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Considerations on methylmercury (MeHg) treatments in *in vitro* studies

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It is quite often that as researchers performing *in vitro* studies we are asked by reviewers of a manuscript or a grant proposal "what is the physiological relevance of the concentrations you use?" Indeed, it is incumbent upon the author to provide and prove the physiological relevance of their studied compound. Having gone through a myriad of these scenarios, I take the liberty to provide you with a justification for the *in vitro* concentrations of one of my favorite compounds, methylmercury. I encourage others with their preferred compound to apply the same exercise. To avoid future criticisms, please feel free to incorporate this description into you grant or paper.

Although a myriad of studies examined MeHg's cellular effects, surprisingly few have measured Hg concentrations. In astrocytes treated for 24 h (hours) with high MeHg concentrations (10 μM) [Hg] were 124 ng/mg protein (Shapiro and Chan, 2008). *In vivo* rat studies demonstrated at birth neuropathologic damage and neurobehavioral alterations at brain Hg concentrations of 4.5 and 0.5 ppm, respectively (Castoldi et al., 2008). Perinatal MeHg treatment resulted in neonatal rat brain Hg concentrations of 3–11 ppm (Burbacher et al., 1990). In rats with behavioral alterations after continual pre-plus postnatal exposure [until postnatal day (PN16)] of 40 μg MeHg/kg/day, brain Hg concentrations were 0.5 ppm at birth and 0.04 ppm at weaning (Newland and Rasmussen, 2000); Six-month exposure to 0.5 ppm MeHg in drinking-water resulted in brain Hg concentrations of 5 ppm (Newland et al., 2006). In PN2 MeHg-treated mice, a single *per os* dose (4 mg/kg) led to cerebellar morphological changes and mean brain Hg concentrations of 1.8 and 0.1 ppm 24 h and 19 days post treatment, respectively (Sager et al., 1984). Forty-eight hours post oral MeHg administration (5 mg/kg/day for 8 days) to juvenile mice, cerebellar and cerebral Hg concentrations were 0.3 ppm (Toyama et al., 2011). In adult mice exhibiting neuropathologic changes after 8-week treatment with 30 ppm MeHg in drinking water, Hg concentrations in cerebellum, cerebral cortex and hippocampus were 10.9, 12.9 and 23.2 ppm, respectively (Fujimura et al., 2009). In children, delayed psychomotor development occurred at brain Hg concentrations <3 ppm (Burbacher et al., 1990). The threshold for observable clinical effects approximated brain Hg concentrations of 1 ppm (Burbacher et al., 1990) and the lowest-observed-adverse-effect-level (LOAEL) was at 0.5–1.0 ppm Hg (Lewandowski et al., 2003). In Seychellois asymptomatic neonates, brain total Hg concentrations ranged from 0.026 to 0.295 ppm (Lapham et al., 1995). Hg concentrations of 0.1–0.4 ppm were detected in infants

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from Minamata (Takeuchi, 1985; Takizawa, 1986) and in two Iraqi babies who died from *in utero* MeHg intoxication, brain Hg concentrations were ~1 and 13.7 ppm (Choi et al., 1978).

We performed the following calculations: protein content in cultured astrocytes is 0.006409 mg/million cells (unpublished data). Assuming an average cell radius of 2.25 μm and a volume of 31.01×10^{-9} μl, and crystalline protein s = ~0.65 (Matthews, 1974), we derive a protein content of 0.2067 mg/μl. This may vary by a factor of 2 dependent upon the cell size. If we apply this estimate, we calculate (using this conversion factor of 206.7 μM/(nmol/mg)) that a total brain Hg concentrations of 0.295 ppm (Lapham et al., 1995) corresponds to 1.48 ngHg/mg protein. The 0.4 ppm brain Hg concentrations detected in Japan (Takeuchi, 1985) corresponds to 2 ngHg/mg protein and the 13.7 ppm Hg concentrations in Iraq (Choi et al., 1978) to 68.5 ngHg/mg protein. If cellular Hg concentrations in your studies capture these sub-threshold and threshold toxic levels reported in the mammalian CNS, your studies are meritorious indeed.

Best of luck with your publications and grants!

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