**How to estimate preliminary CORT doses**

* Range reported in Hanover et al. (2019) as ~5 – 10 pg/mg
* Average delicata egg mass = 0.157 g
* Figure out concentration of CORT per egg

5.0 pg/mg = X pg/157mg

X = 785 pg / egg = 0.785 ng / egg

10.0 pg/mg = X pg/157mg

X = 1570 pg / egg = 1.570 ng / egg

* Want to dose in 5 ul aliquots

**Low dose: 0.785 ng / 5 ul = 0.157 ng/ul = 157 pg/mL**

**High dose: 1.57 ng / 5 ul = 0.314 ng/ul = 314 pg/mL**

**Accounting for transfer**

* Crews et al. (1991) showed that the highest count per minute of topically applied radiolabelled hormone is detectable 25 hours after topical administration
* Crews et al. (1991) show that about 0.7% of the dose of radiolabelled hormone is detected in whole embryo homogenates 25 hours after administration (estimated from figure)

**CORT stock solution**

Molecular weight = 346.46 g/mol

1M = mol/L

Concentration = 1.0 mol/L \* 346.46 g/mol

1M concentration = 346.46 g/L = 346.46 mg/mL

1mM = 0.34646 mg/mL

3mM = 0.34646 mg/mL \* 3

=1.0394 mg/mL

10mM = 0.34646 mg/mL \* 10

= 3.4646 mg/mL

To make 5 mL of 10mM stock solution (3.4646 mg/mL):

* Weigh 17.323 mg of CORT powder (3.4646 mg \* 5)
* Add to 5 mL of 100% EtOH

Note: it is not possible to weigh exactly 17.323 mg of powder. To calculate actual amount of EtOH, use:

17.323 mg / 5.0 mL = Actual amount of CORT powder (mg) / x mL of EtOH

**To make CORT doses using 10mM stock (3.4646mg/mL) or 3464.6 ug/mL**

High dose: **314 pg/mL**

Low dose: **157 pg/mL**

**CORT High dose**

Want: 314 pg/mL

3.4646 mg/mL \* X mL = 314 pg/mL \* 4mL

X = 362.52 mL

X = 307.2 ul of Dilution 1

To make: 307.2 ul of Dilution 1 into 4.6928 mL of 5% DMSO diluent

**T4 Low dose**

Want: 238.6 ng/mL = 0.2386 ug/mL

7.7687 ug/mL \* X mL = 0.2386 ug/mL \* 5mL

X = 0.15356 mL of Dilution 1

X = 153.6 ul of Dilution 1

To make: 153.6 ul of Dilution 1 into 4.8464 mL of 5% DMSO diluent