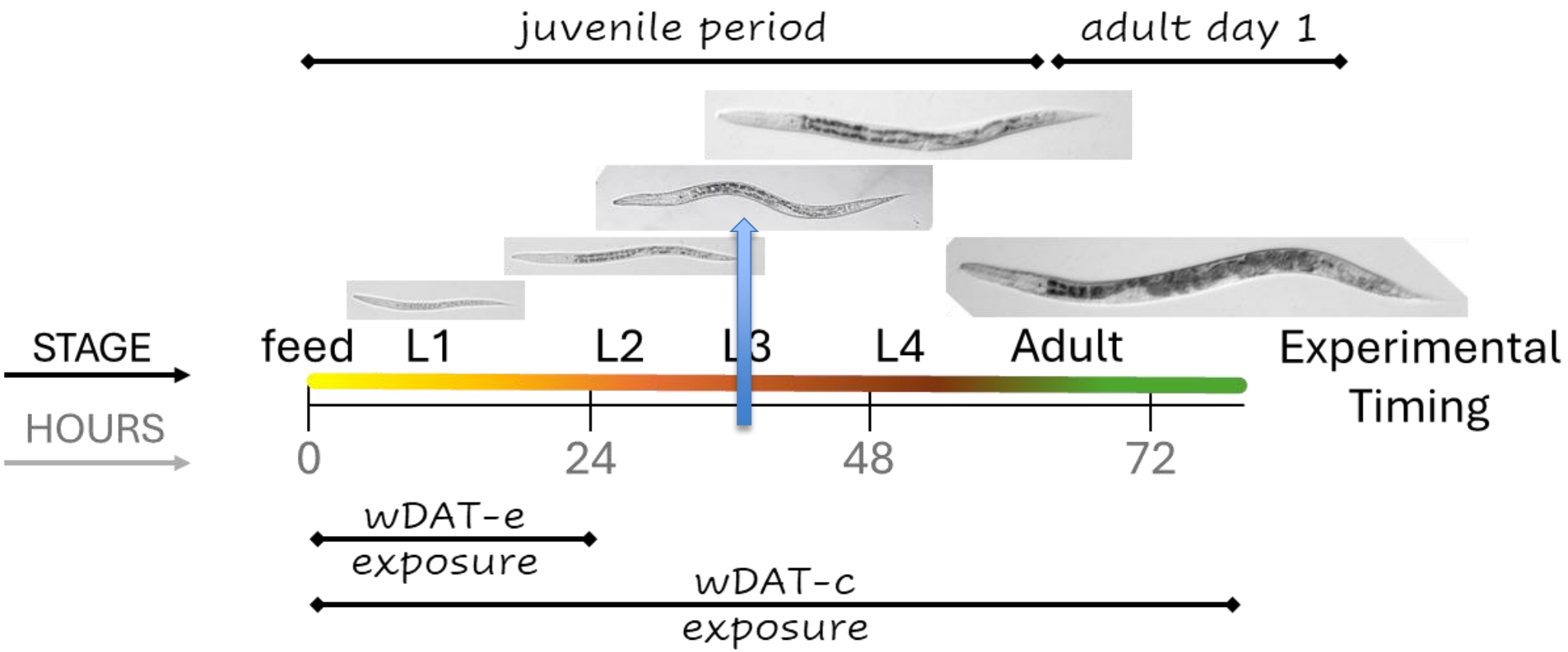
~ -22mV

~ 0.5 x 2µm



(Gubert, P., G. Gubert, R.C. Oliveira, I.C.O. Fernandes, I.C. Bezerra, B. Ramos, . . . and D.H. Mosca. **2023**. *Caenorhabditis elegans* as a Prediction Platform for Nanotechnology-Based Strategies: Insights on Analytical Challenges. Toxics. 11(3). DOI:10.3390/toxics11030239)

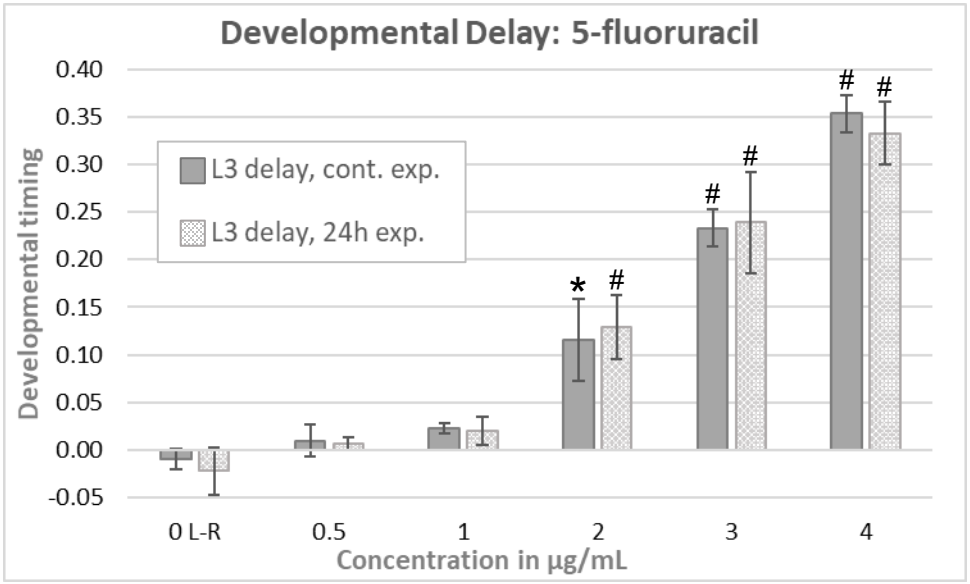
The worm Development & Activity Test (wDAT)



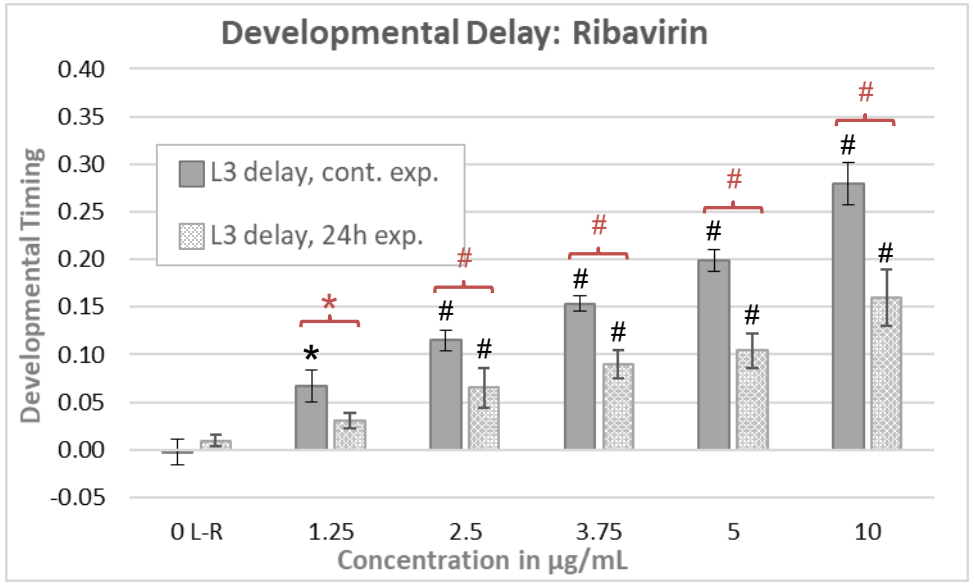
The worm Development & Activity Test (wDAT)

24h vs. continuous exposures from L1 first feeding after hatching

Irreversible Developmental Toxicant



Partially Reversible Effects

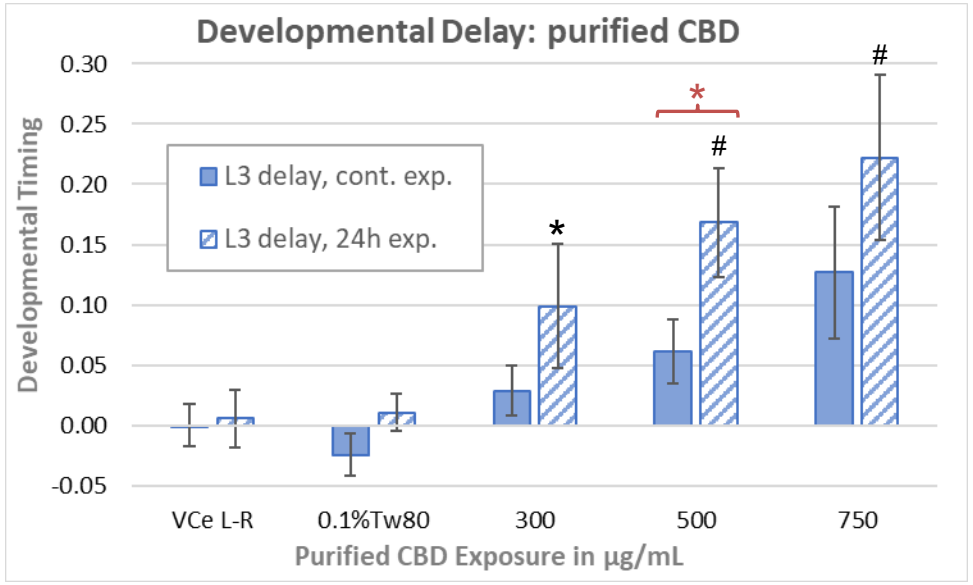


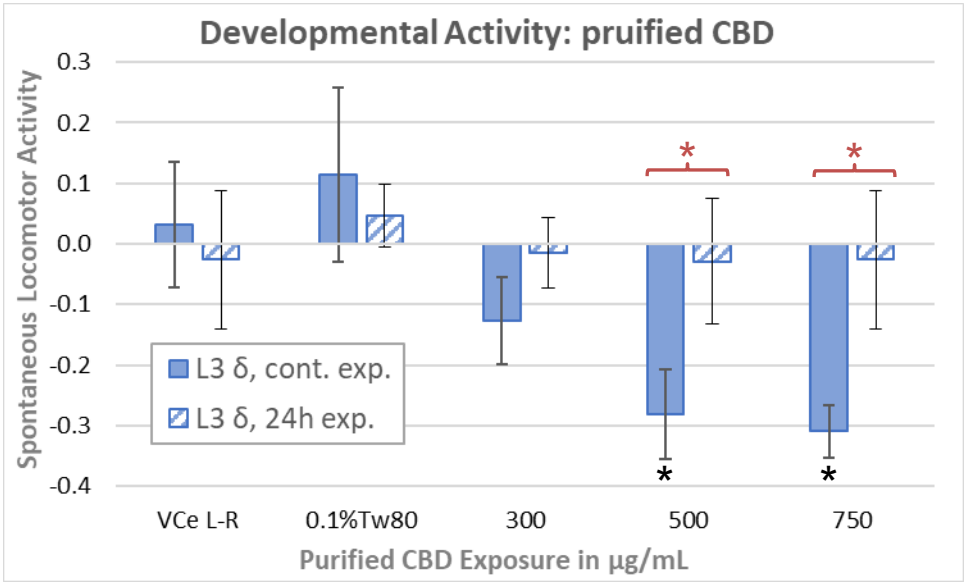
(Hunt et al, 2025)

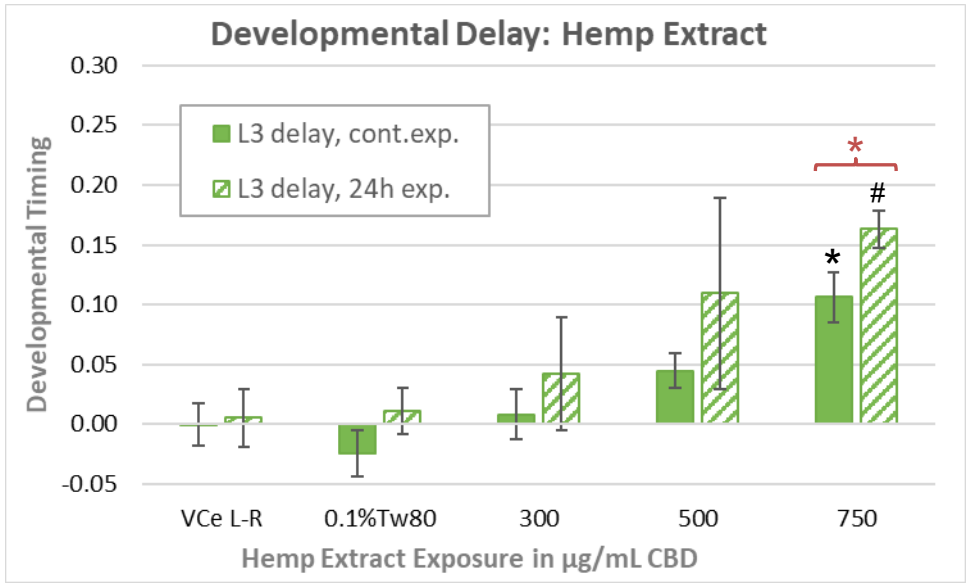
* p-value ≤ 0.0500
# p-value ≤ 0.0050

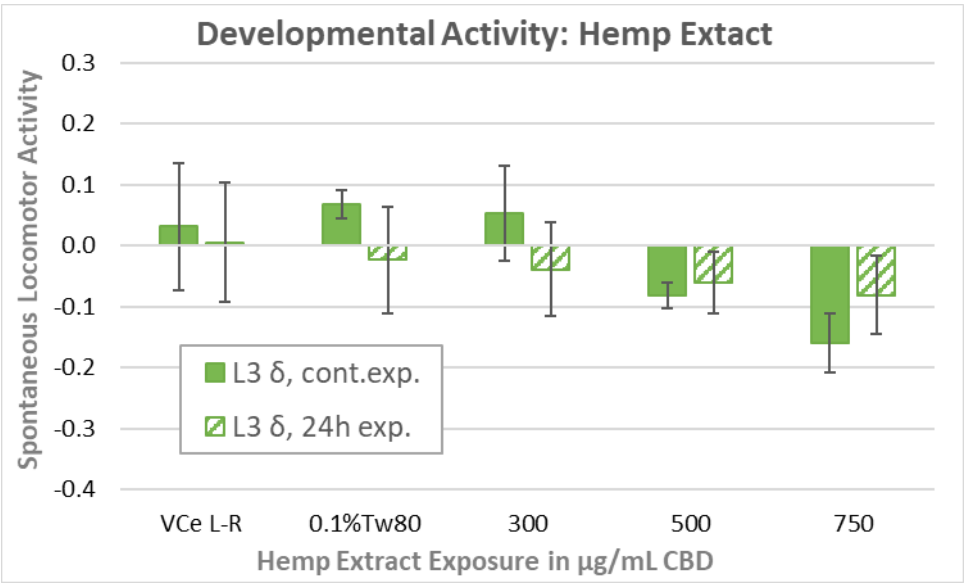
(Hunt, P.R., Olejnik, N., Yourick, J., and Sprando, R.L. 2025. The *Caenorhabditis elegans* worm Development and Activity Test (wDAT) can be used to differentiate between reversible and irreversible developmental effects. Toxicol Rep. 15: p. 102124. DOI:10.1016/j.toxrep.2025.102124.)

The worm Development & Activity Test (wDAT)









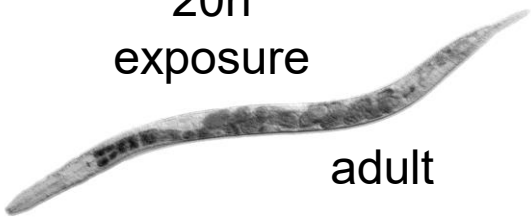
24h vs. continuous exposures from L1 first feeding after hatching

* p-value ≤ 0.0500
\# p-value ≤ 0.0050

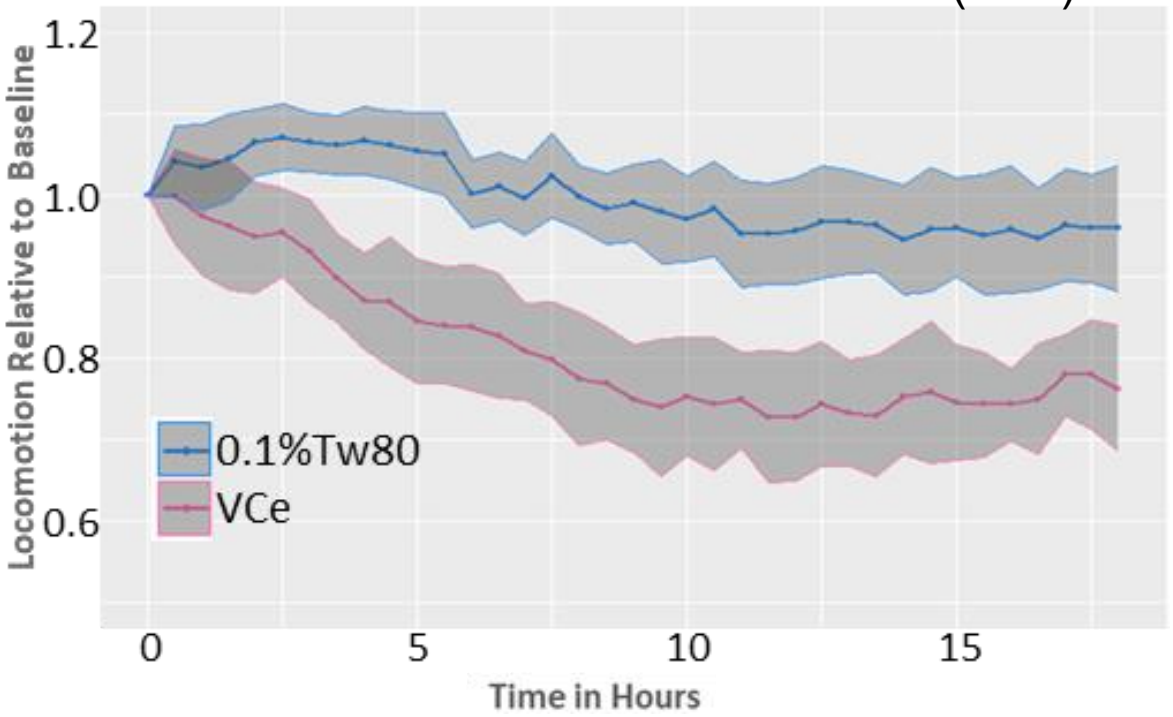
CBD Counteracts HFD-induced Adult Hypoactivity

1. High fat diets (HFD) are associated with ↓ activity in humans & mice (causation not defined)
2. CBD ameliorates induced hypolocomotion in rodent models of arthritic inflammation, chronic liver disease, and Parkinson's disease (Camacho et al. 2023)

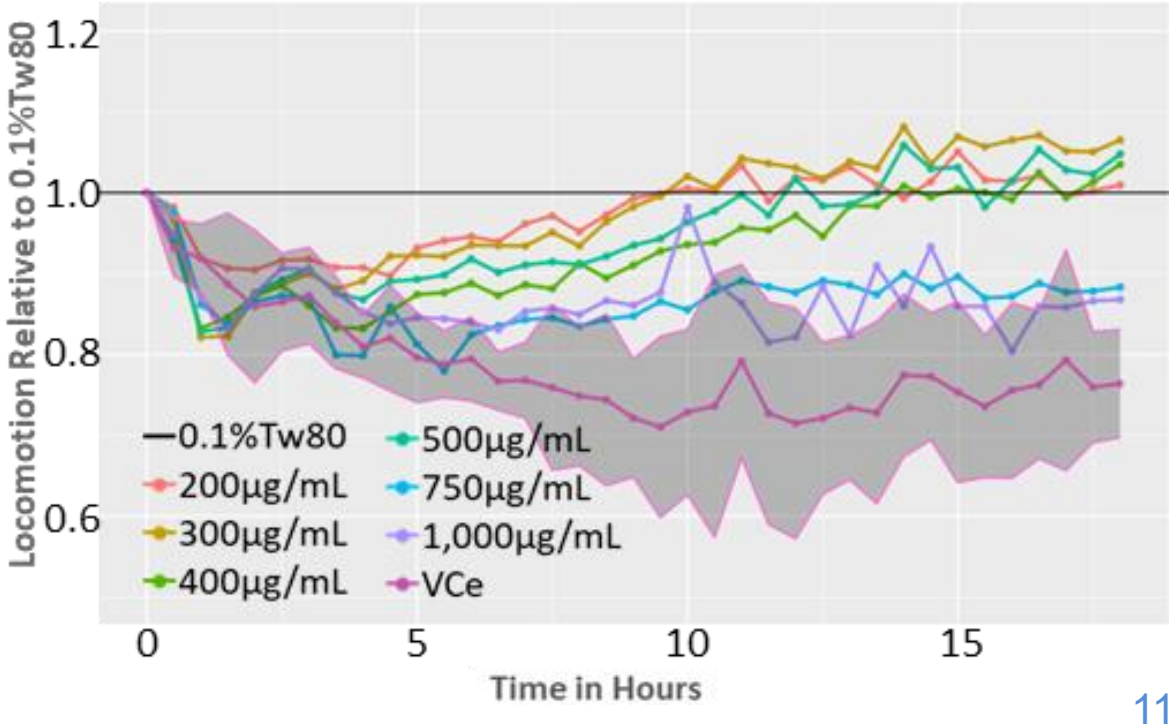
1. HFD induced hypoactivity in adult *C. elegans*
2. CBD counteracted HFD-induced hypoactivity in adult *C. elegans*



Oil-Free vs. Vehicle Carrier Emulsion (VCe)



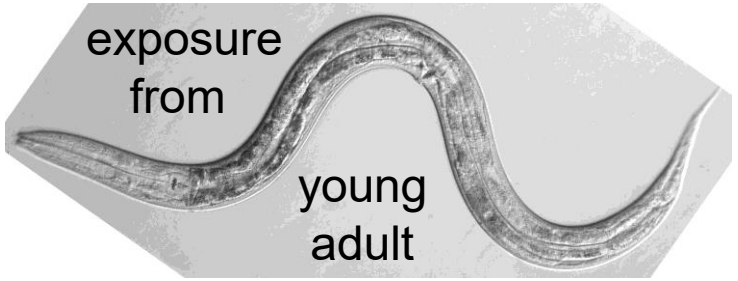
CBD

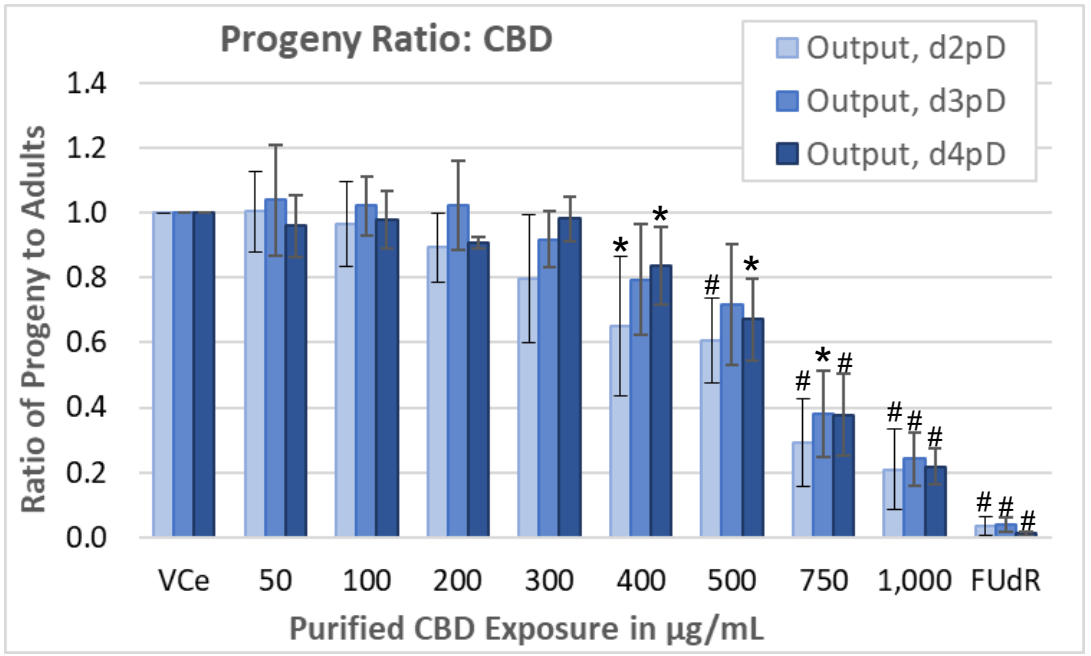


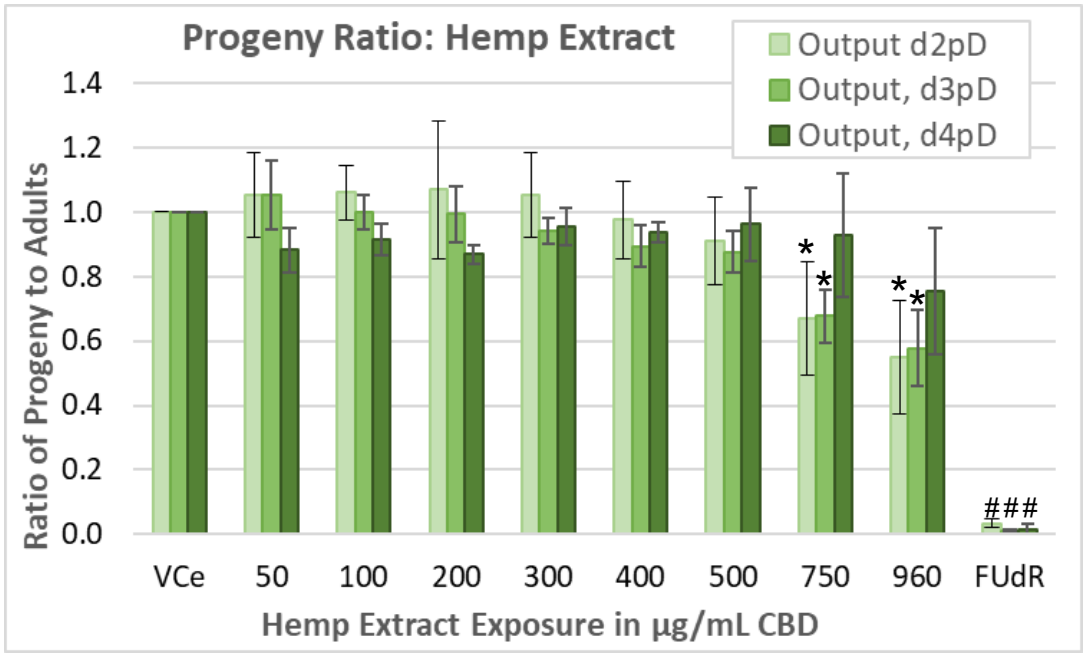
(Camacho, J.A., Welch, B., Ferguson, M., Sepehr, E., . . . **2024**. Assessment of the effects of cannabidiol and a CBD-rich hemp extract in *Caenorhabditis elegans*. Front Toxicol. DOI:10.3389/ftox.2024.1469341)

CBD inhibits *C. elegans* reproduction at concentrations toxic to adults

- *C. elegans* populations exposed from initiation of reproductive competence
- CBD reduced *C. elegans* reproductive output at 'maternally' toxic concentrations
- Hemp extract had less of an effect on reproductive output than purified CBD



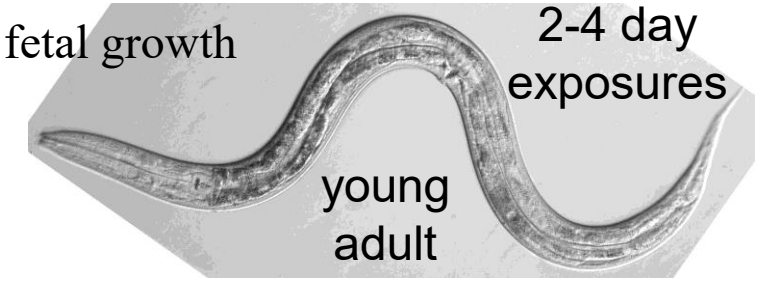


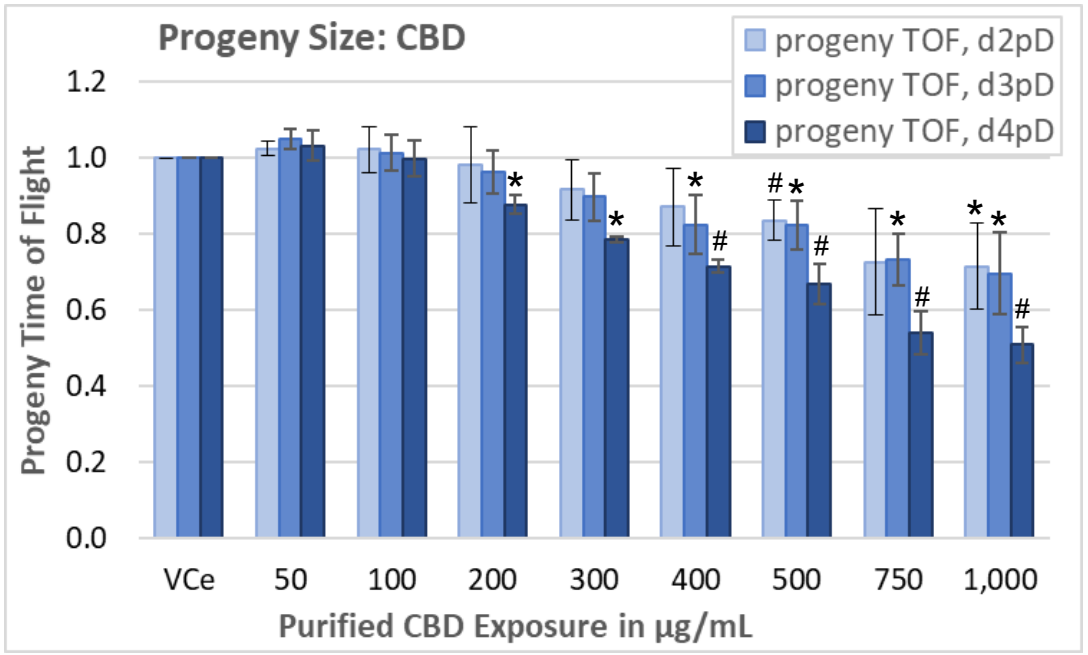


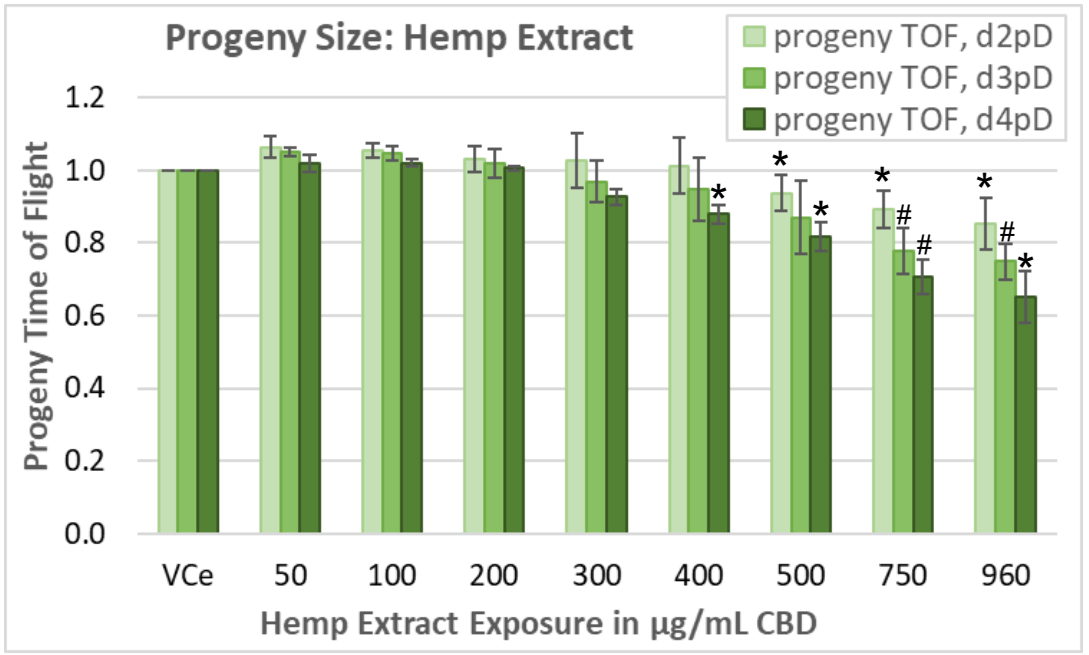
* p-value ≤ 0.0500
\# p-value ≤ 0.0050

CBD inhibits growth of *C. elegans* progeny

- In humans and laboratory mammals, high maternal cannabinoid exposures inhibit fetal growth
- CBD reduced the mean body size of *C. elegans* progeny in a cumulative manner
- Hemp extract had less of an effect on mean progeny body size than purified CBD



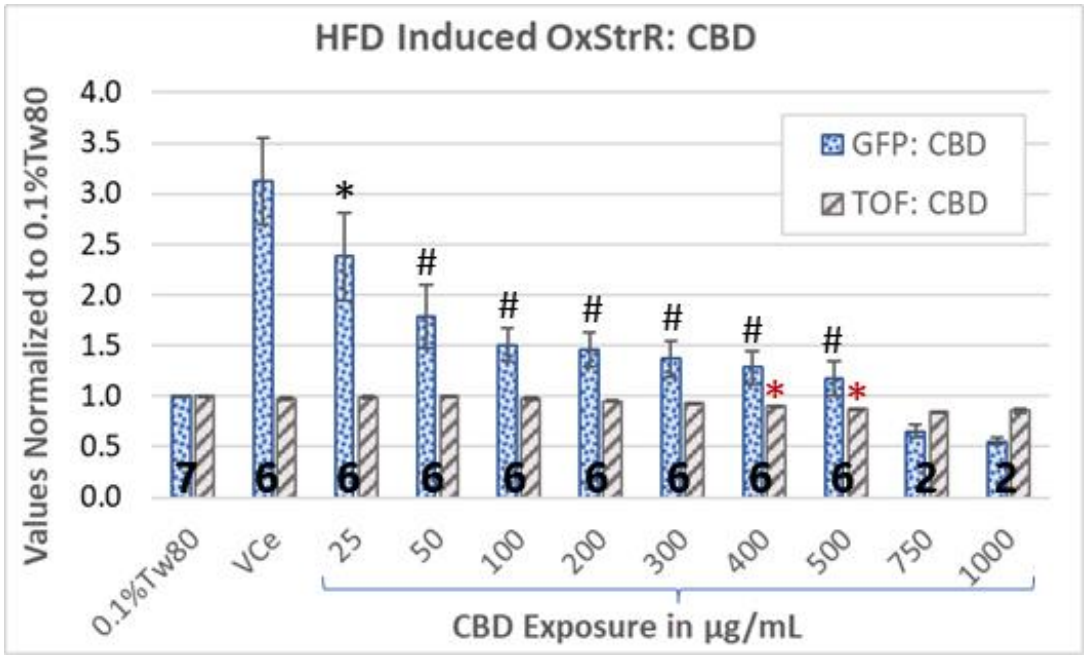


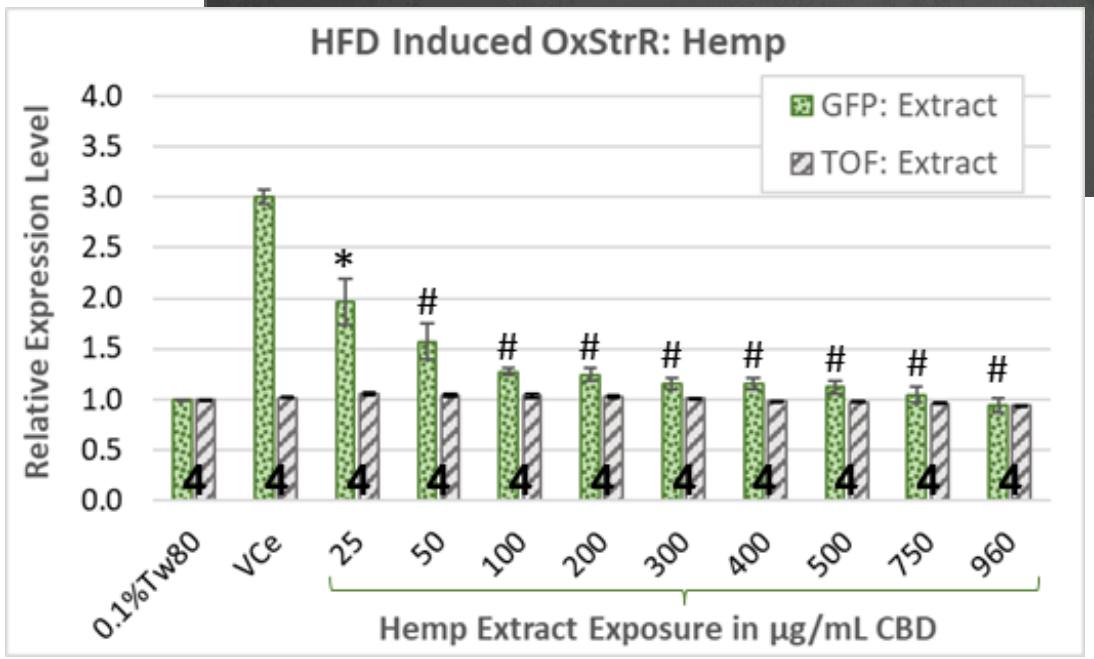


* p-value ≤ 0.0500
\# p-value ≤ 0.0050

CBD Reduces Oxidative Stress Responses (OxStrR)

1. High fat diets (HFD) = ↑oxidative stress in humans & other mammals
2. CBD reduces oxidative stress in rodents & human cell cultures
1. Purified CBD reduced HFD-induced OxStrR transgene expression
2. Effective OxStrR exposures = 25 to 100µg/mL purified CBD
   - Toxicity (↓ body size) = ≥ 400µg/mL purified CBD
   - Hemp extract less toxic than purified CBD





OxStrR Body Size

* p-value ≤ 0.0500
# p-value ≤ 0.0050

OxStrR Body Size

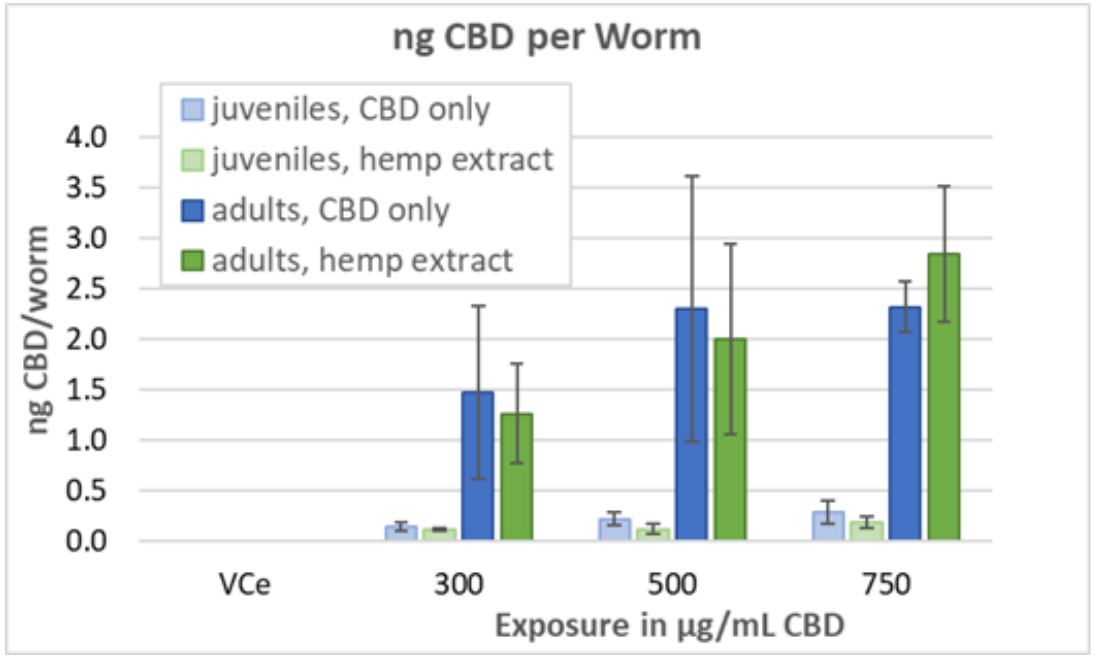
Dosimetry: Internal CBD Concentrations

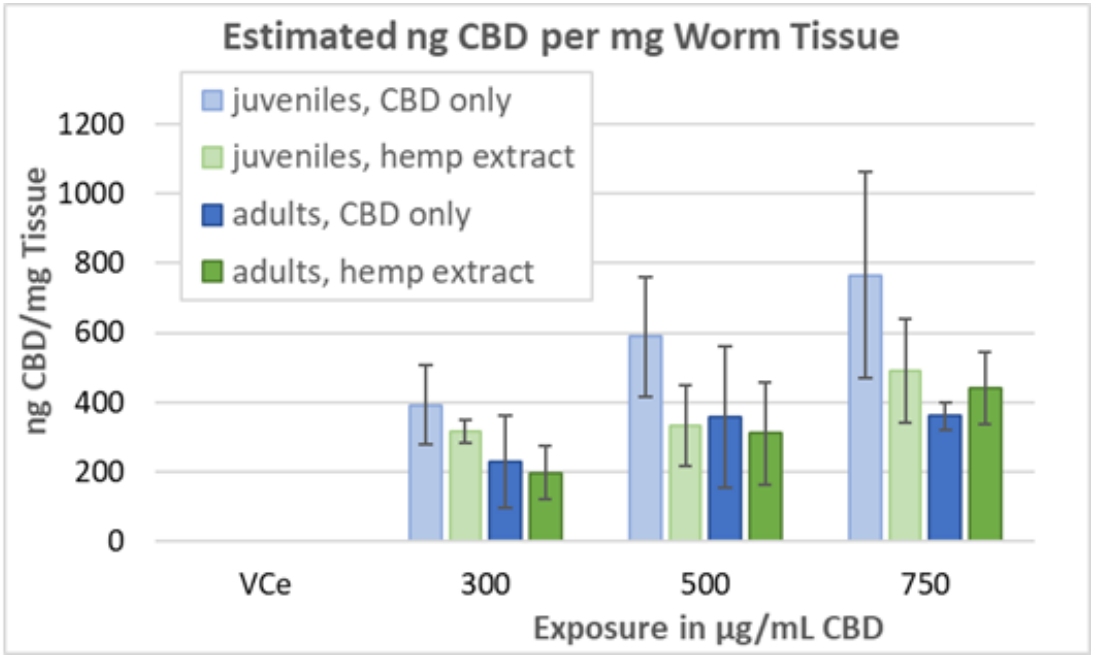
- Humans: Epidiolex dose = 5 to 25mg/kg/day
- Rats: 115 or 230 mg CBD/kg/day for 28 days
  - → ~ 110mg/kg CBD in adipose tissue

Dose	Tissue CBD Concentration (mg/kg)		
	Adipose	Muscle	Liver
Low (30 mg/kg/day)	5.30 ± 4.24	0.27 ± 0.25	0.07 ± 0.03
Medium (115 mg/kg/day)	116.17 ± 61.27 *	0.64 ± 0.17 **	0.95 ± 0.18 **
High (230 mg/kg/day)	108.60 ± 86.34 *	0.93 ± 0.31 ***	1.15 ± 0.34 ***

(Child, R.B., and M.J. Tallon. **2022**. Cannabidiol (CBD) Dosing: Plasma Pharmacokinetics and Effects on Accumulation in Skeletal Muscle, Liver and Adipose Tissue. *Nutrients*. 14. doi: 10.3390/nu14102101.)

- At 300µg/mL CBD exposures, tissue concentrations were 200 to 400 mg/kg *C. elegans* lysate
- CBD concentrations that are toxic in *C. elegans* exceed human recommended dosing





Summary

- Adult effective range for dose-responsive OxStrR reduction was well below toxic exposure levels
- Life Stage Disparity in Effects:
  - ✓ LOAEL concentrations for toxicity in adults 2x that for juveniles
  - ✓ CBD induced hypoactivity in juveniles but normalized adult HFD activity
- Similar tissue uptake levels of CBD from purified CBD and hemp extract
- Hemp extract was slightly less toxic than purified CBD for all evaluated *C. elegans* endpoints
- **Juveniles: developmental delays increased post-removal of CBD = a withdrawal-like effect**
- **All toxic exposures exceeded human-recommended dosing**

Questions?

http://www.**wormatlas.org**/hermaphrodite/introduction/mainframe.htm?mobify=0