Case study using transcriptomics from acute *in vivo* and *in vitro* exposures to inform points of departure

Genetics and Environmental Mutagenesis Society

Leah Wehmas, US EPA

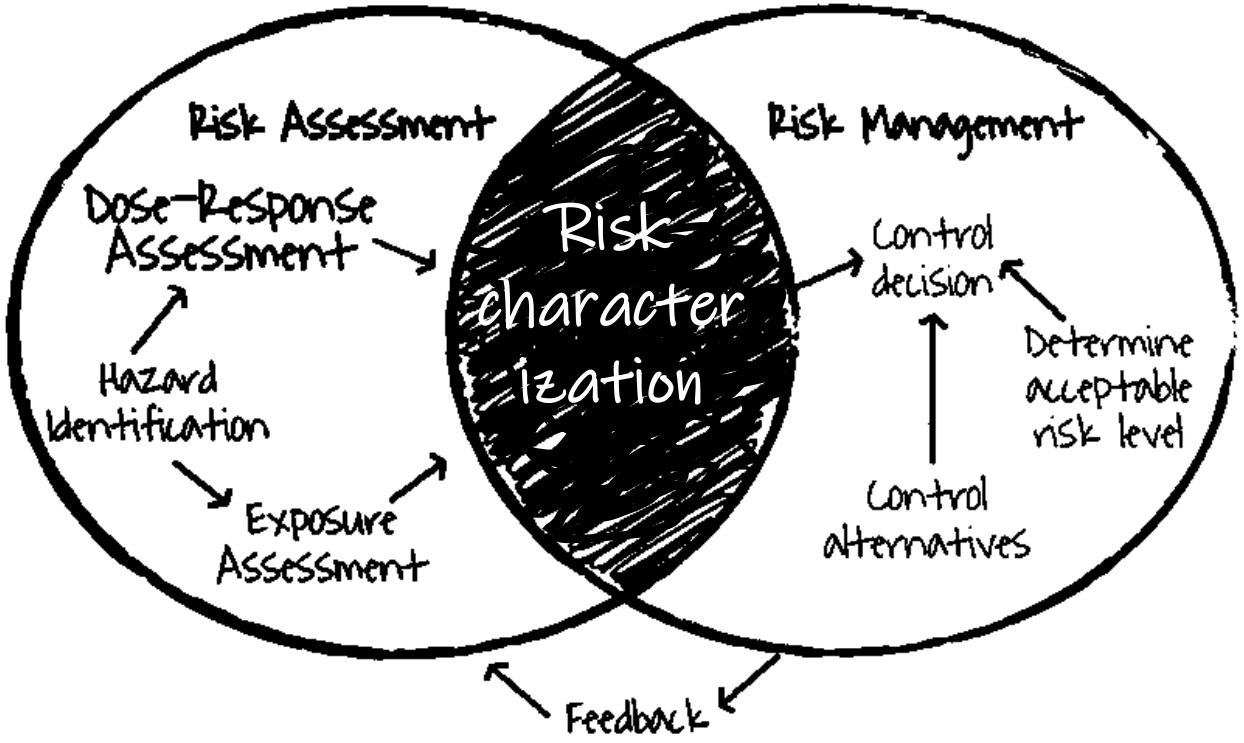
Spring Meeting 2021-05-24

Acknowledgments: Susan Hester, Nyssa Tucker, Brian Chorley, Hisham El-Masri, Lake et al. 2016 contributors, Amanda Brennan, Jermaine Ford and Chemical Safety for Sustainability

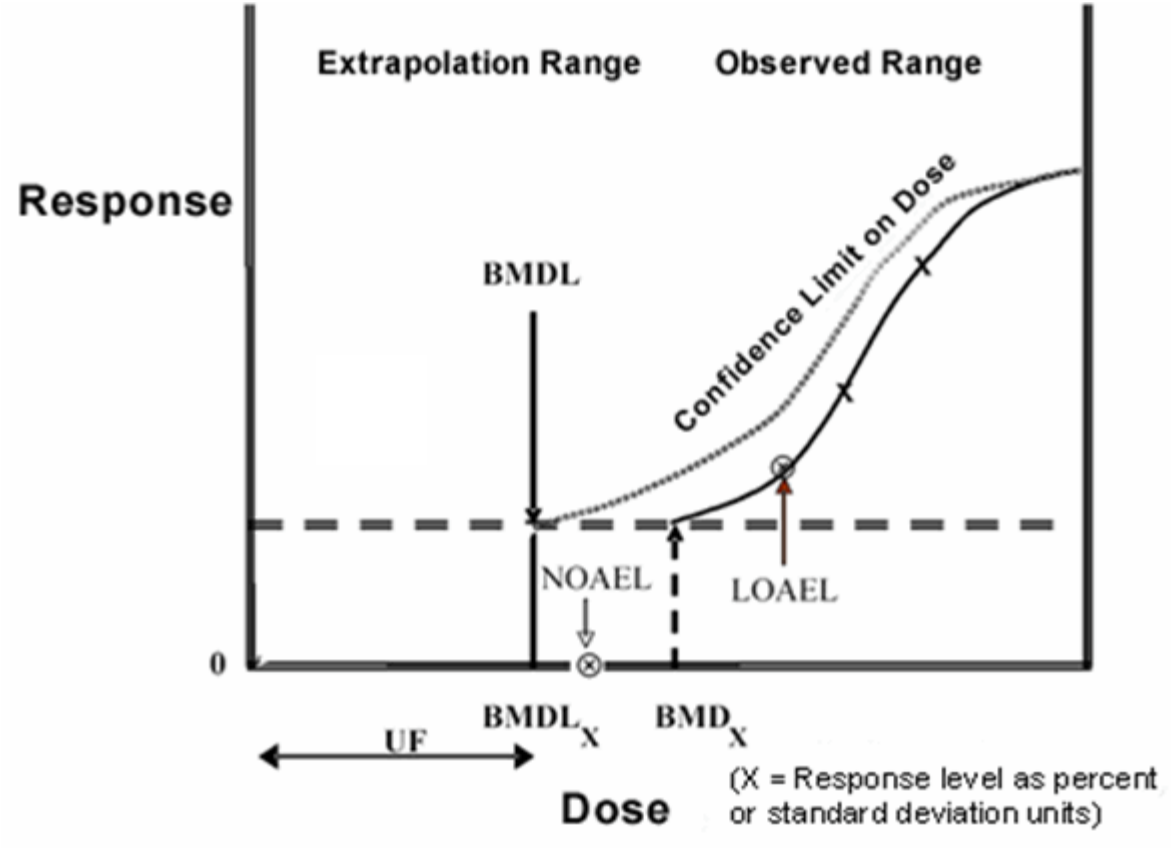
Disclaimer

The views in this presentation do not represent the US EPA

Points of departure help set the risk threshold for chemicals

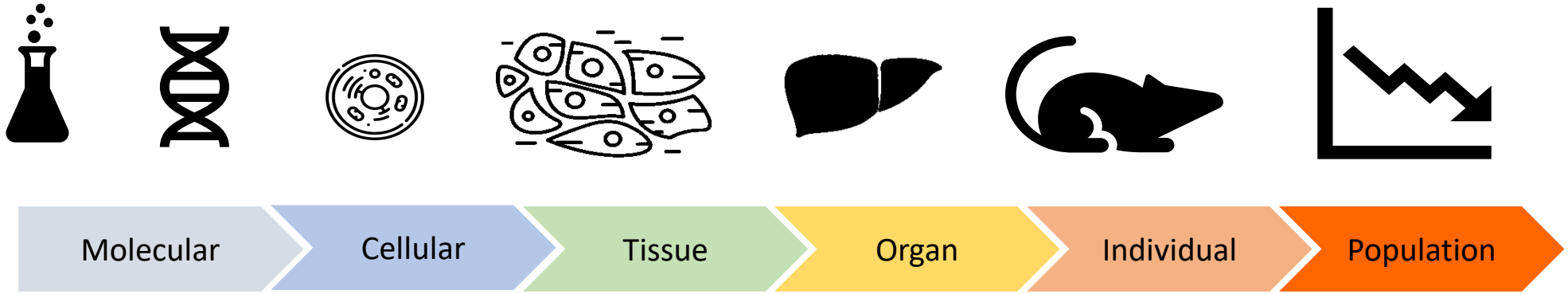


Dose-response assessment is a step in the risk assessment process (Image Source: ORAU, ©)



Extrapolated values using the benchmark dose method reflect the shape of a dose-response curve (Image Source: EPA)

Using new approach methods and the AOP framework to understand toxicity



Using new approach methods and the AOP framework to understand toxicity

Molecular | Cellular | Tissue | Organ | Individual | Population

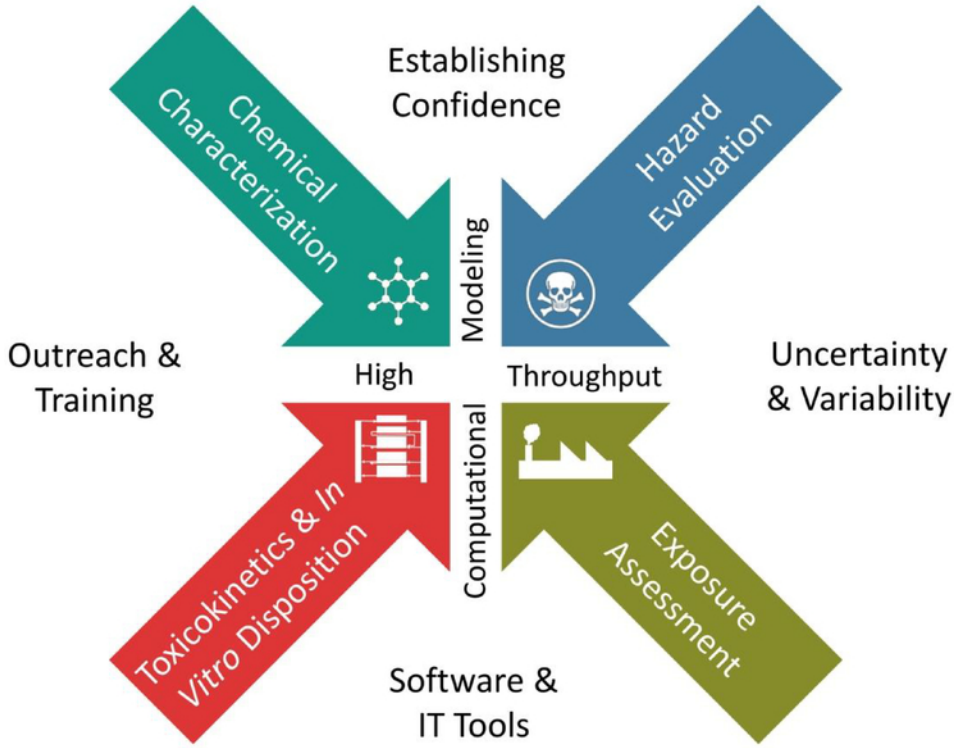
Molecular Endpoints
(identify relationships)

Traditional Endpoints
(associate with adverse effects)

Existing acute *in vivo* studies can bridge high throughput *in vitro* gene response with later adverse outcomes

Potency

Metabolism



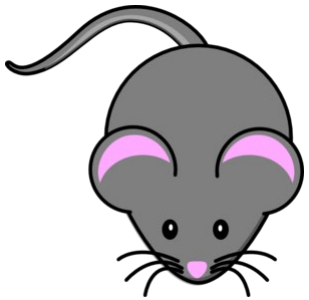
Chemical delivery

Differences in transcriptomic response

Thomas *et al.* 2019 doi:10.1093toxsci/kfz058

In vivo experiment 1.

Male B6C3F1 Mouse



- Liver
- 0, 1500, 3000, 6000 ppm DEHP
- Dietary exposure
- 7 days
- n=4/dose level
- RNA

Lake et al. 2016 doi:10.1093/toxsci/kfv236
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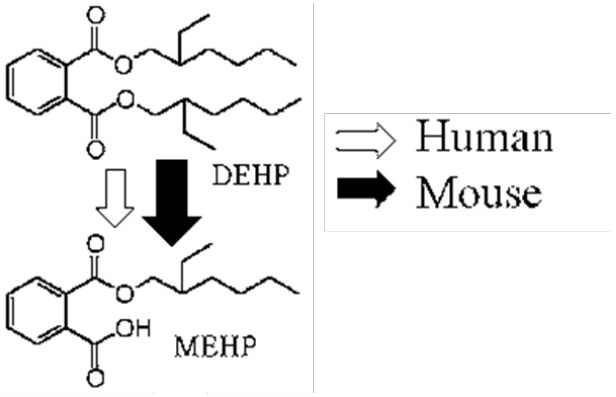
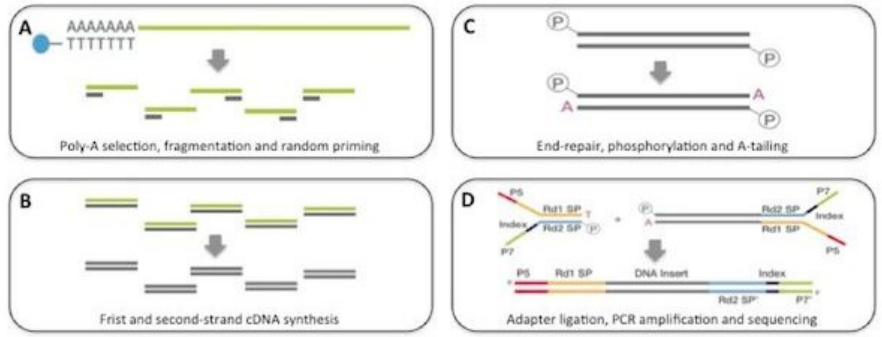


Di(2-ethylhexyl)phthalate
Mono(2-ethylhexyl)phthalate

Total RNA-seq



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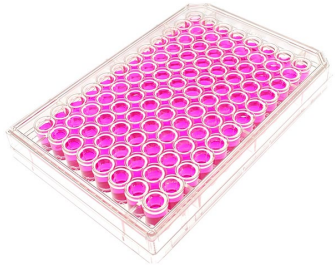
In vitro experiment 2.

B6C3F1 primary liver cells



- 0.016-10 µM DEHP
- 0.056-35 µM MEHP
- Five-fold dilution
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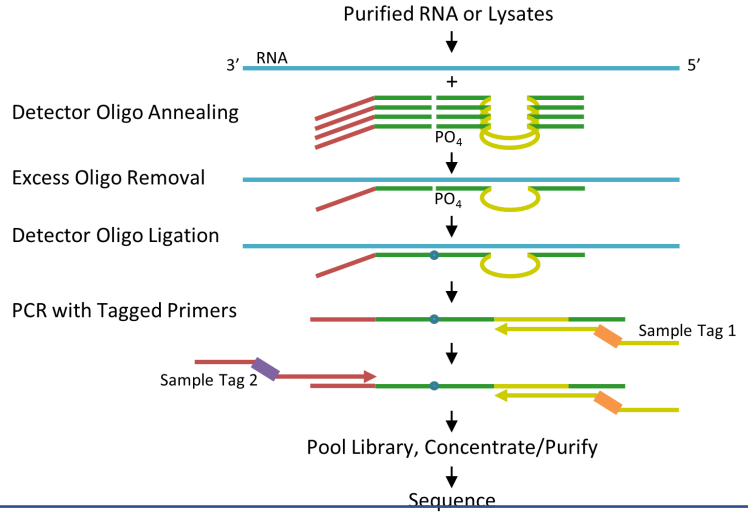
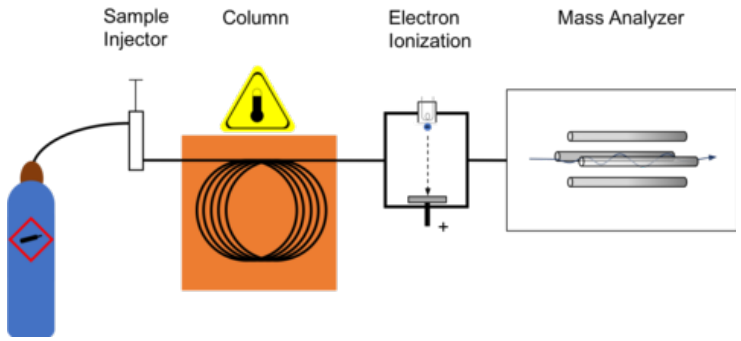
- 1 day
- n=4 assays
- Cell lysates
- Cell medium

Mouse Whole Transcriptome TempO-seq



Analytical Chemistry



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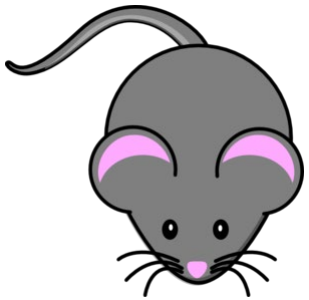

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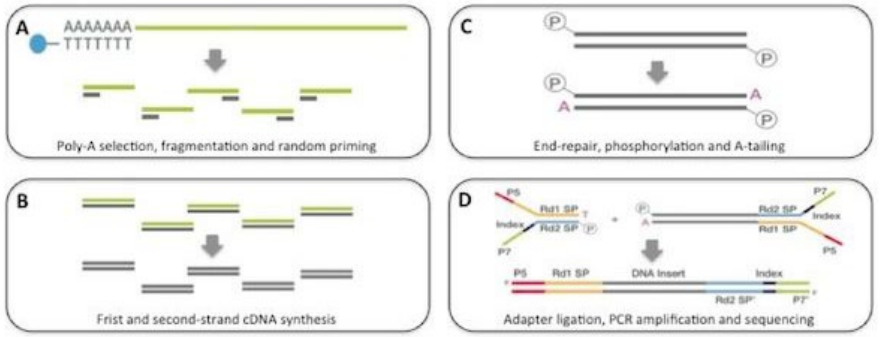

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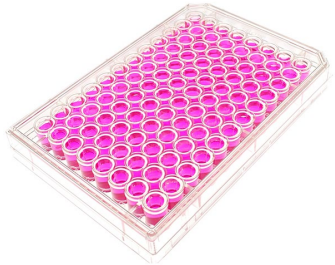
1. **Exploratory analysis**
2. **Significant gene response**
3. **BMDExpress**
4. **Compare across experiments**

In vitro experiment 2.

B6C3F1 primary liver cells



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- 1 day
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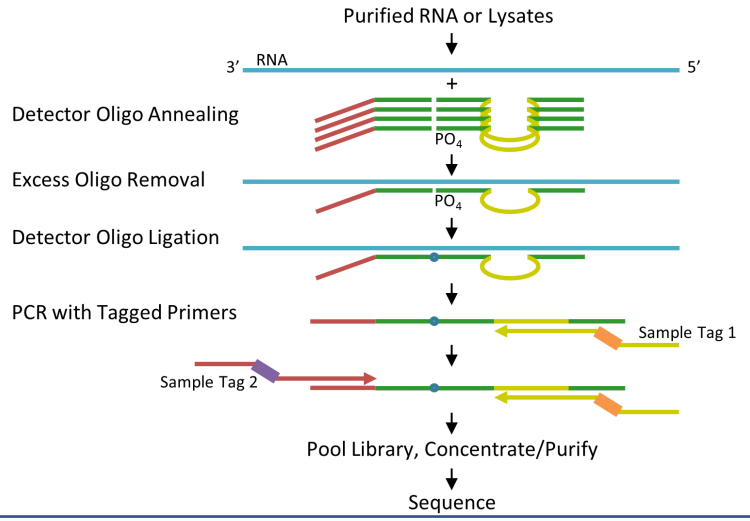
- Cell medium

Mouse Whole Transcriptome TempO-seq



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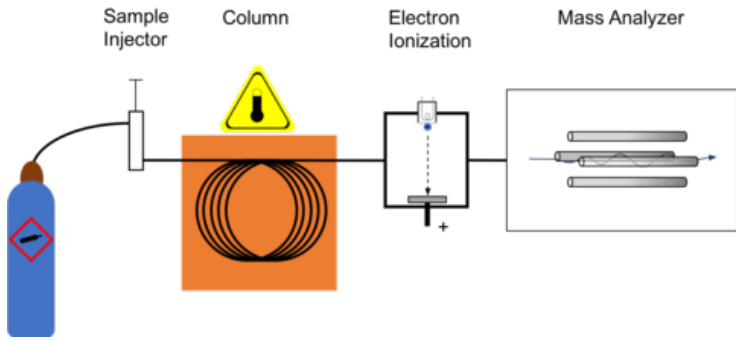
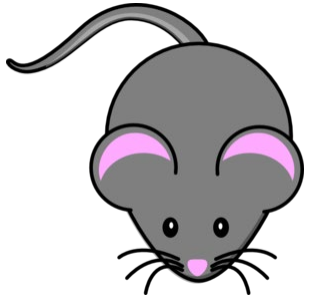


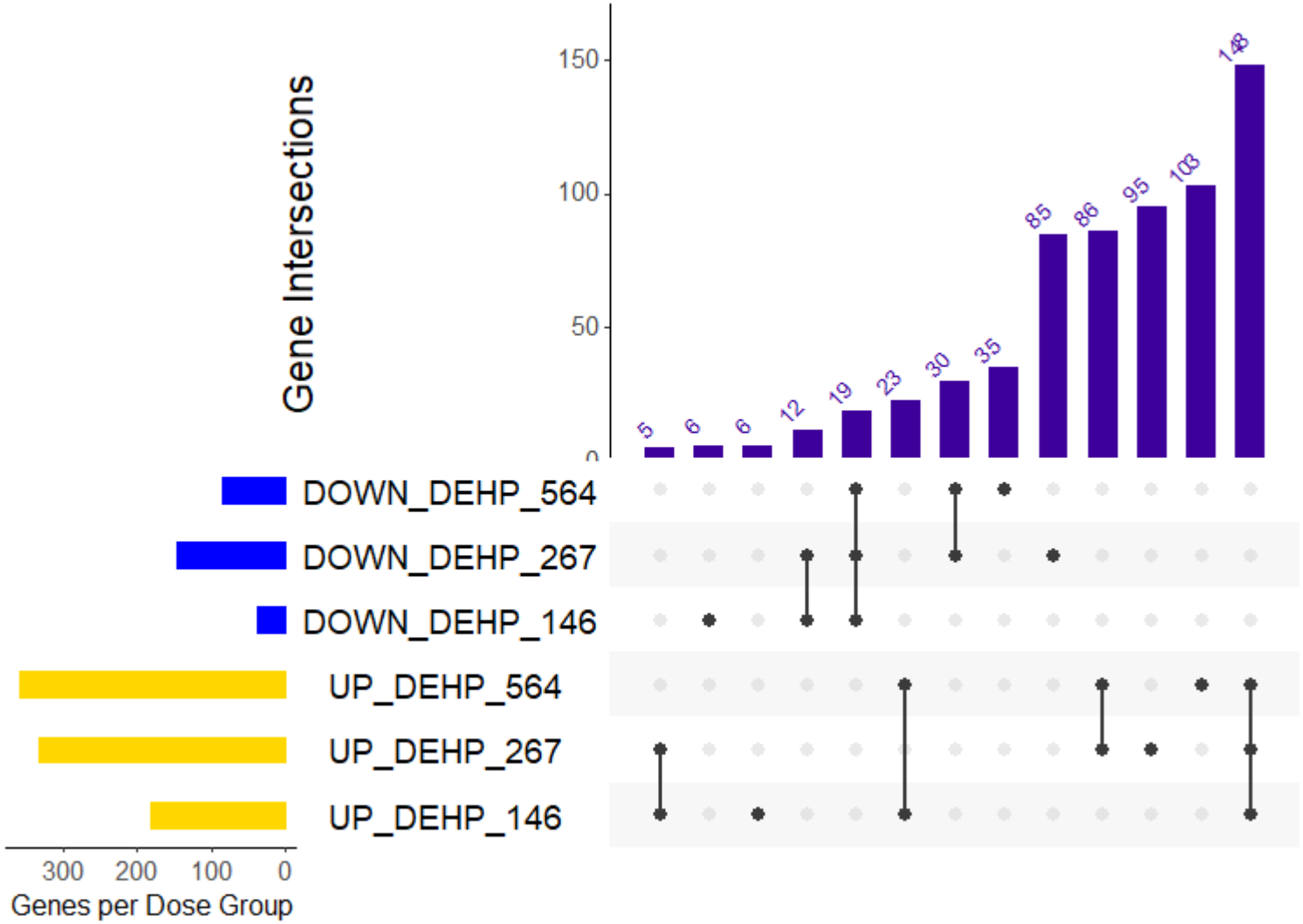
In vivo DEHP results in dose dependent change in genes

Male B6C3F1 Mouse



Lake et al. 2016 doi:10.1093/toxsci/kfv236
Hester et al. 2016 10.1093/toxsci/kfw161

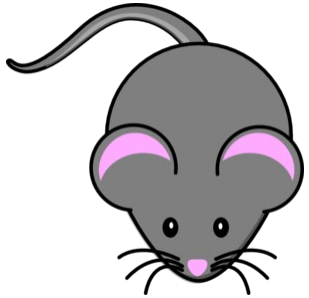
- Liver
- 0, 1500, 3000, 6000 ppm DEHP
- Dietary exposure: 0, 145.5, 266.6, and 564.3 mg/kg/d
- 7 days
- n=4/dose level
- RNA



Significant genes: FDR adjusted p-value <0.05, absolute fold-change >=2

In vivo DEHP results in dose dependent change *Acot*

Male B6C3F1 Mouse



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- n=4/dose level
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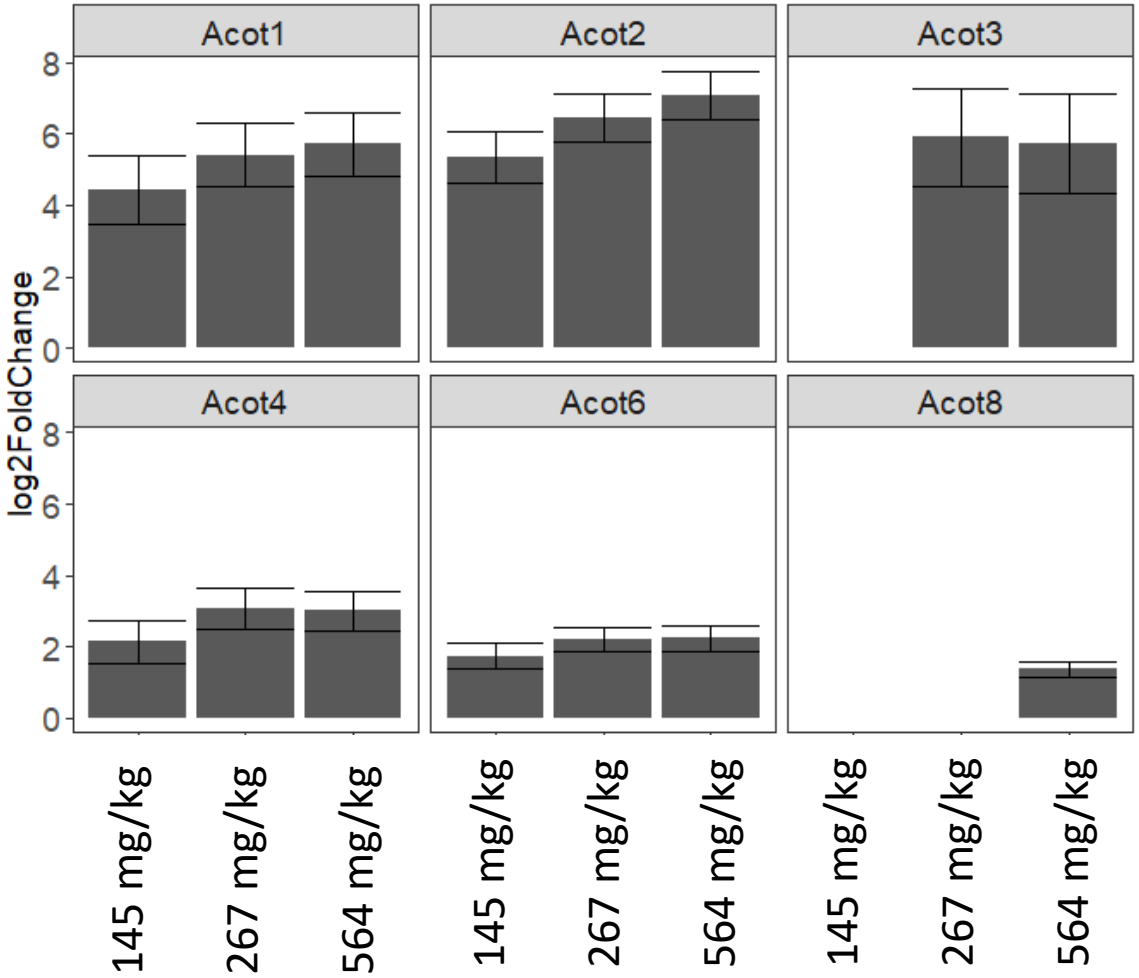
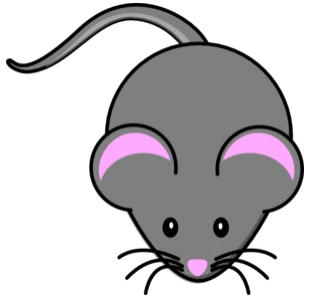


Significant genes: FDR adjusted p-value <0.05, absolute fold-change >=2

Lowest median gene set BMD (24.2 mg/kg/d) is 1.4-fold lower than the BMD for hepatocellular adenoma or carcinoma (35 mg/kg-day) at 2 years

Male B6C3F1 Mouse



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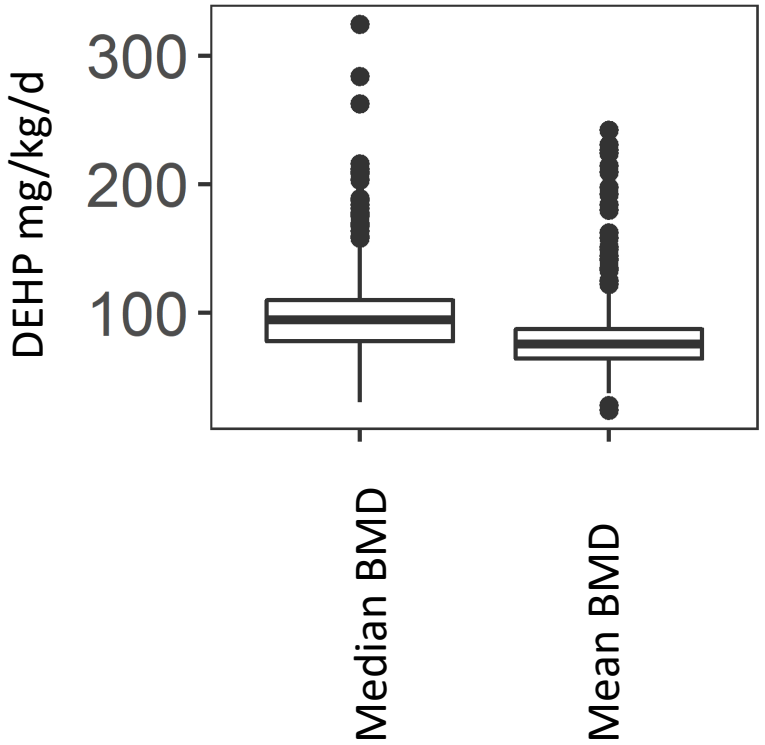
Hester et al. 2016 10.1093/toxsci/kfw161

- Dietary exposure: 0, 145.5, 266.6, and 564.3 mg/kg/d
- 7 days
- n=4/dose level
- ANOVA p-value < 0.05
- Max fold change >= 2
- BMR=10%
- Best BMD <= 564.3 mg/kg/d
- Best BMDU/Best BMDL < 40

GO Gene-set level



Chemical	Lowest Median BMD GO (mg/kg/d)	BMDL (mg/kg/d)	GO Description
DEHP	24.2	13.7	acyl-CoA metabolic process

In vivo experiment 1.

Male B6C3F1 Mouse

- Liver
- 0, 1500, 3000, 6000 ppm DEHP
- Dietary exposure
- 7 days
- n=4/dose level
- RNA

Lake et al. 2016 doi:10.1093/toxsci/kfv236
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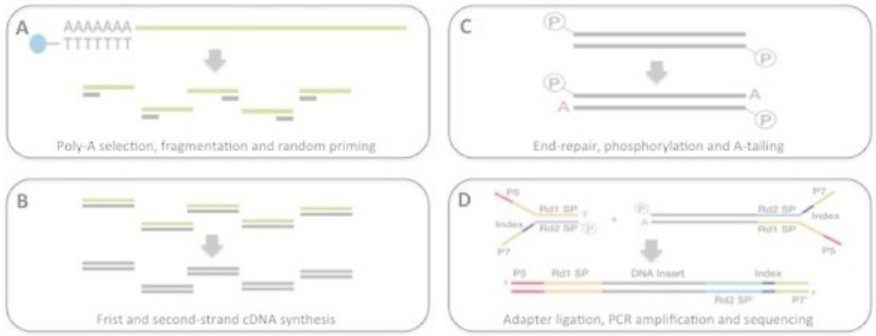
Total RNA-seq



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In vitro experiment 2.

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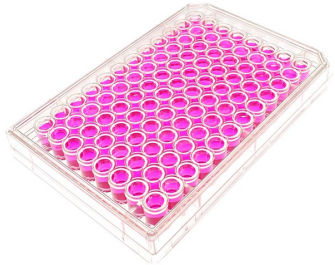

- 0.016-10 µM DEHP
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- Five-fold dilution
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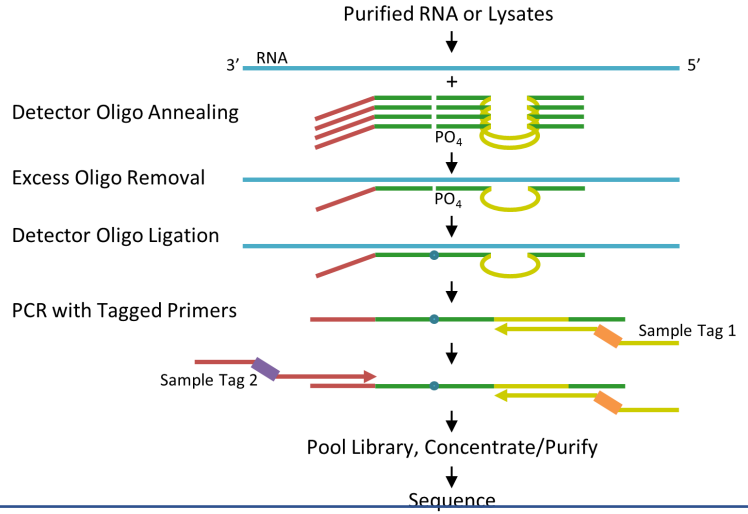
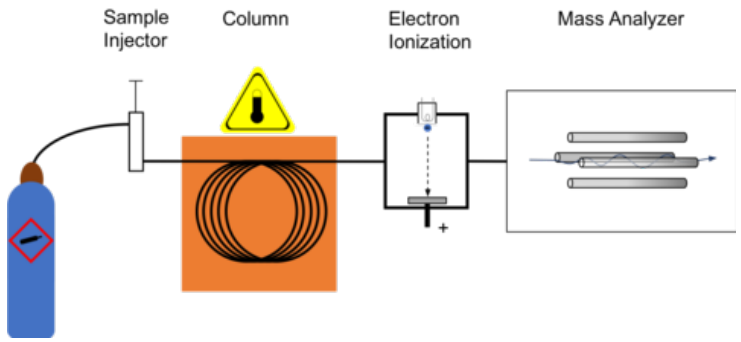
- 1 day
- n=4 assays
- Cell lysates
- Cell medium

Mouse Whole Transcriptome TempO-seq

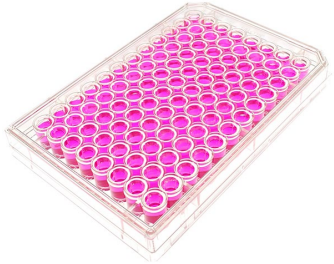


Analytical Chemistry



In vitro response to DEHP with a few common genes across all concentration groups

B6C3F1 primary liver cells



- 0.016-10 µM DEHP
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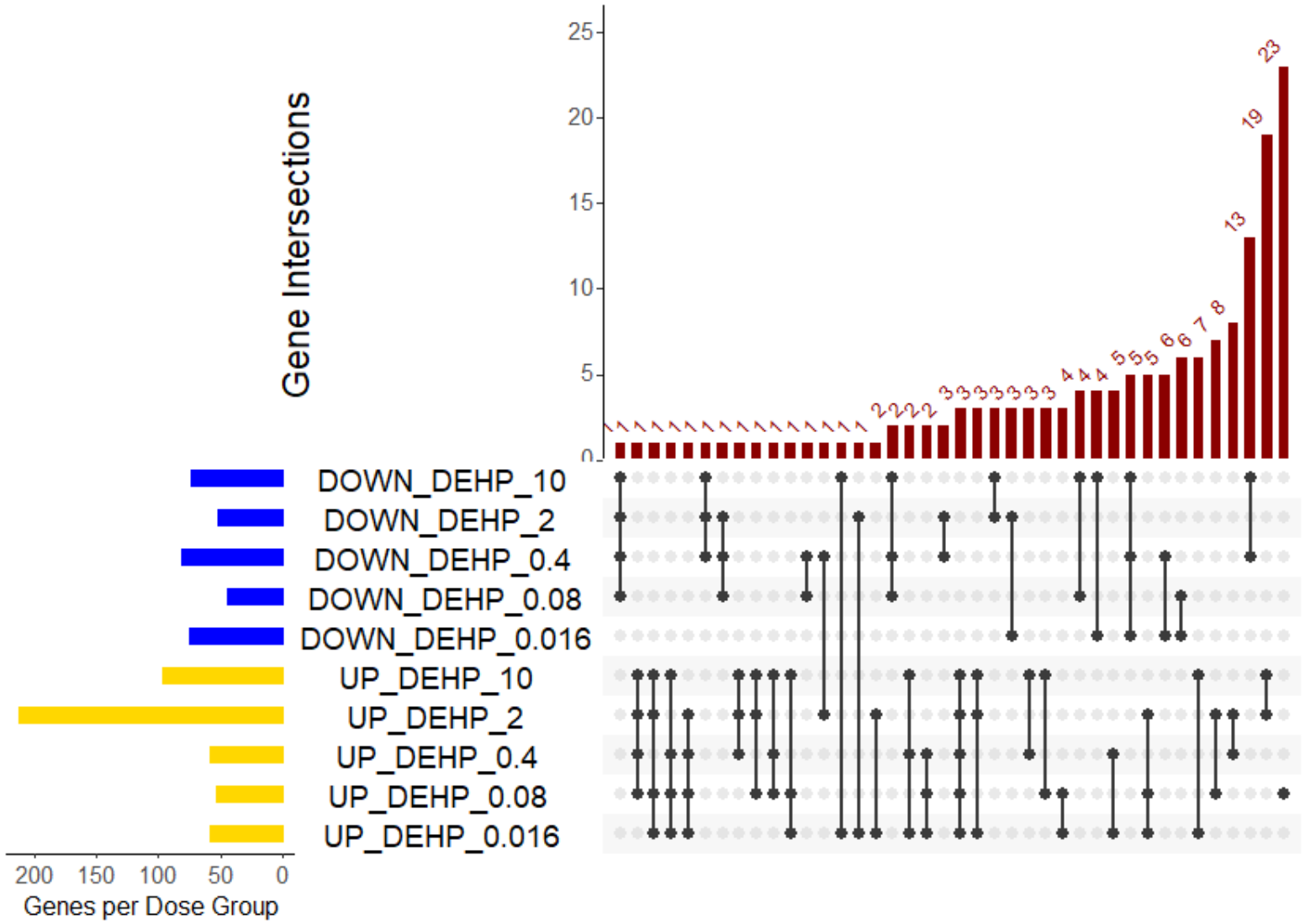
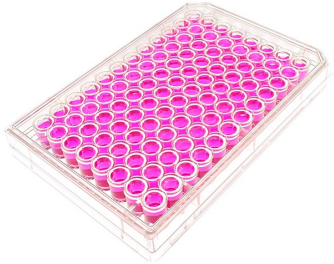


Significant genes: p-value <0.05, absolute fold-change >=2

In vitro response to MEHP showing some concentration dependent changes

B6C3F1 primary liver cells



- 0.016-10 µM DEHP
- 0.056-35 µM MEHP
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- 0.1% DMSO vehicle
- 1 day
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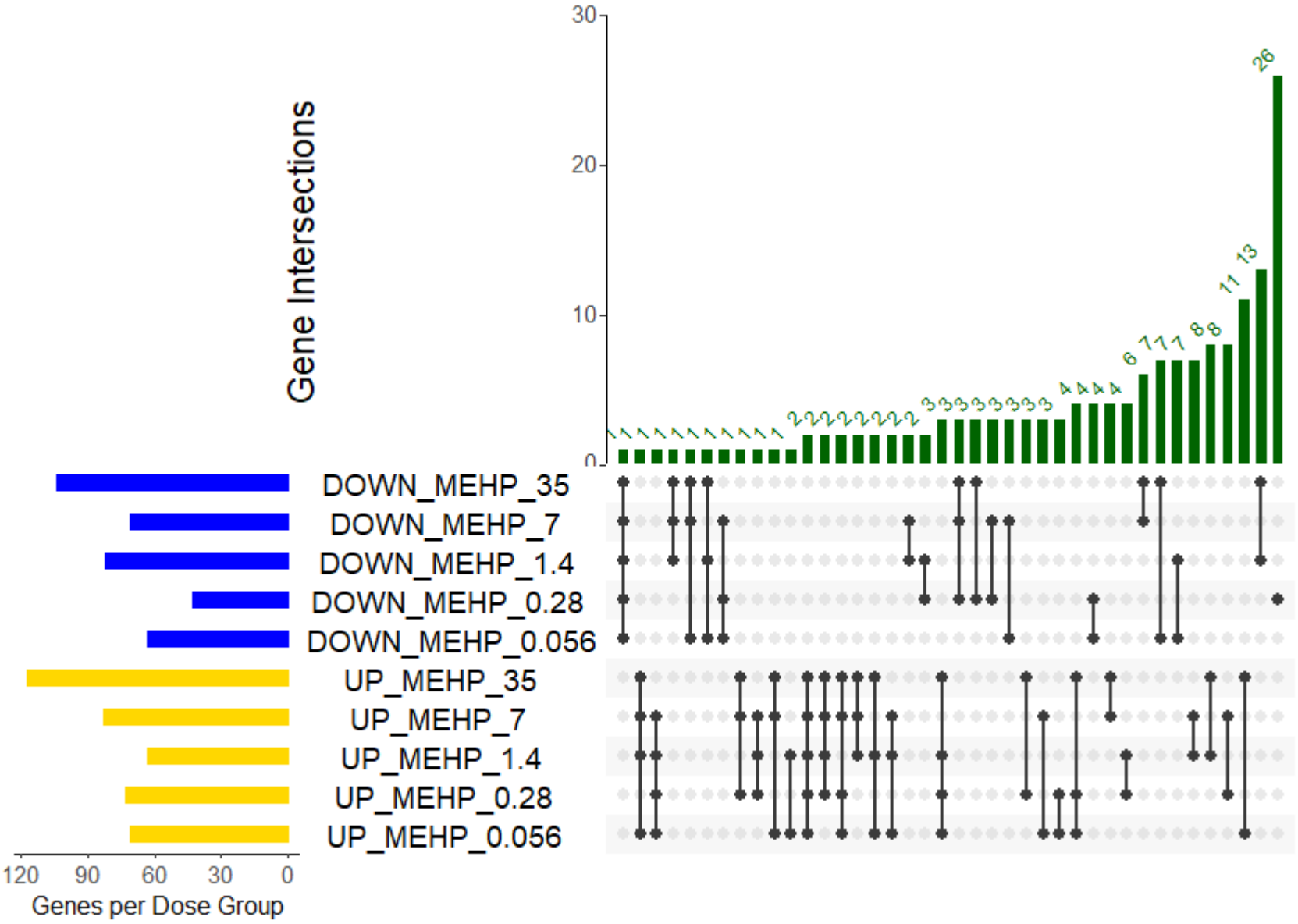
- Cell medium



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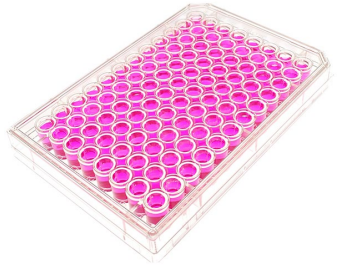
Overlap between DEHP & MEHP high concentration genes

B6C3F1 primary liver cells



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- 1 day
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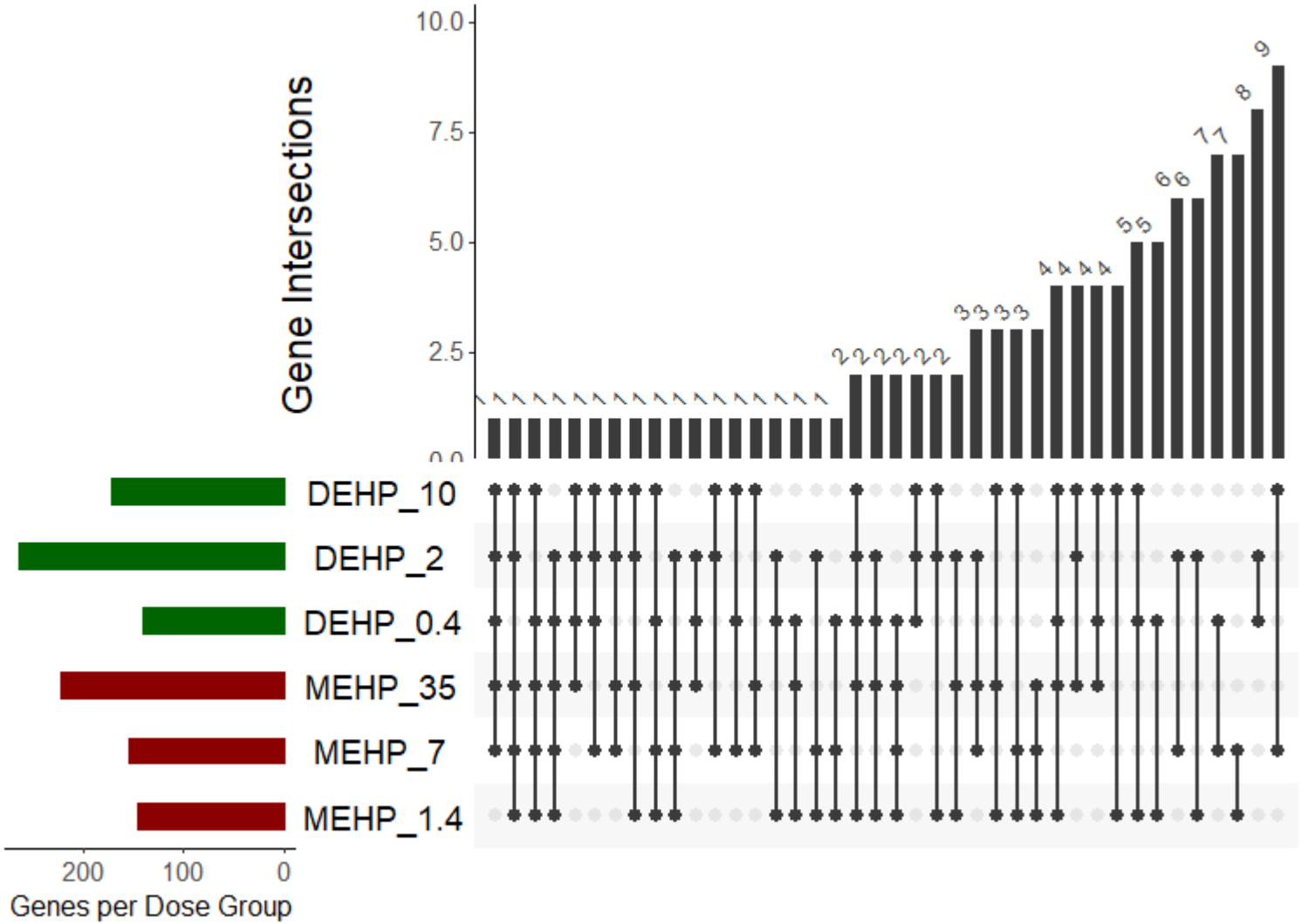
- Cell medium



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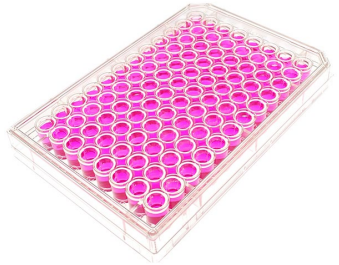
Significant *Acot* response only in high MEHP

B6C3F1 primary liver cells



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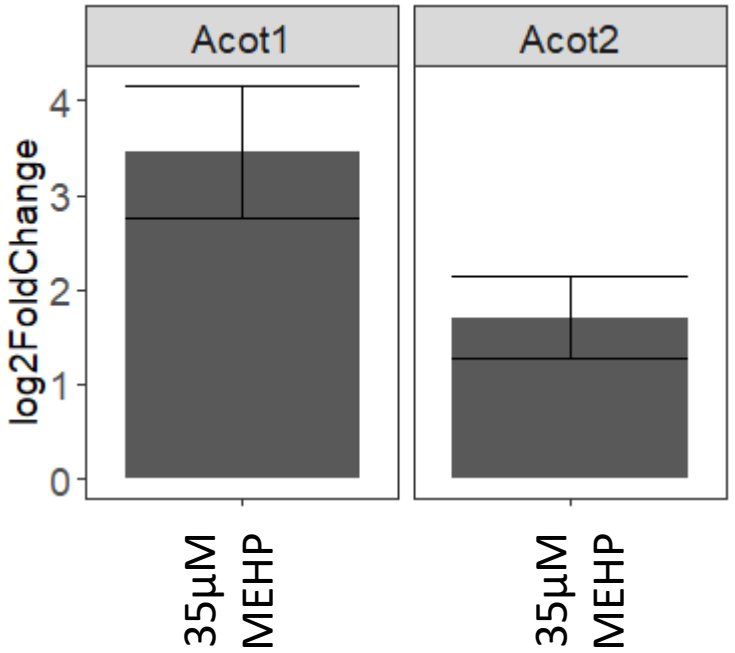

Level of induction of *Acot1* is similar to 145 mg/kg/d DEHP
Level of *Acot2* is ~2.8 fold less than 145 mg/kg/d DEHP

Significant genes: p-value <0.05, absolute fold-change >=2

Gene set benchmark concentration response 0.5 (DEHP) and 11.7 (MEHP) µM

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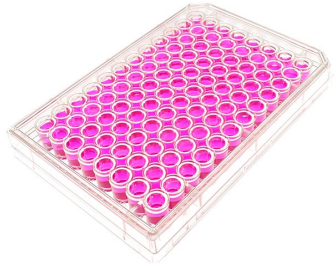

- 0.016-10 µM DEHP
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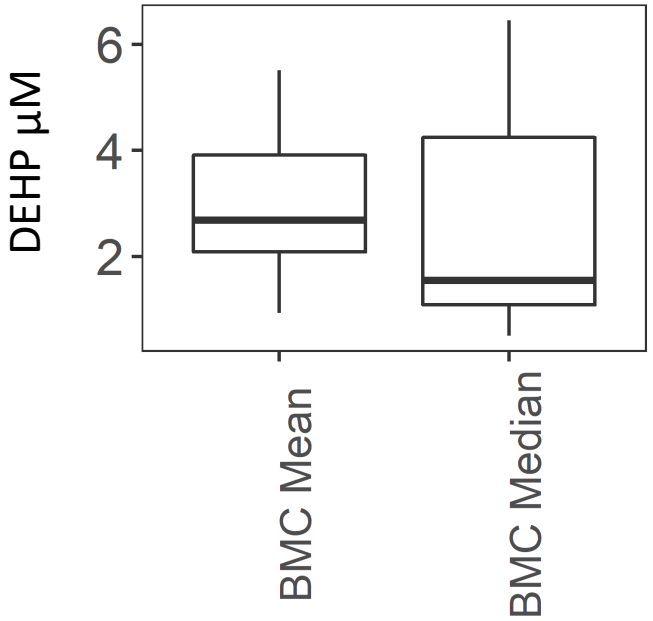
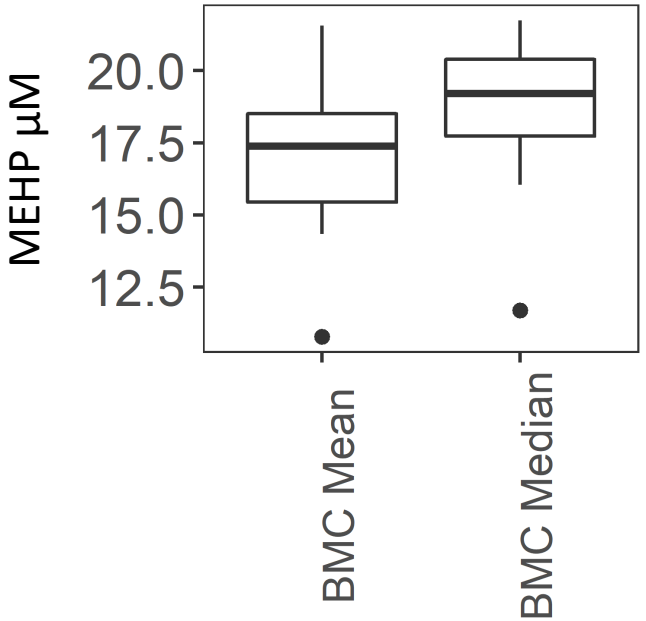
- n=4 assays
- Fisher's Exact Two-Tailed p-value < 0.05
- >= 3 genes enriched

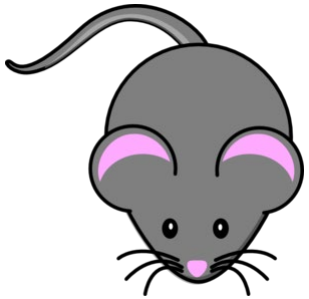




Chemical	Lowest Median BMC GO (µM)	BMCL (µM)	GO Description
DEHP	0.5	0.2	in-utero embryonic development
MEHP	11.7	8.8	transmembrane receptor protein tyrosine kinase signaling pathway

In vivo experiment 1.

Male B6C3F1 Mouse



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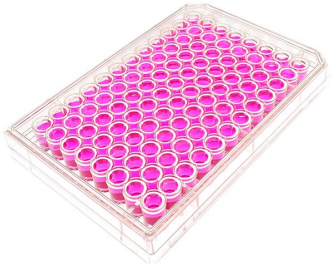
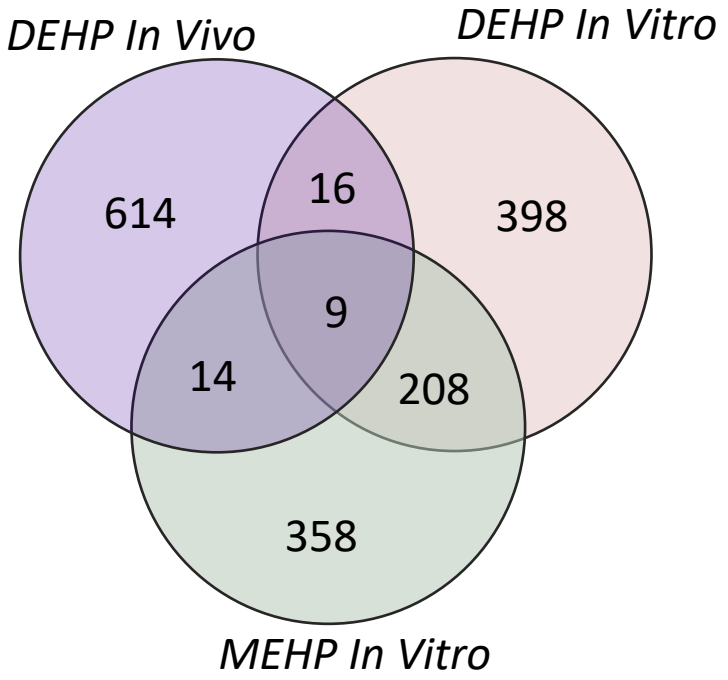


- 0.016-10 µM DEHP
- 0.056-35 µM MEHP
- Five-fold dilution
- 0.1% DMSO vehicle
- 1 day
- n=4 assays
- Cell lysates
- Cell medium

Modest overlap in significant genes



Exp	Assay	Chemical	Lowest Median BMC/BMD GO	BMCL/BMDL	GO Description
1	in vivo	DEHP	24.2 mg/kg/d	13.7 mg/kg/d	acyl-CoA metabolic process
2	in vitro	DEHP	0.5 uM	0.2 uM	in-utero embryonic development
2	in vitro	MEHP	11.7 uM	8.8 uM	transmembrane receptor protein tyrosine kinase signaling pathway

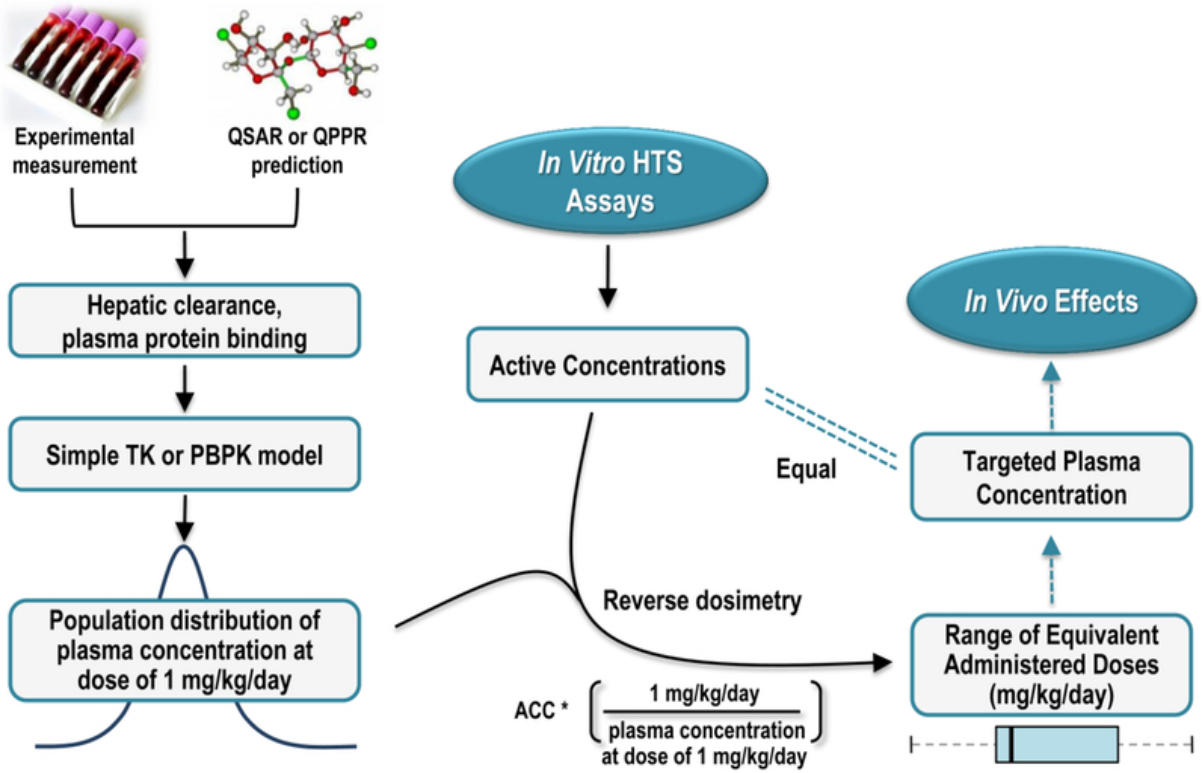
Future Work and Conclusions

Future

- Confirm exposure concentrations
- Estimate blood concentration and relate to *in vivo* dose for IVIVE

Conclusions

- Cell culture response less robust
- Gut metabolism may be an important factor
- Heavy use of plastics can create added challenges



Bell et al. 2018, doi: 10.1016/j.tiv.2017.11.016

Questions

Molecular | Cellular | Tissue | Organ | Individual | Population

Molecular Endpoints
(identify relationships)

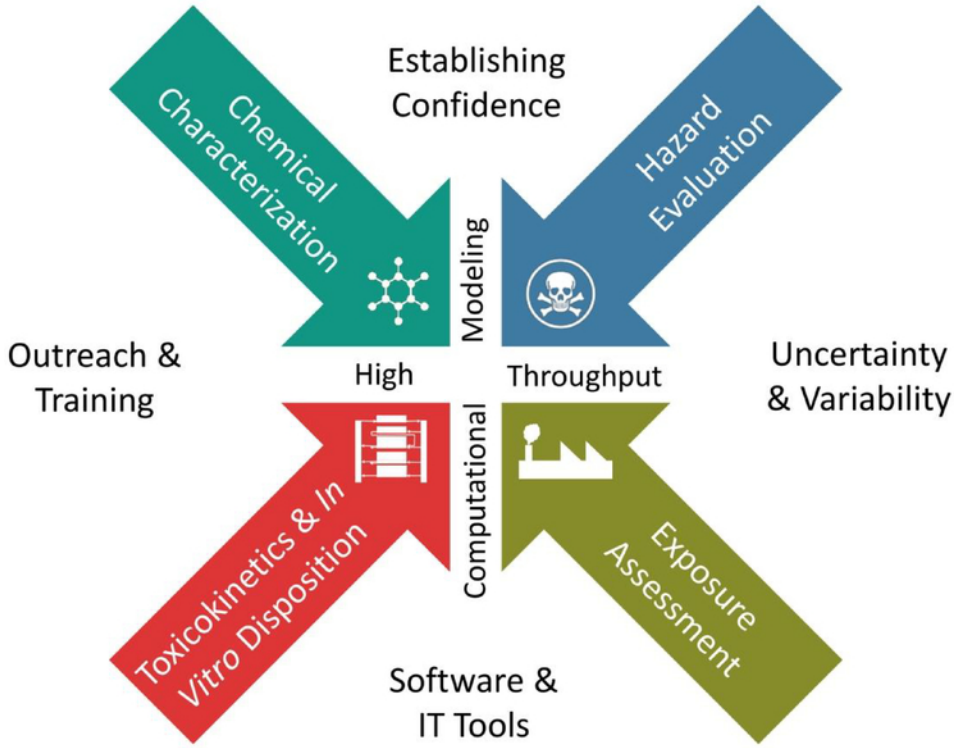
Traditional Endpoints
(associate with adverse effects)

Molecular Initiating Event ▶ Key Events ▶ Adverse Outcome

Existing acute *in vivo* studies can bridge high throughput *in vitro* transcriptomics with chronic adverse outcomes

Potency

Metabolism

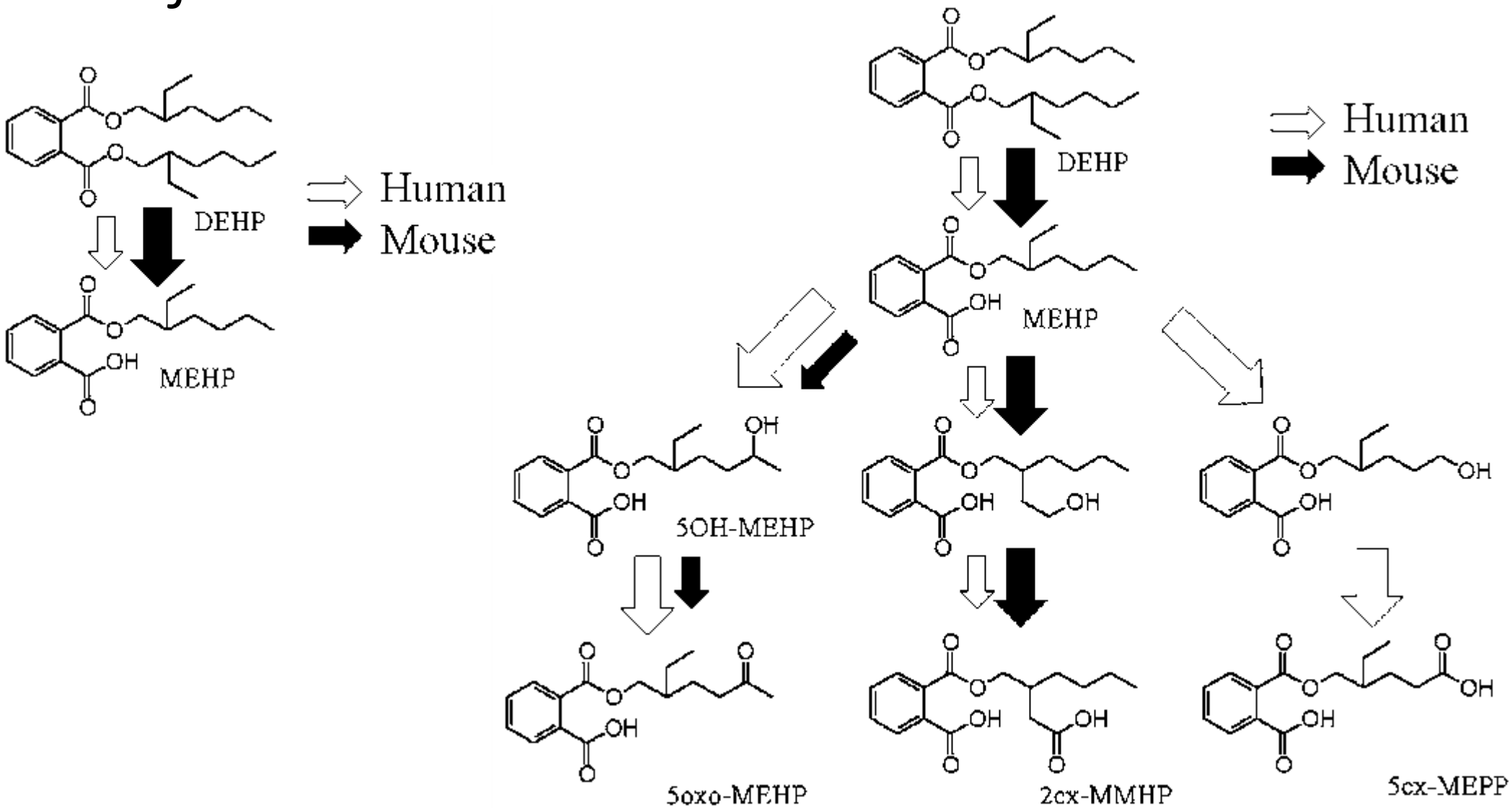


Chemical delivery

Differences in transcriptomic response

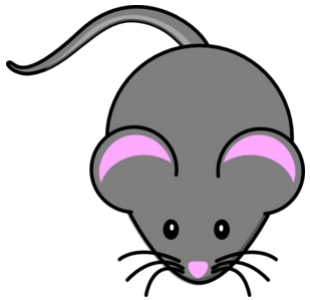
Thomas *et al.* 2019 doi:10.1093toxsci/kfz058

Major metabolites of DEHP



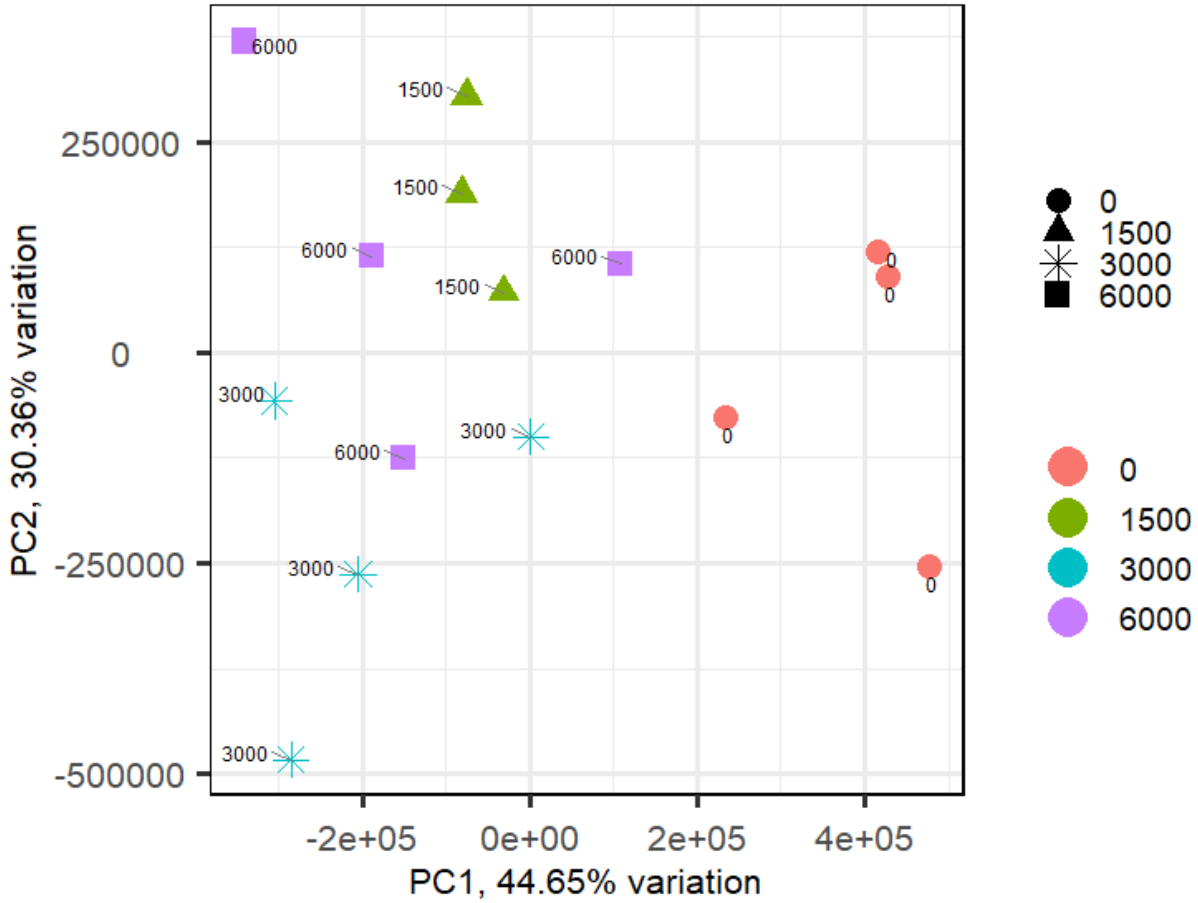
In vivo DEHP results in clear dose separation

Male B6C3F1 Mouse



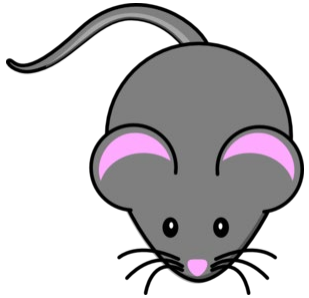
Lake et al. 2016 doi:10.1093/toxsci/kfv236
Hester et al. 2016 10.1093/toxsci/kfw161

- Liver
- 0, 1500, 3000, 6000 ppm DEHP
- Dietary exposure: 0, 145.5, 266.6, and 564.3 mg/kg/d
- 7 days
- n=4/dose level
- RNA



In vivo DEHP results in dose dependent change in genes

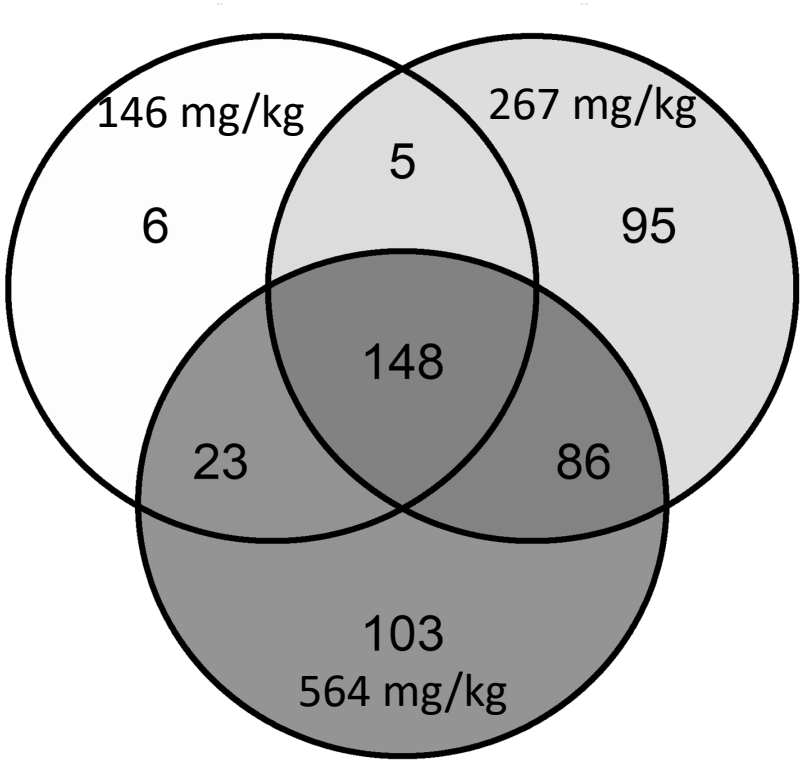
Male B6C3F1 Mouse



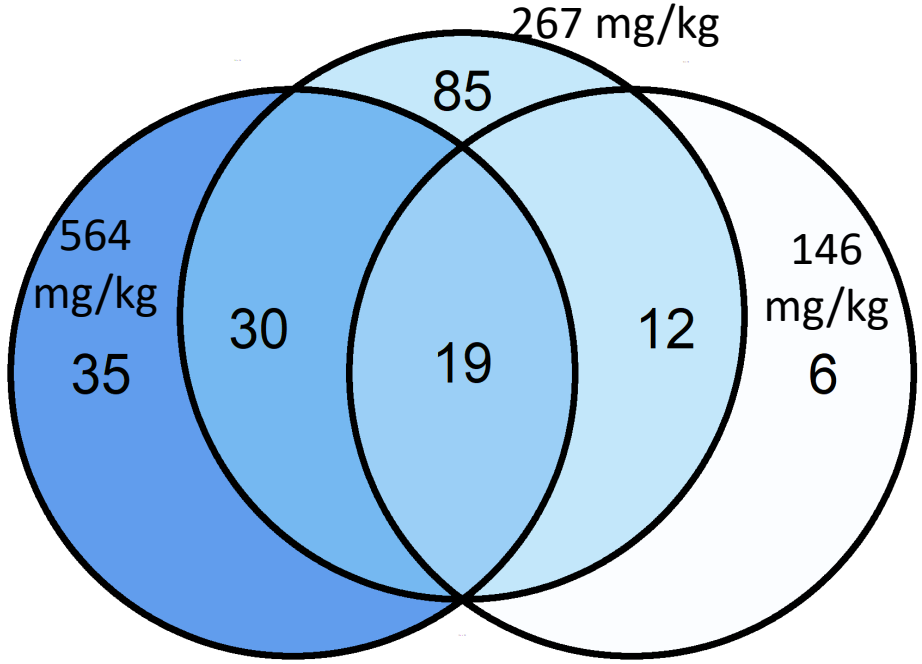
Lake et al. 2016 doi:10.1093/toxsci/kfv236
Hester et al. 2016 10.1093/toxsci/kfw161

- Liver
- 0, 1500, 3000, 6000 ppm DEHP
- Dietary exposure: 0, 145.5, 266.6, and 564.3 mg/kg/d
- 7 days
- n=4/dose level
- RNA

Up-Regulated Genes



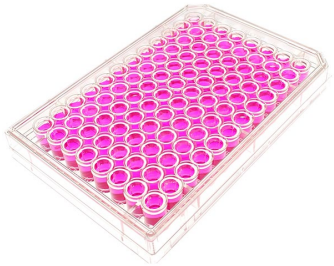
Down-Regulated Genes



Significant genes: FDR adjusted p-value <0.05, absolute fold-change >=2

In vitro response to DEHP & MEHP less robust but concentration dependent changes present

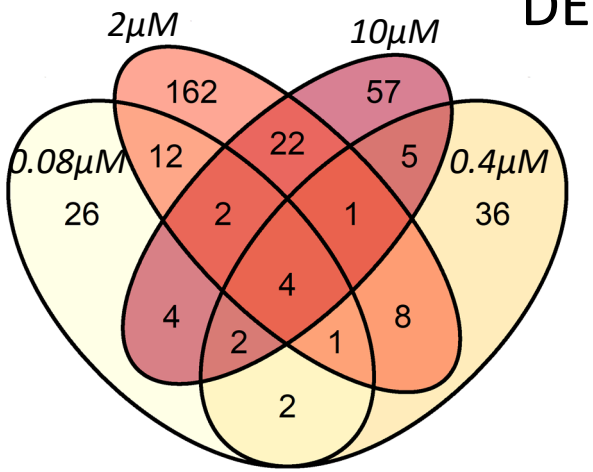
B6C3F1 primary liver cells



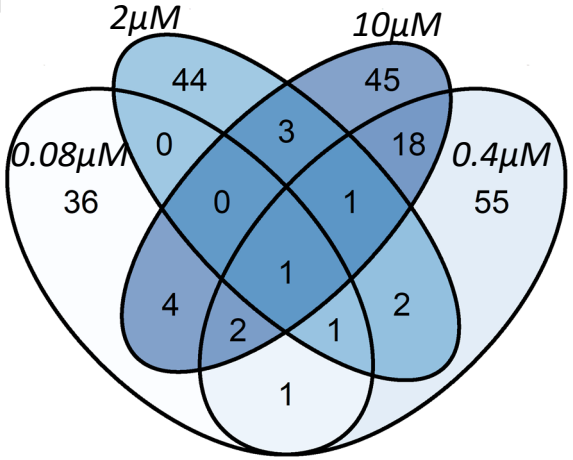
- 0.016-10 µM DEHP
- 5.6-35 µM MEHP
- Five-fold dilution
- 0.1% DMSO vehicle
- 1 day
- n=4 assays
- Cell lysates
- Cell medium

Up-Regulated Genes

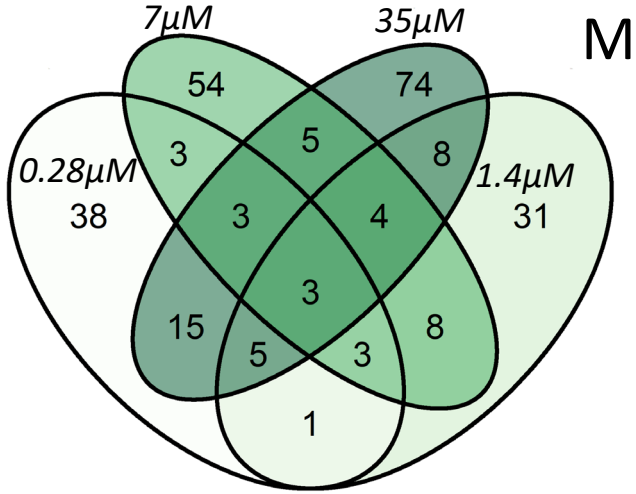
DEHP

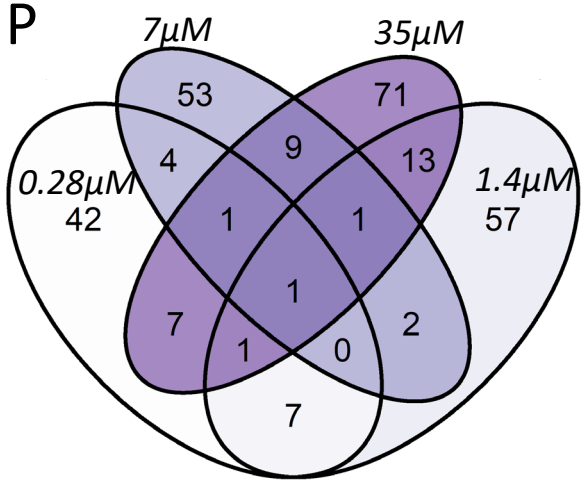


Down-Regulated Genes



MEHP

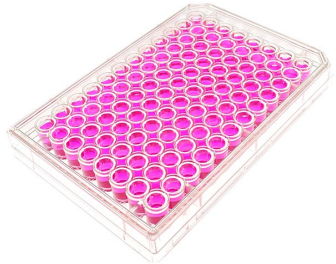




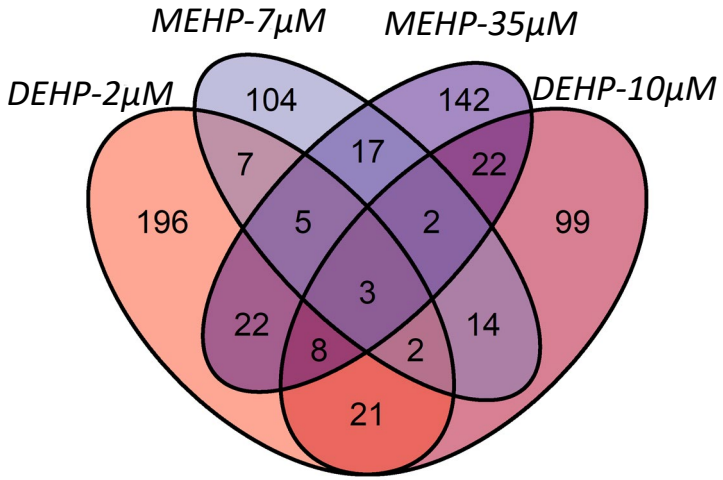
Significant genes: p-value <0.05, absolute fold-change >=2

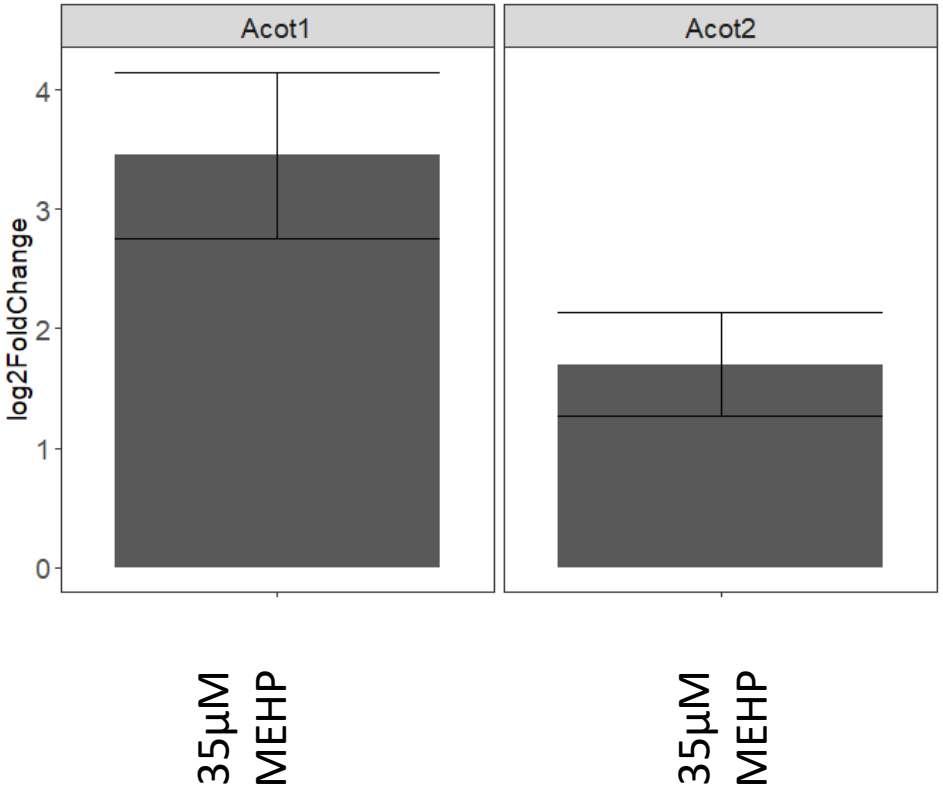
Overlap between DEHP & MEHP high concentration genes
Significant *Acot* response only in high MEHP

B6C3F1 primary liver cells



- 0.016-10 µM DEHP
- 0.056-35 µM MEHP
- Five-fold dilution
- 0.1% DMSO vehicle
- 1 day
- n=4 assays
- Cell lysates
- Cell medium





Significant genes: p-value <0.05, absolute fold-change >=2