

# Considerations for valid exposure designs to generate data that is relevant for an Ecological Risk Assessment

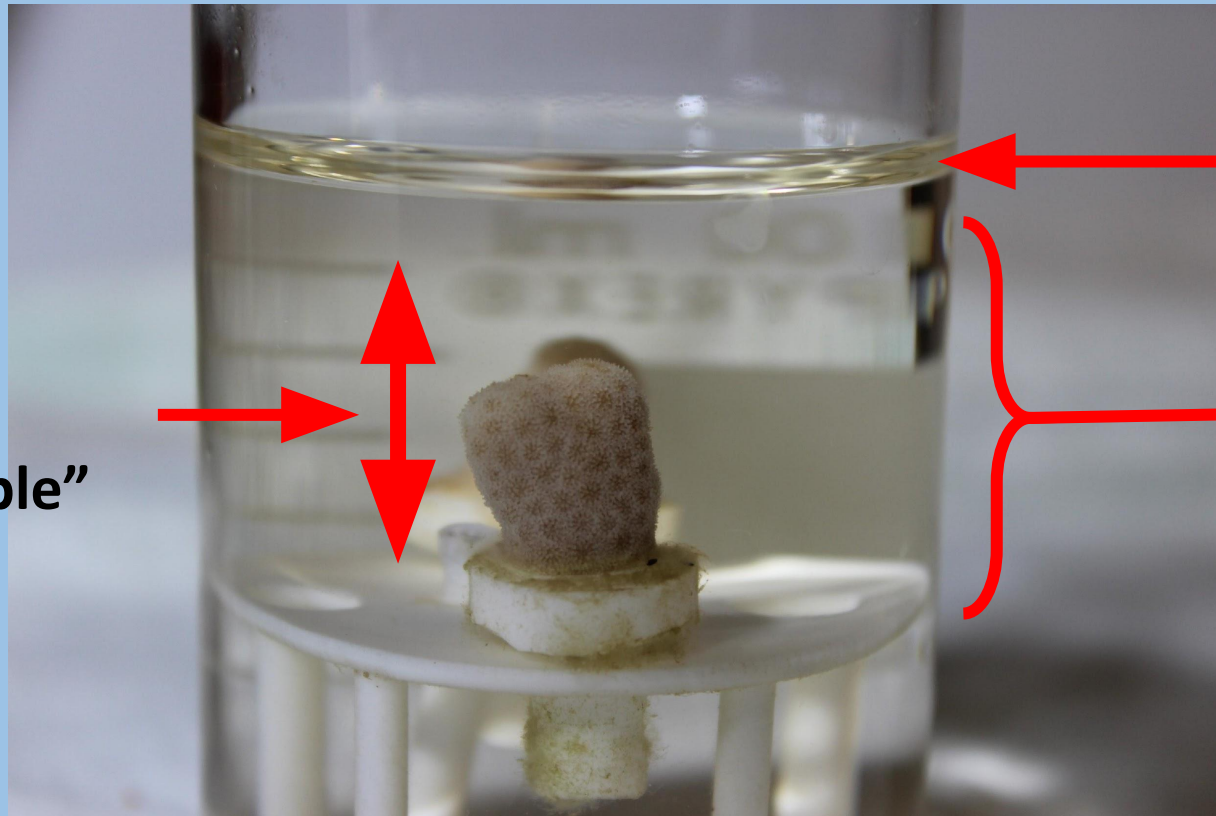
Craig Downs, Ph.D.

# Factors for valid exposure design for Ecological Risk Assessment relevance

1. Actual Concentration vs. Assumed Concentration
2. Static vs semi-static depending on length of exposure
3. Exposure Medium (formula)
4. Light: Necessary for realistic and valid photo-toxicity and non-confounding plant/animal culturing

# Actual Concentration vs. Assumed Concentration

**Water Column  
(homogeneous)  
“Biologically available”**



**Meniscus**

**Wall Surface  
(adhesion)**

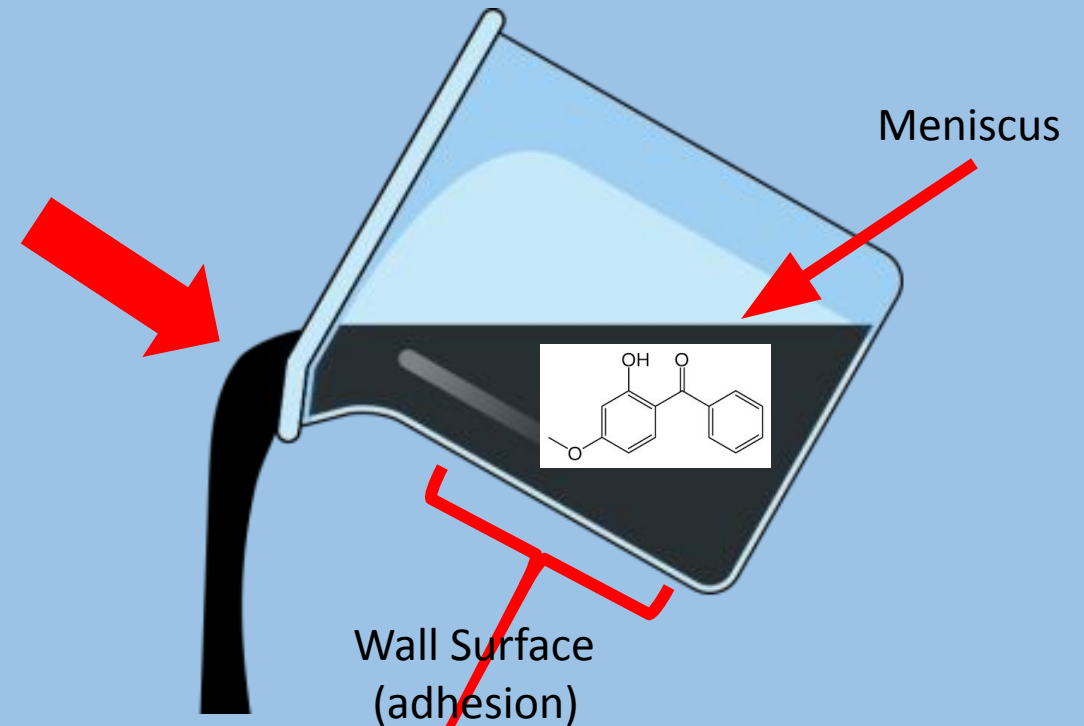
# Actual Concentration vs. Assumed Concentration

## “Measured vs. Nominal Concentration”

**Table 1** Measured BP-3 concentrations in the experimental solutions (mean  $\pm$  standard error)

Nominal concentration	Measured concentration ( $\mu\text{g/L}$ )	
	0 h	48 h
Solvent control	n.d.	n.d.
EE2	n.d.	n.d.
10 $\mu\text{g/L}$	$8.80 \pm 0.28$	$2.17 \pm 0.04$
100 $\mu\text{g/L}$	$87.10 \pm .027$	$22.65 \pm 1.70$
1000 $\mu\text{g/L}$	$887.67 \pm 0.95$	$219.22 \pm 1.52$
<b>“Actual” Concen.</b>	<b>-12%</b>	<b>-75%</b>

Chen, TH., Wu, YT. & Ding, WH. UV-filter benzophenone-3 inhibits agonistic behavior in male Siamese fighting fish (*Betta splendens*). *Ecotoxicology* 25, 302–309 (2016).  
<https://doi.org/10.1007/s10646-015-1588-4>



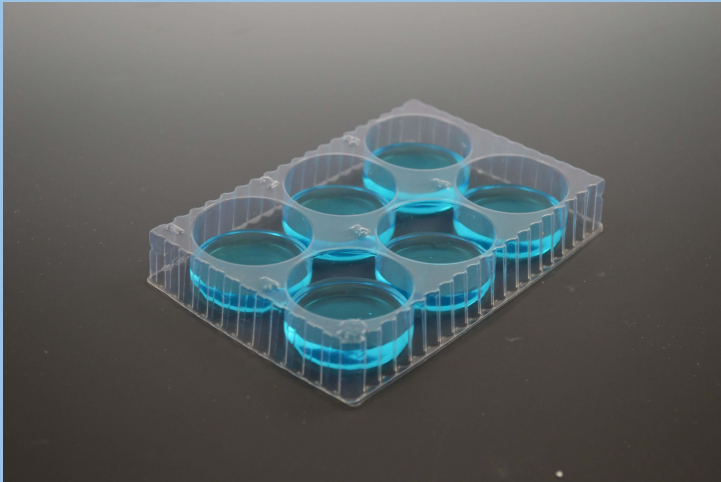
40 CFR § 158.300 *Nominal concentration* means the amount of an ingredient which is expected to be present in a typical sample of a pesticide product at the time the product is produced, expressed as a percentage by weight.

# Actual Concentration vs. Assumed Concentration



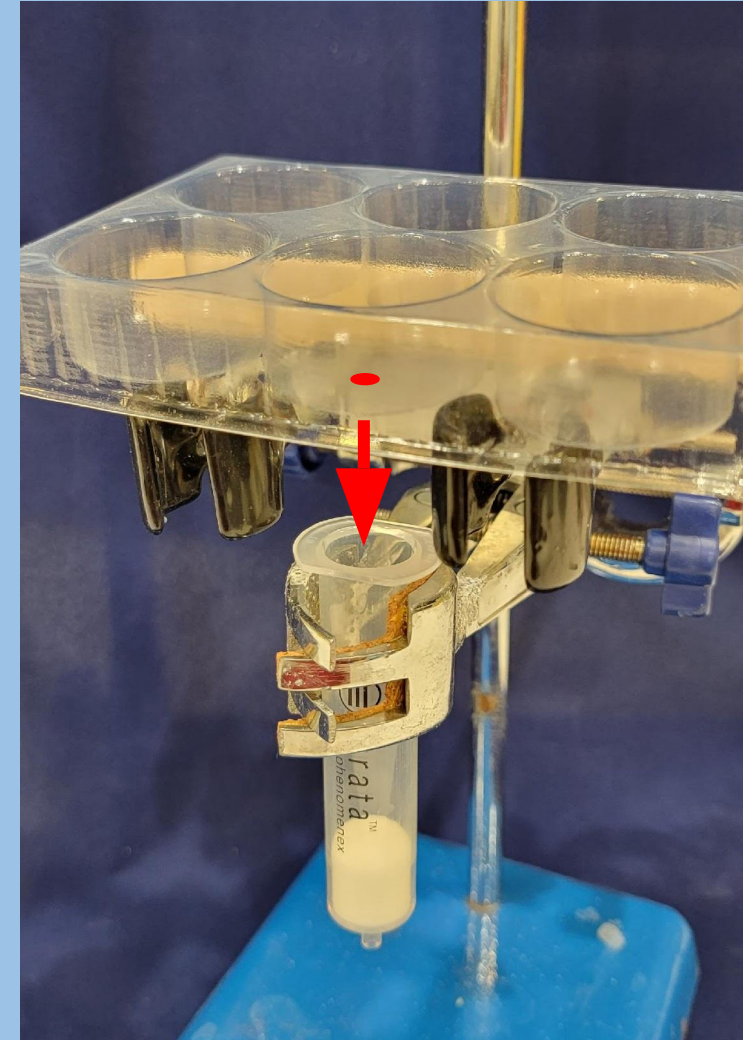
## Virgin Teflon Liners

- No Additives
- EPA Cleaned



Pre-screen to ensure no confounding leachates with exposure media

- Bisphenols
- Organofluorines
- Slip agents (nonylphenol)
- UV filters, including  $\text{TiO}_2$



# Exposure Medium

- Cannot be filtered seawater – not replicable, no idea of composition
  - Confounding xenobiotics, variable total organic carbon (dissolved, particulate), amount of natural organic matter, etc
- Artificial seawater –
  - HPLC-grade water
  - >99% salts (watch out for confounding contamination; heavy metals, PFAS)
  - Fundamentals – pH buffering capacity, ion availability (e.g., Ca<sup>++</sup>)
  - Biological & organic components (increasing biological availability of xenobiotic)
    - Consider drug discovery/*in vitro* toxicity designs (culture media)
    - Consider physiological media recipes



# Static vs semi-static depending on length of exposure

**Table 1** Measured BP-3 concentrations in the experimental solutions (mean  $\pm$  standard error)

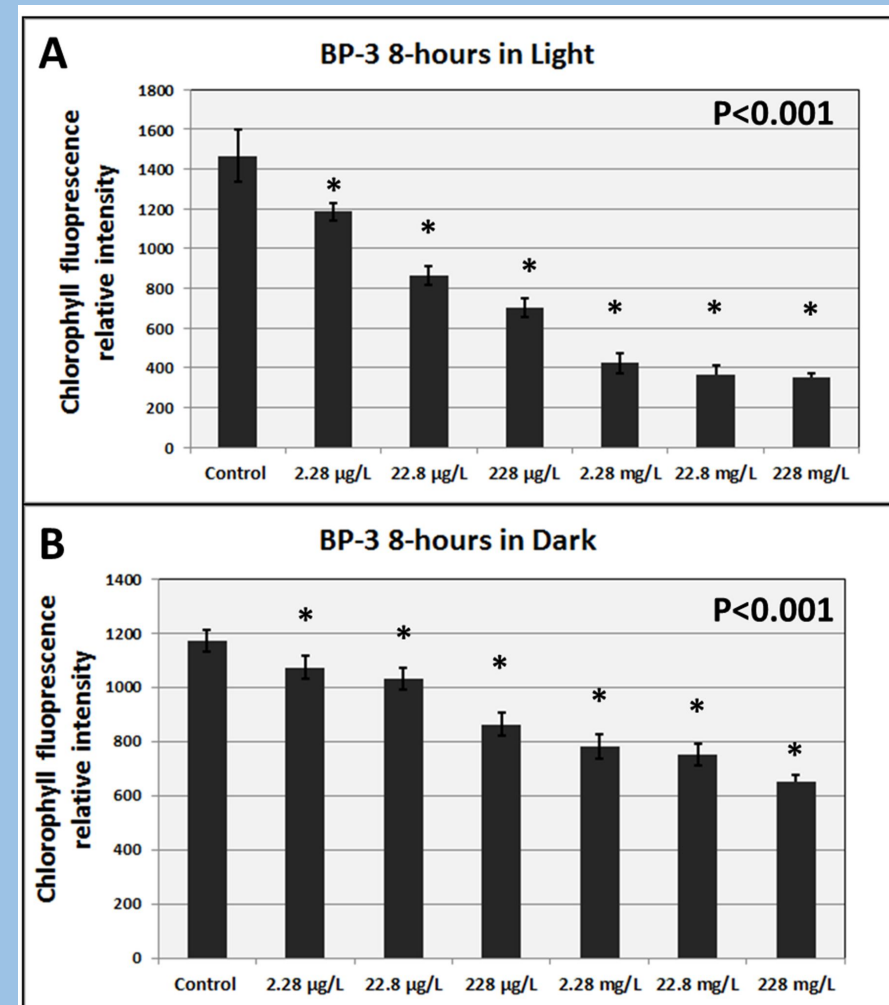
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## Exposure Validation Design

- Determine ACTUAL concentration in water column in exposure system
- Run exposure with model organism
- Measure ACTUAL concentration at 8 hr, 12 hr & 24 hr, etc
- If ACTUAL concentration decreases by 15% at a time point, then need to refresh exposure-media before it reaches -15% of ACTUAL concentration

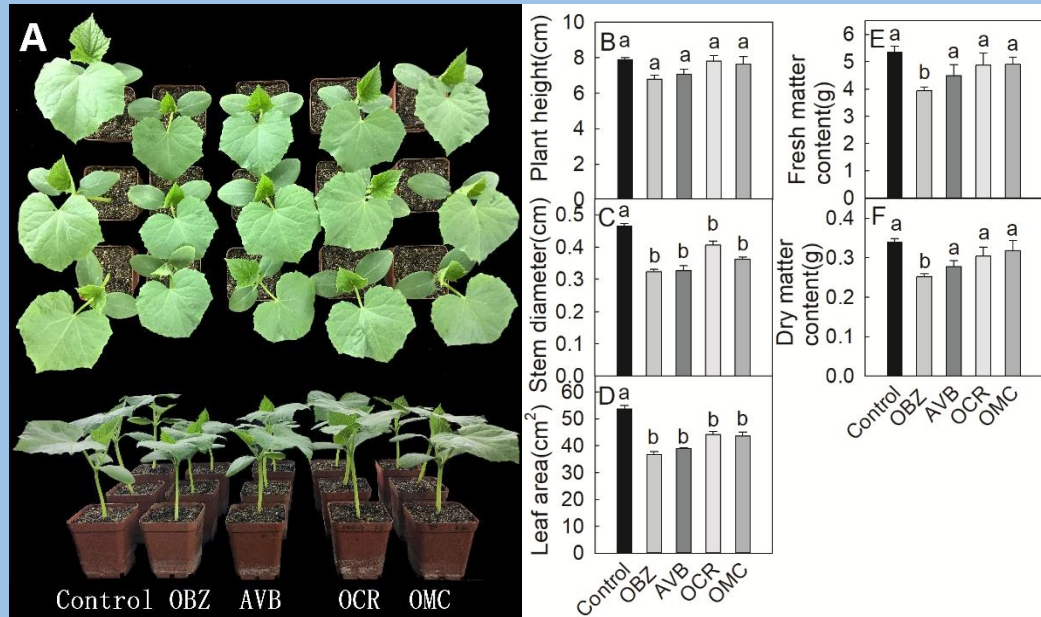
# Can't ignore light as both a driver of toxicity and as a necessary requirement for non-confounding culturing/exposure



Downs, C.A., Kramarsky-Winter, E., Segal, R. et al. Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands. Arch Environ Contam Toxicol 70, 265–288 (2016). <https://doi.org/10.1007/s00244-015-0227-7>



# Can't ignore light as both a driver of toxicity and as a necessary requirement for non-confounding culturing/exposure



Science of the Total Environment 714 (2020) 136879



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)

Comparison of toxicological effects of oxybenzone, avobenzone, octocrylene, and octinoxate sunscreen ingredients on cucumber plants (*Cucumis sativus* L.)

Xin Zhong<sup>a,b</sup>, Craig A. Downs<sup>c</sup>, Yuting Li<sup>a,d</sup>, Zishan Zhang<sup>a,d</sup>, Yiman Li<sup>a,b</sup>, Binbin Liu<sup>a</sup>, Huiyuan Gao<sup>a,d</sup>, Qingming Li<sup>a,b,\*</sup>

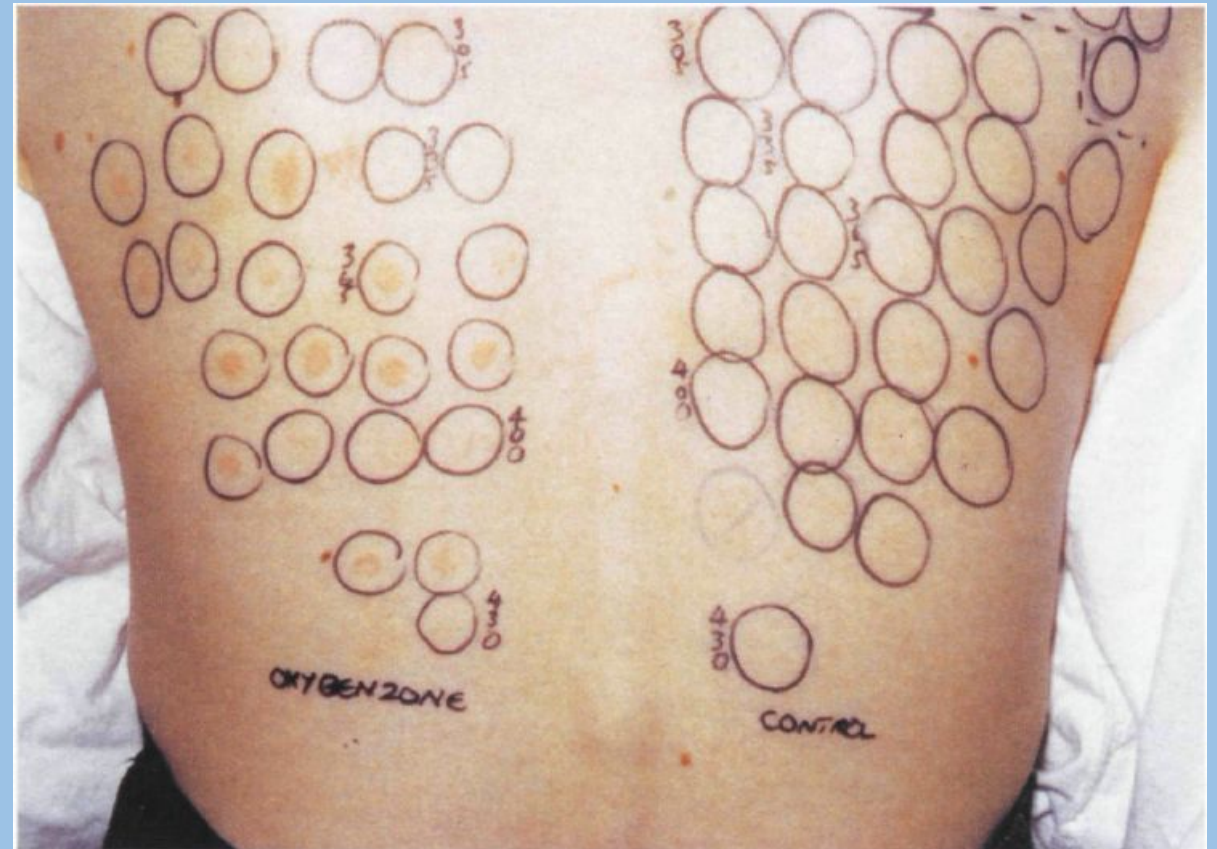


Figure 3. Mirror-image monochromator phototest (right) and photopatch test (oxybenzone 2%) responses on back skin at 24 h. *British Journal of Dermatology* (1994) **131**, 124–129.

## Photoallergic contact dermatitis to oxybenzone

P. COLLINS AND J. FERGUSON

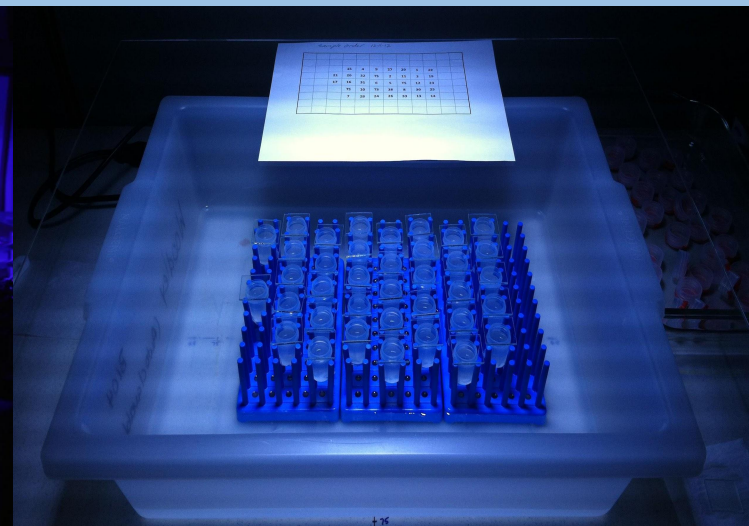
Photobiology Unit, Department of Dermatology, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

Accepted for publication 23 November 1993



# Light

- “Lab light” (fluorescent light fixtures that gives  $\sim 20 \mu\text{moles } m^{-2} s^{-1}$ ) with unrealistic spectrum composition is unacceptable
- Require actual solar incidence attenuated with neutral density filter to provide a justified PPFD intensity, as well as justified UV spectrum
- Replicable LED array with justified simulated solar spectrum at model species depth
  - light intensity and composition differences between 3m and 20m depth



# **Considerations for valid exposure designs to generate data that is relevant for an Ecological Risk Assessment**

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