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# Passive dosing for the zebrafish embryo test using silicone O-rings

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## Background

### Zebrafish Embryo Toxicity (ZFET) Test: OECD TG236

Hydrophobic organic compounds (HOCs) pose a challenge due to poorly defined exposure conditions.

#### Passive dosing

- Silicone O-rings
- Equilibrium partitioning

HOC(s) in methanol



Loading of O-rings



HOC(s) in test medium

Continuously replenish test medium



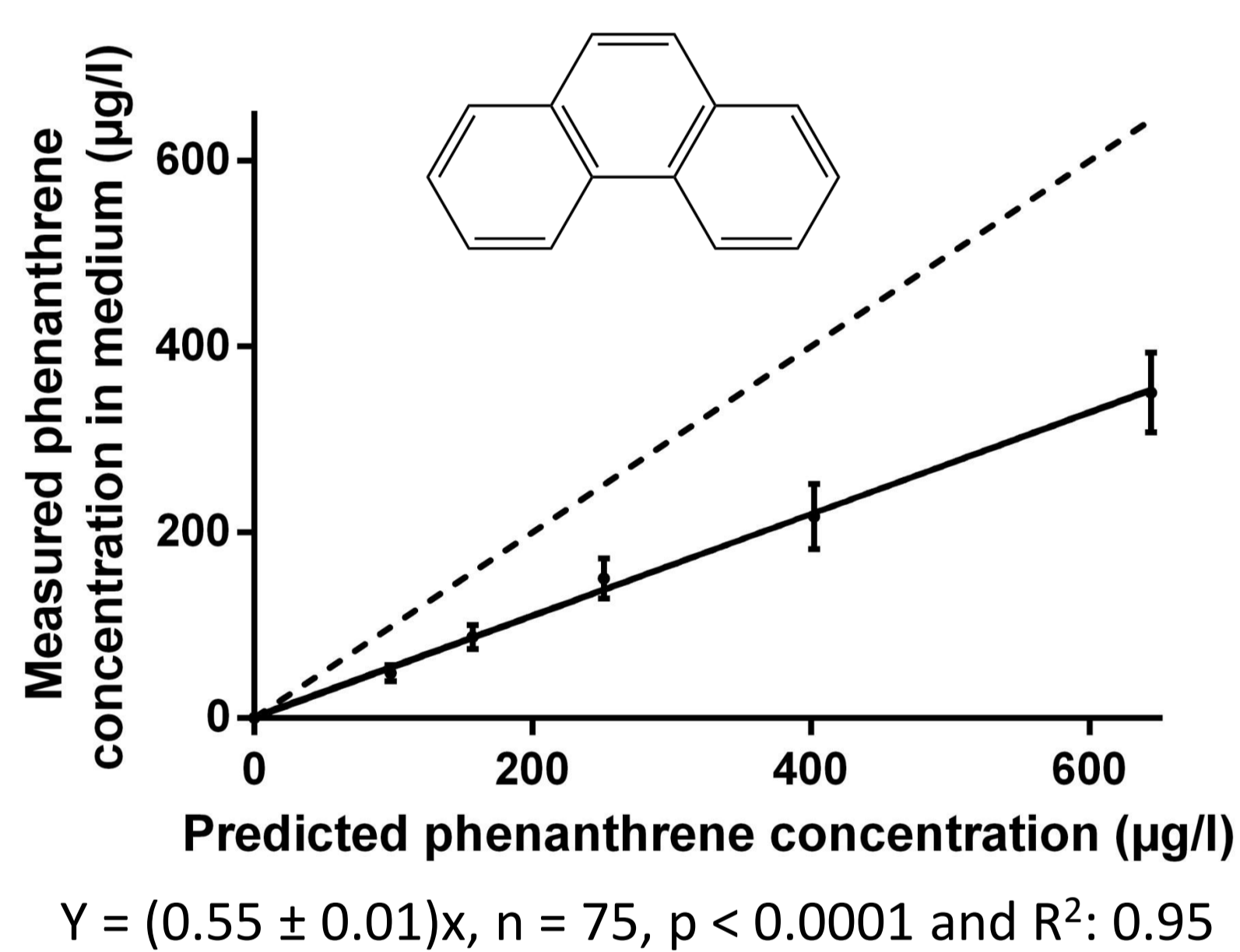
#### Loading principle:

Loading from a saturated methanol solution results in saturation of the test medium during exposure.

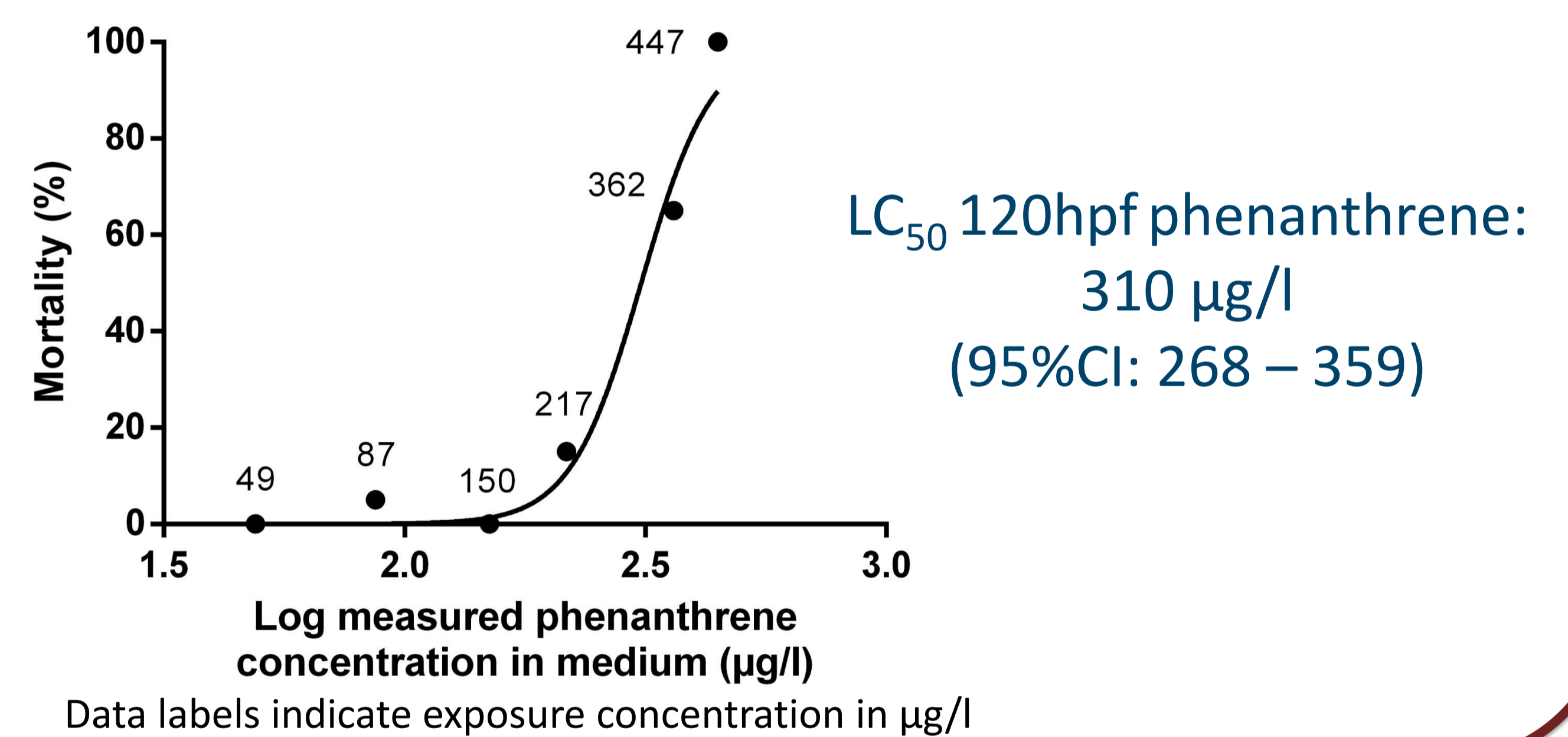
Loading from dilutions of the saturated methanol solution results in corresponding dilutions of the test medium.

## Results & Discussion

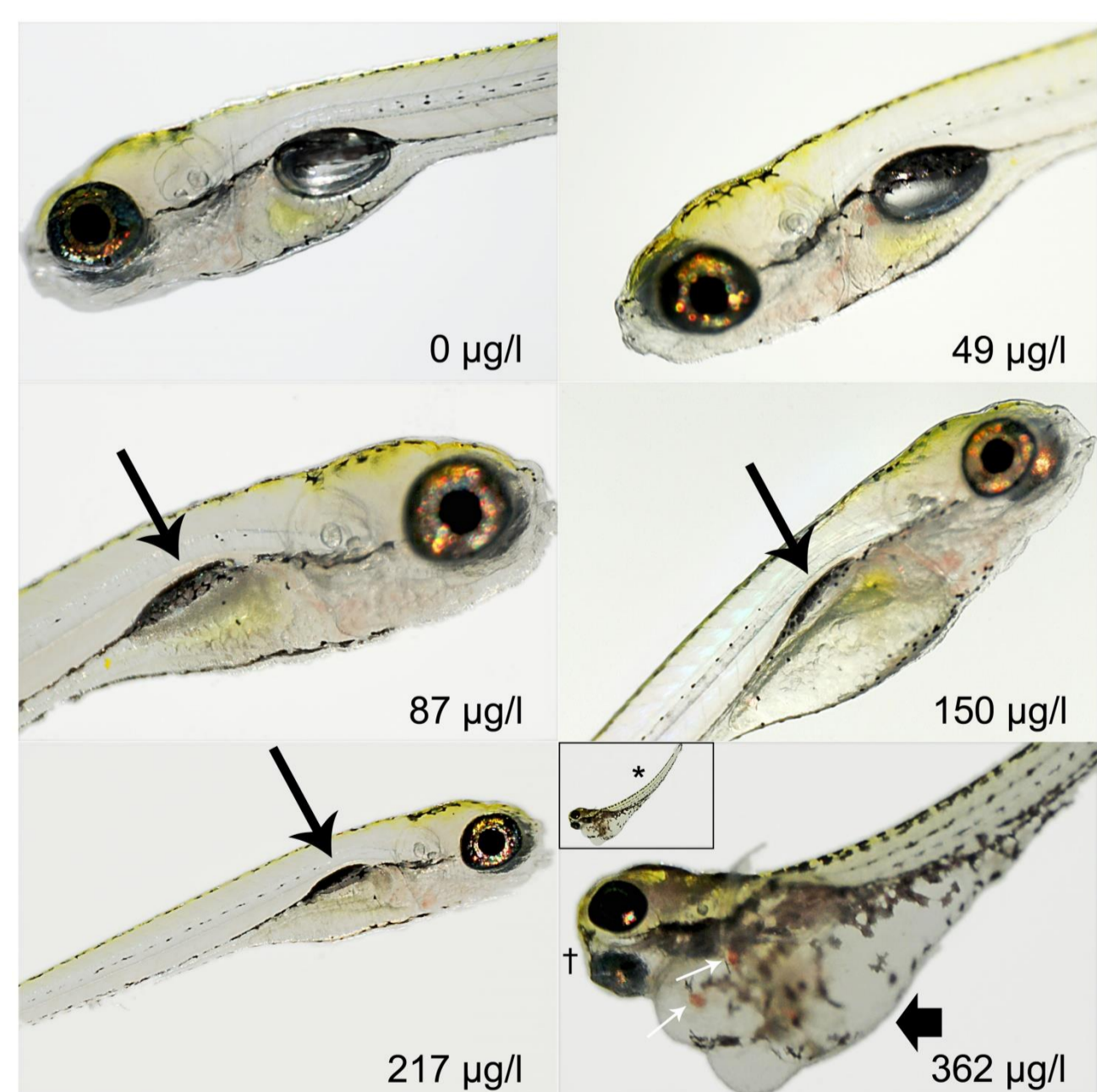
### Silicone O-rings produce a reliable and stable exposure concentration series



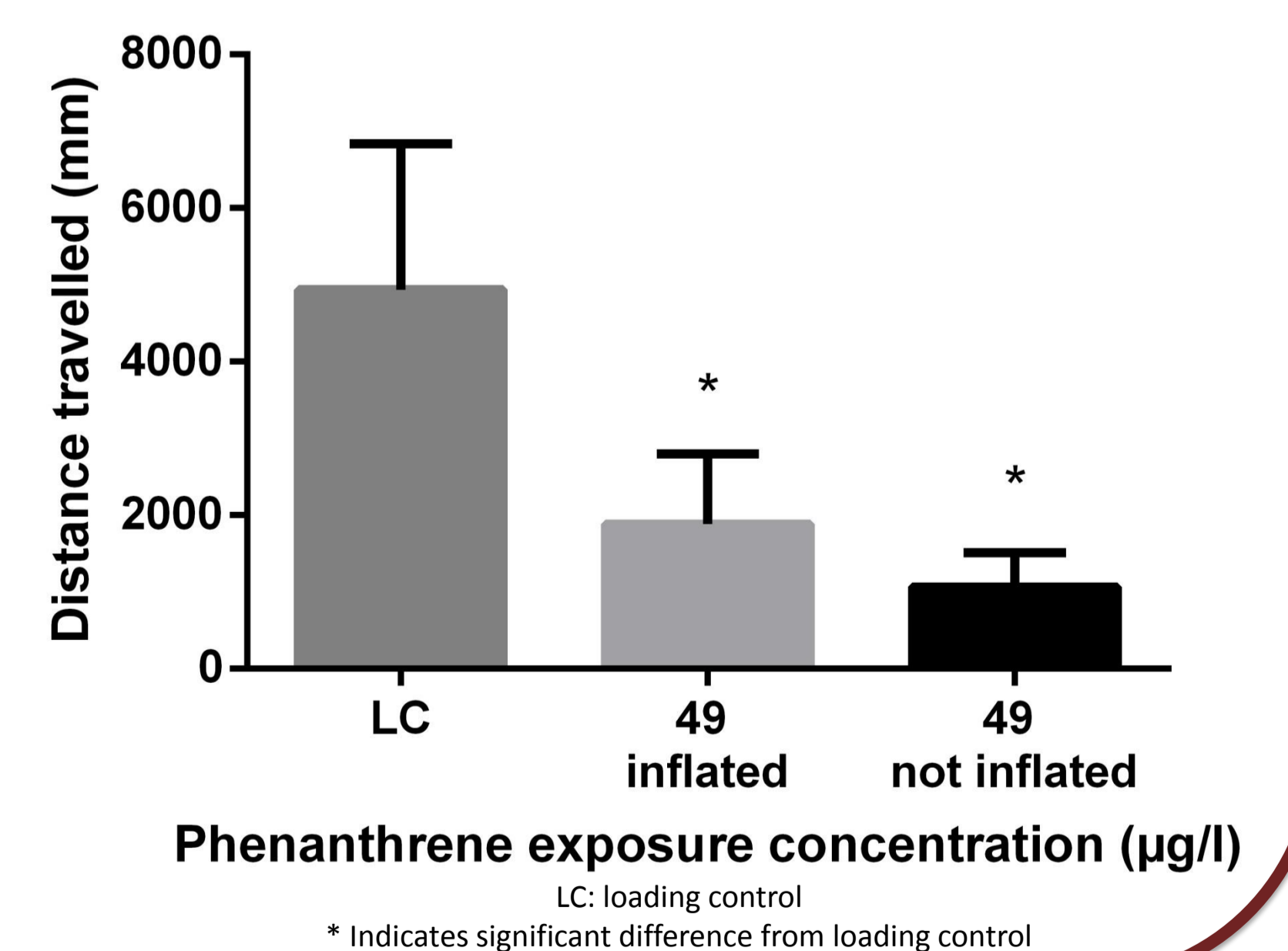
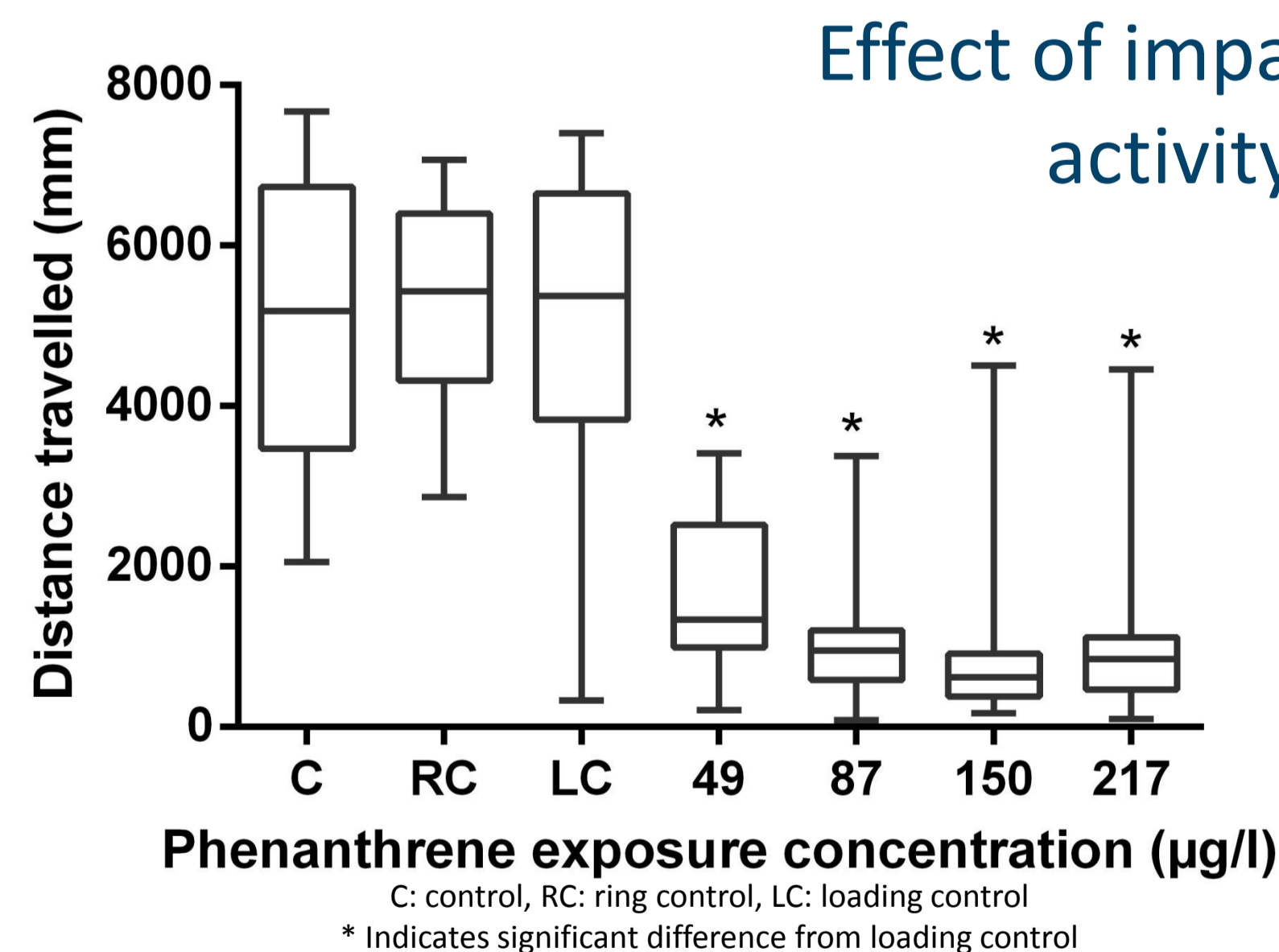
### Controlled exposure concentrations facilitate precise determination of effect concentrations



### Silicone O-rings are practical and allow for obtaining mechanistic toxicity information.



Impaired inflation of posterior swim bladder chamber is most important sublethal effect.



thin black arrow: swim bladder not inflated; thick black arrow: oedema of yolk (extension) and pericard; white arrow: blood accumulation in heart and yolk; †: malformation of eyes and mouth; asterisk in inset: curvature of the tail

#### Advantages

- Reliable exposure when analytically confirmed
- Compatible with standard 24-well plate format
- Allows for individual follow-up
- Loading principle does not require prior knowledge of chemical partitioning behaviour

#### Practical considerations

- Cleaning and loading procedure time-consuming
- Testing at saturation would require loading at experimental temperature

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