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Short communication

Dose-response analysis indicating time-dependent neurotoxicity caused by organic and inorganic mercury—Implications for toxic effects in the developing brain

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ABSTRACT

A latency period preceding neurotoxicity is a common characteristic in the dose-response relationship induced by organic mercury. Latency periods have typically been observed with genotoxicants in carcinogenesis, with cancer being manifested a long time after the initiating event. These observations indicate that even a very small dose may cause extensive adverse effects later in life, so the toxicity of the genotoxic compound is dose and time-dependent. In children, methylmercury exposure during pregnancy (in utero) has been associated with delays in reaching developmental milestones (e.g., age at first walking) and decreases in intelligence, increasing in severity with increasing exposure. Ethylmercury exposure from thimerosal in some vaccines has been associated, in some studies, with autism and other neurological disorders in children. In this paper, we have examined whether dose-response data from *in vitro* and *in vivo* organic mercury toxicity studies fit the Druckrey-Küpfmüller equation $c \cdot t^n = \text{constant}$ (c = exposure concentration, t = latency period), first established for genotoxic carcinogens, and whether or not irreversible effects are enhanced by time of exposure ($n \geq 1$), or else toxic effects are dose-dependent while time has only minor influence on the adverse outcome ($n < 1$). The mode of action underlying time-dependent toxicity is irreversible binding to critical receptors causing adverse and cumulative effects. The results indicate that the Druckrey-Küpfmüller equation describes well the dose-response characteristics of organic mercury induced neurotoxic effects. This amounts to a paradigm shift in chemical risk assessment of mercurial compounds and highlights that it is vital to perform toxicity testing geared to investigate time-dependent effects.

1. Introduction

Organic mercury induced neurotoxicity has typically been observed after a preceding latency period. Even severe and fatal ethylmercury intoxications in humans featured a latency period between cessation of exposure and onset of first symptoms of 10 days to 7 weeks (Cinca et al., 1980; Magos, 2001). For methylmercury, latencies in intoxications in Iraq and Japan ranged from weeks to more than a year (Bakir et al., 1973; National Research Council, 2000; Weiss et al., 2002) and effects were proceeding even after exposure had ended 20–30 years before (Rice and Barone, 2000). When monkeys were exposed to low levels of methylmercury during their developmental phase, neurotoxicity appeared after several years (Rice, 1996). Latency periods have typically been observed with genotoxicants in carcinogenesis, with cancer being manifested a long time after the initiating event. These observations indicate that even a very small dose may cause extensive adverse effects later in life, so the toxicity of the genotoxic compound is dose and time-dependent.

Methylmercury is widely distributed throughout the environment, particularly in estuarine and marine sediments (Bryan and Langston, 1992; Compeau and Bartha, 1985; Morel et al., 1998) and accumulates in fish and birds (Greichus et al., 1973; Harris et al., 2007; Henny et al., 2005; Houserová et al., 2007; Lam et al., 2005; Polak-Juszczak, 2012; Wren, 1986). Therefore, people are likely to be continuously exposed to small amounts of methylmercury through consumption of contaminated food (Chan et al., 2010; Lin et al., 2012). Ethylmercury is used as preservative in vaccines that may be administered to pregnant women. Toxicokinetic evidence confirms that alkyl mercury compounds cross the placental barrier (Aschner and Clarkson, 1988; Bridges and Zalups, 2005; Dórea,

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2011; Dórea et al., 2013; Geier et al., 2007; Kajiwara et al., 1996; Kerper et al., 1992) and the blood-brain barrier (Apostoli et al., 2006; National Research Council, 2000).

A myriad of scientific papers have been published on the toxicology of inorganic and organic mercury compounds. However, limited data are available for quantitative analysis of time-dependent toxicity. Most animal studies did not examine effects observed at different time points over extended exposure periods. Studies of human poisoning cases widely observed a latency period but an accurate estimation of dose or concentration at the target site is difficult to obtain. Data on Iraqi poisonings presented by Bakir et al. (1973) outlined the onset of symptoms after a mean latency period in relation to mercury concentrations in the blood of intoxicated individuals. These blood samples were obtained up to 115 days after the onset of symptoms. However, animal studies demonstrate a great divergence between blood and brain concentrations after exposure has ceased, suggesting that mercury persists in the brain while clearance from the blood occurs faster (Burbacher et al., 2005; Evans et al., 1977; Magos, 2001; Vahter et al., 1995). Therefore, blood concentrations taken a long time after exposure has ceased may not be a good parameter for assessing dose- and time-dependent neurotoxicity.

2. Dose and time-dependent toxicity of alkyl mercury compounds: data analysis

Wobeser et al. (1976) published data on the relationship between methylmercury dose and the occurrence of neurotoxic clinical signs in mink. A latency period was observed in all animals exhibiting toxicity. The higher the administered dose the earlier adverse effects appeared. When examining the total dose administered to the animals in the different dose groups it becomes apparent that low doses given continuously over a long period until the occurrence of ataxia add up to a lower total dose than the total dose from higher doses over a shorter period of time (Table 1). Decreasing total doses and increasing adverse effect induction times could reflect dose and time dependencies according to the Druckrey-Küpfmüller equation (Druckrey and Küpfmüller, 1949):

$$dt^n = \text{constant} \quad (1)$$

where the exponent $n > 1$ can be regarded as an exposure-time-reinforcement factor (Tennekes and Sánchez-Bayo, 2013). A dose-time-effect analysis of the ataxia data provided by Wobeser et al. (1976) results in a value of $n = 1.5$ ($r^2 = 0.93$), as illustrated in Fig. 1.

Another dataset confirming that neurotoxic effects from methylmercury exposure are reinforced over time was established using data obtained by Grant (1973) in cats (Table 2). In this case, the Druckrey-Küpfmüller equation described the dose-response relationship with $n = 2.9$ ($r^2 = 0.71$). This high n-value may be related to the observation that cats were particularly sensitive to methylmercury exposure (Charbonneau et al., 1976). A latency period of at least 59 days was noted and clinical signs observed included ataxia and convulsions. Interestingly, when brain concentrations are correlated to the latency period in the onset of clinical signs a lower r^2 value is obtained. Mercury is known to accumulate in specific brain regions which may not have been taken into account when brain concentrations were determined.

For ethylmercury-induced effects, the Druckrey-Küpfmüller equation describes the dose-response relationship in human cortical neurotoxicity observed by Baskin et al. (2003), (Table 3) with an exposure-time-reinforcement factor of 2.81 ($r^2 = 0.8$), (Fig. 2) for concentrations ranging from 1 to 250 μM at time points between 2 and 24 h. The extremely high value of n obtained in this case indicates pronounced reinforcement of toxicity by exposure time.

Table 1
Effect of dose on onset of methylmercury-induced ataxia in mink (after data from Wobeser et al., 1976).

Dose (d) in feed (ppm)	Median time (t50) to onset of ataxia (days)	Total dose (ppm) = d·t50
1.8	69	124.2
4.8	30.5	146.4
8.3	20	166
15	17.75	266.25

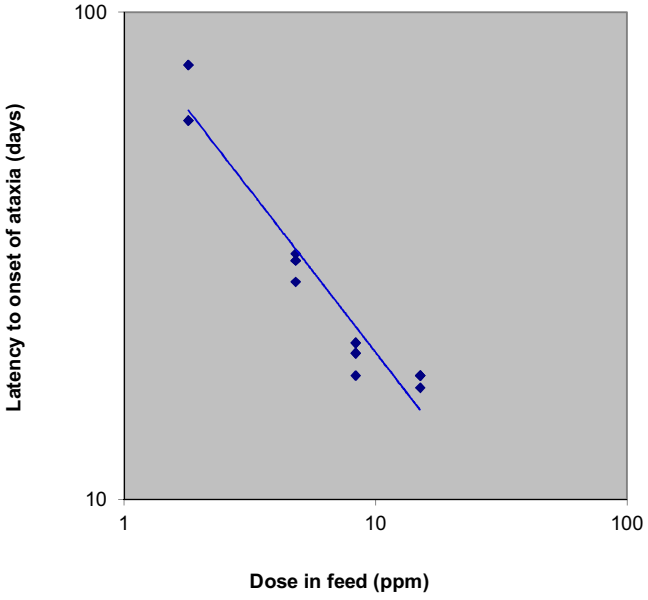

Fig. 1. Onset of ataxia in mink exposed to various doses of methylmercury in feed over time.