

Exploration of *in vitro* methods for investigating mechanism of action of Sulfur Mustard (HD)

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Background

- Half-life of HD in water at 25°C is 8 min
- HD degradation is highly concentration dependent
- Cell culture exposures use HD in an organic solvent, such as dimethylsulfoxide (DMSO), that is then added to media (water, amino acids, salts, glucose)
- Human Dermal Fibroblasts (HDF) are an abundant cell type in the dermis



Methods

Assay Validation

- Normal HDF passage 5 monolayer
- HD in DMSO and media: **12.54 mM, 6.27 mM, 3.13 mM, and 1.57 mM, 0.783, DMSO vehicle control**
- Incubated with treatment for **24 h**

Viability/Cytotoxicity Reagent Reactivity to HD

- HD into DMSO, then FGM™-2 BulletKit™ (Lonza, CC-3132)
- Reagents added well-by-well
- MTT, alamarBlue®, and LDH by manufacturer instructions

Analytical Chemistry of HD in Various Solvents

- Gas chromatography/mass spectrometry (GC/MS) of HD in corn oil with/without media
- Nuclear Magnetic Resonance (NMR) of HD, DMSO, and media alone and mixed

Assay Validation

- Viability **disagreed between assays**
- LDH gave unrealistic viability results

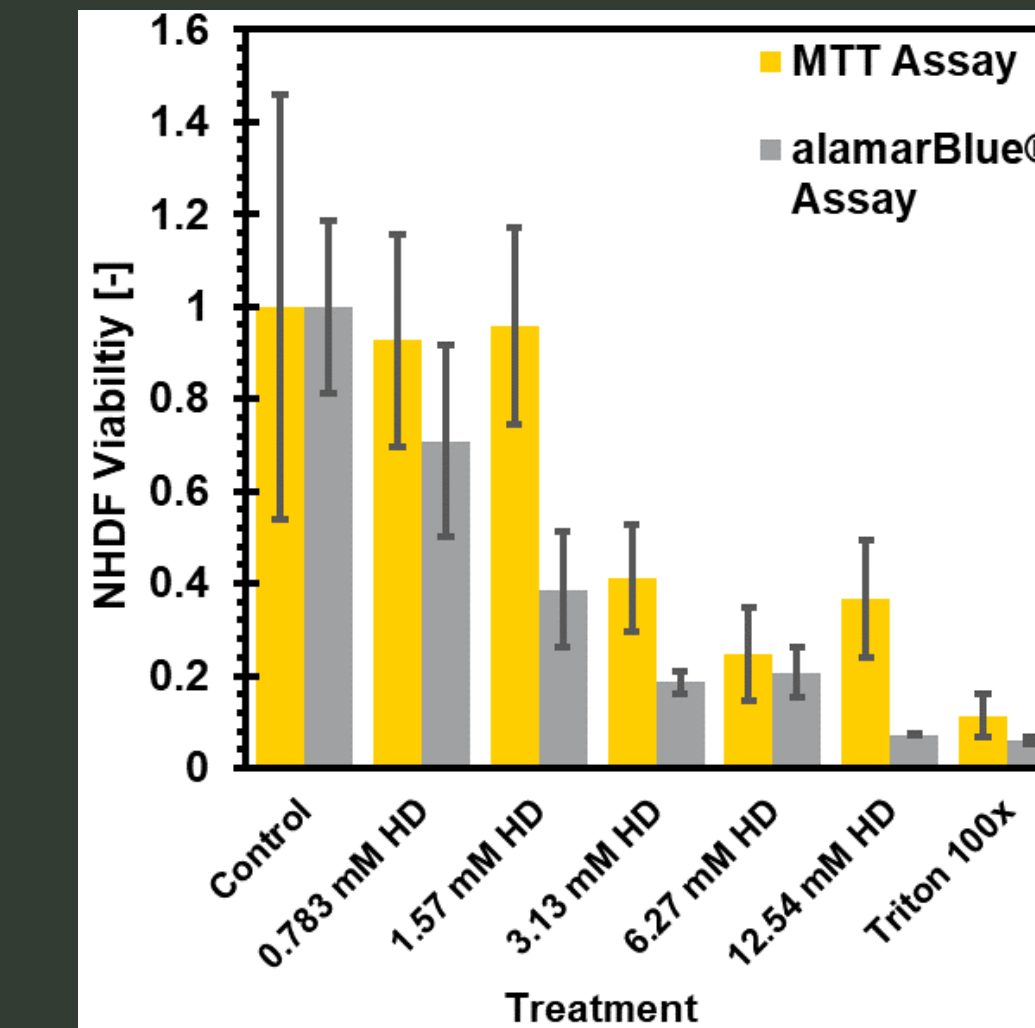
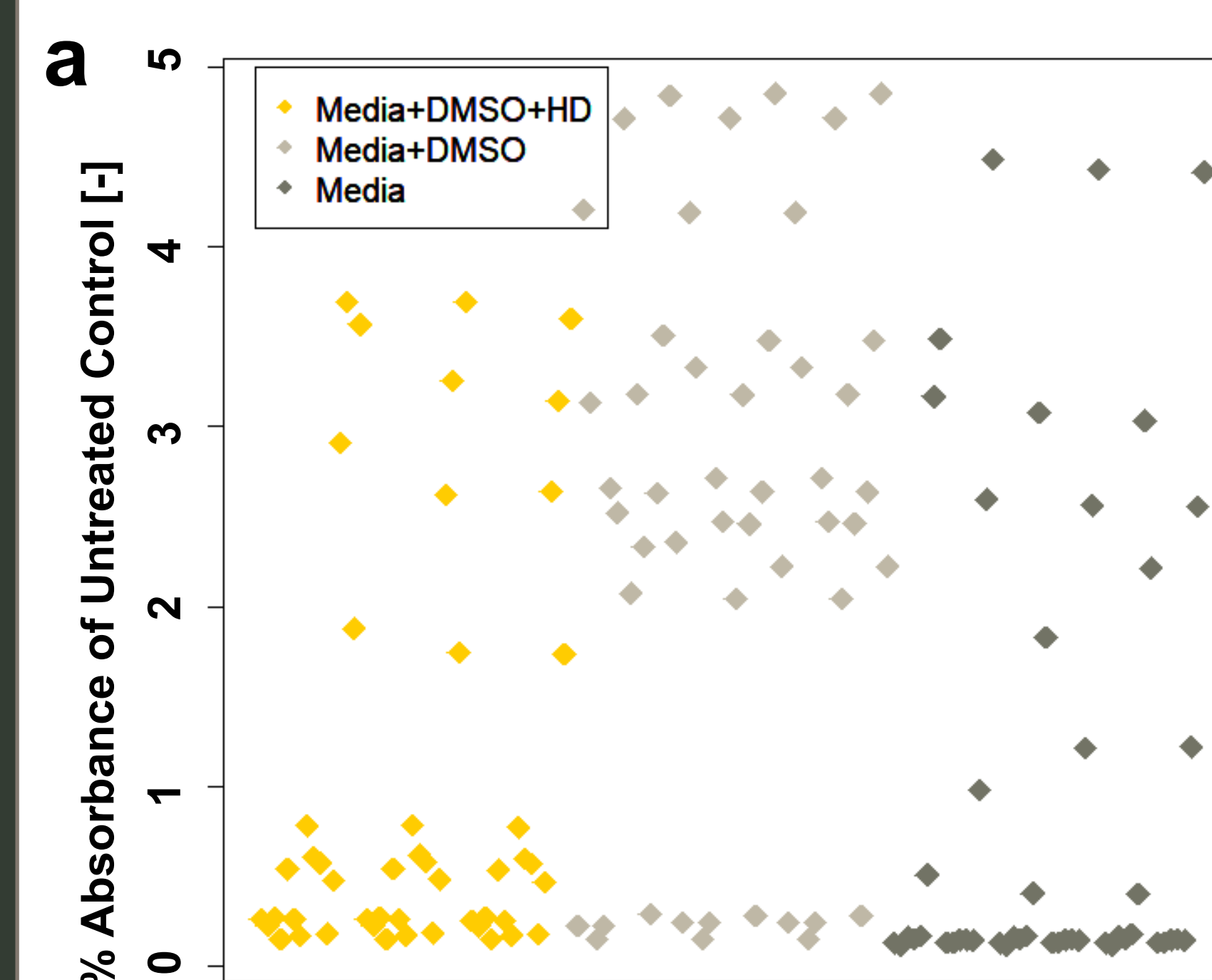


Figure 1. Mean viability of NHDF P5 to HD diluted in DMSO and media, measured by MTT and alamarBlue assays. Error bars are one standard deviation.

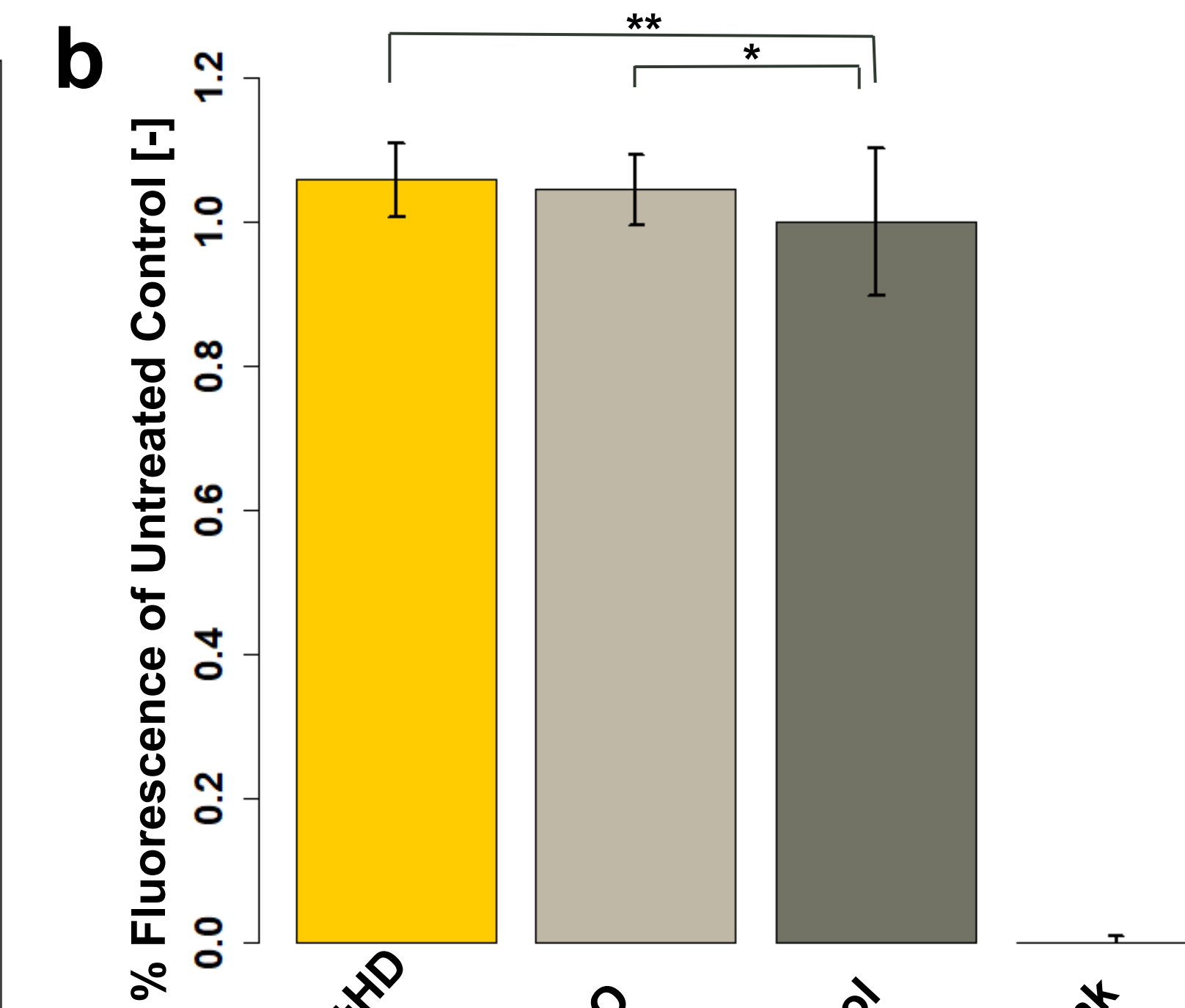
Reagent Reactivity



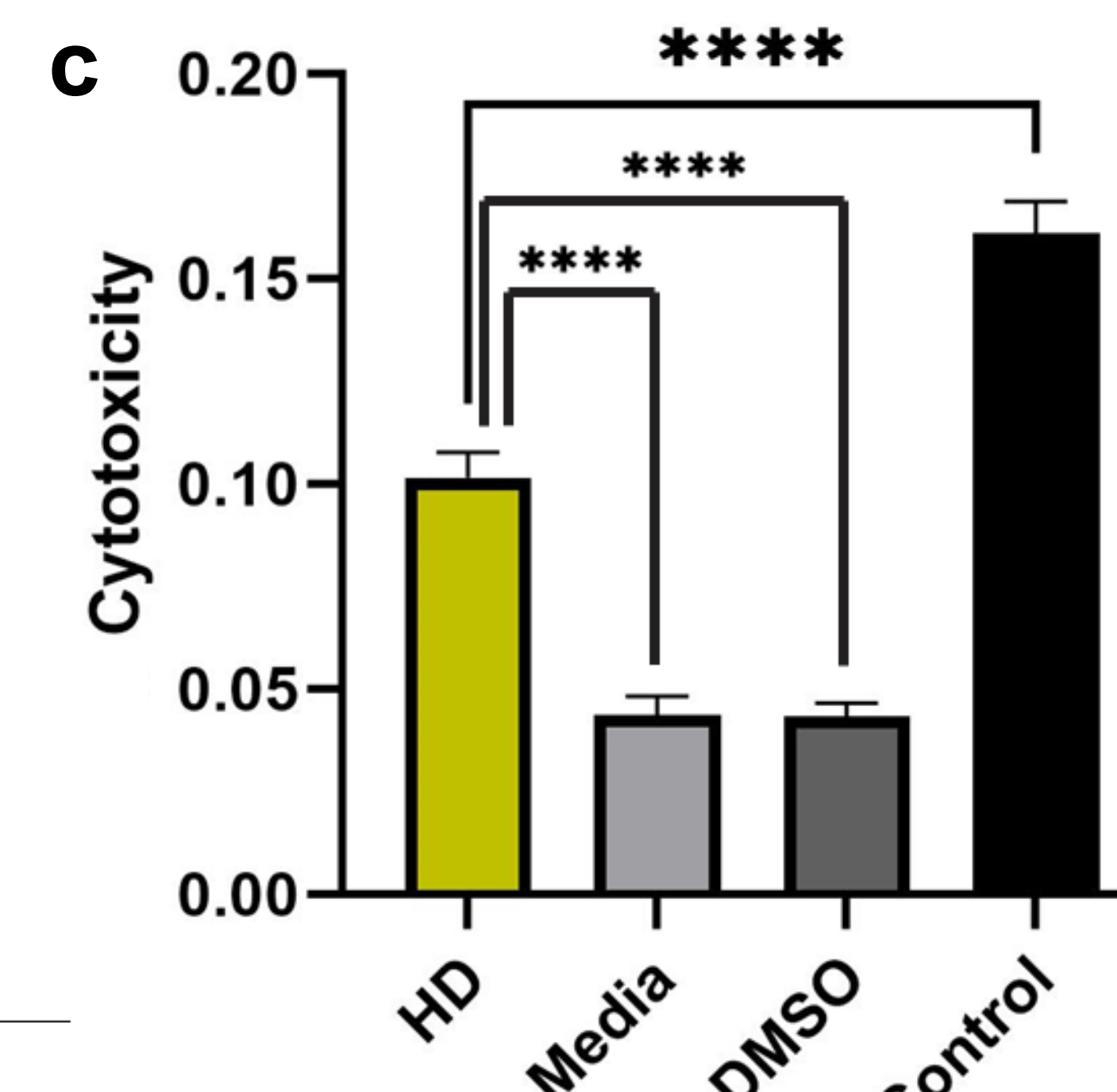
- “Worst case scenario” was simulated, normally HD is removed and cultures washed before analysis
- DMSO interfered with MTT, can not determine if HD interfered with MTT
- HD interferes **least** with alamarBlue®
- HD interfered with LDH assay



MTT raw data, triplicate repeated measures



alamarBlue Conditions



LDH Conditions

Preliminary Analytical Chemistry

- HD degraded to 50% of the starting concentration in <30 min in corn oil and media
- NMR indicates that HD reacts with cell culture media within minutes, and it is dependent on type of media (preliminary data not shown)

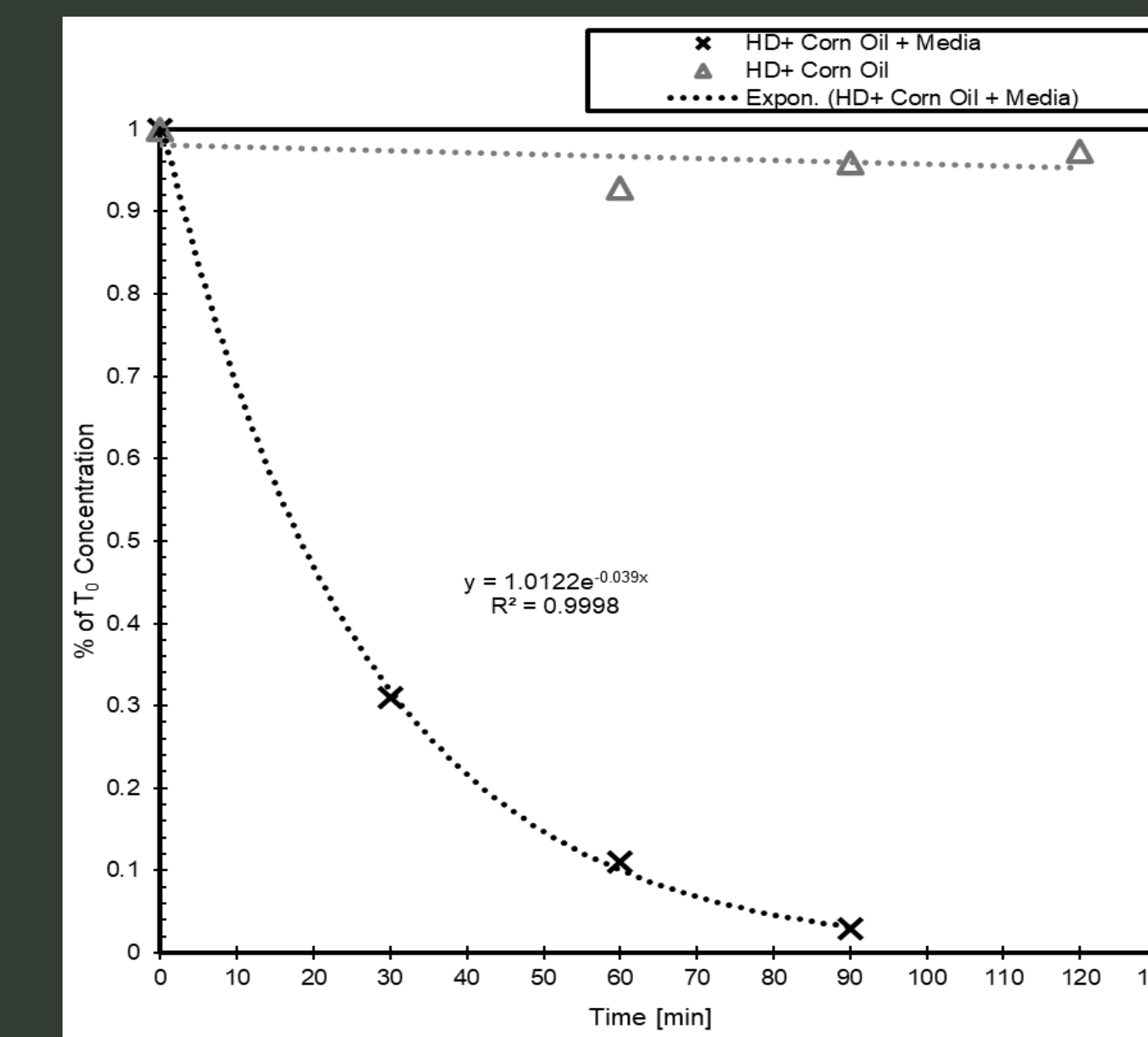


Figure 3. Preliminary GC/MS of HD in corn oil and cell culture media (N=1).

We must reexamine how *in vitro* HD exposures are conducted

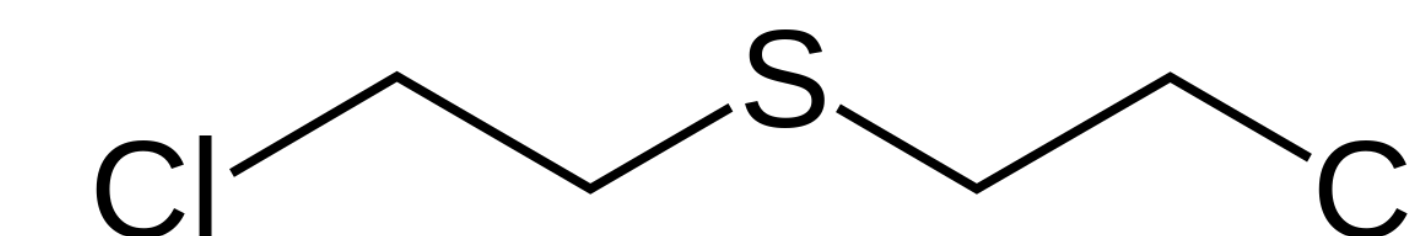
- Next Steps: Repeat analytical experiments with larger sample sizes**

Figure 2. The effect of HD and DMSO on (a) MTT assay, (b) alamarBlue® assay, N= 48, non-parametric test, error bars are 95% confidence intervals, and (c) LDH assay, N= 48, error bars are 95% confidence intervals. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$.



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