Fly Zinc Dichloride Treatment Protocol

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Protocol reference: For larval treatments, Yepiskoposyan et al. 2006. Treatments for adults followed advice from Richard Burke, Monash University, Australia.

Treatment feeding schedule for Larvae:

For each treatment, approximately 50 (mixed sex) young mated adults were transferred to each fresh food vials and maintained for 12 hours. Vials were cleared and allowed to age 3.5 to 4 days. Vials were then rinsed into a series of sieves using tepid water; feeding third instar larvae were collected form the #40 sieve and transferred to a hard agar plate with a pot of yeast to induce crawling. Prior to reaching the yeast, larvae were captured and 50 larvae were transferred to new food vials containing the treatment of interest (details below), and larvae were allowed to feed for 4 hours. Treated larvae were captured and transferred to 2 ml vials, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. The number of survivors was recorded and the mean lethality calculated for each treatment.

Treatment feeding schedule for Adults:

For each treatment, 40 newly eclosed males and females (1:1) were transferred to fresh food (BDSC corn meal agar) vials and maintained at 25° C for two days. To treat flies, two Kimwipes were folded into a square and put in the bottom of a one-pint glass bottle. Kimwipes were saturated with 4 ml of the treatment solution, (10% sucrose solution and one drop of green vegetable coloring per 50 ml solution, plus the treatment of interest). Harvesting time for adults varied by treatment. Upon harvesting, flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations.

Zinc treatment:

Raise larvae on standard media. Transfer third instar larvae to media supplemented with Zinc dichloride (Simply add the metal solutions directly to the fly food while it is still hot). Allow larvae to feed for 6 hours. Recover larvae and immediately snap freeze in liquid nitrogen.
Raise adult flies on standard media. Transfer adults to media supplemented with zinc dichloride. Allow adults to feed for 48 hours to make sure they eat some of it. Recover adults and immediately snap freeze in liquid nitrogen. Take note of the following table concerning % lethality of $ZnCl_2$:

Treatment	Stage	% Lethality	Notes
4.5mM $ZnCl_2$	Adults	39.4%	48 HR Feeding
5mM $ZnCl_2$	Larvae	22%	12 HR Feeding

Adults were fed 4.5 mM zinc for 48 hours. Larvae were fed the published concentration of 5mM $ZnCl_2$ for 6 hours. Zinc appears to cause a neuromuscular defect in both adults and larvae.