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The dose makes the poison

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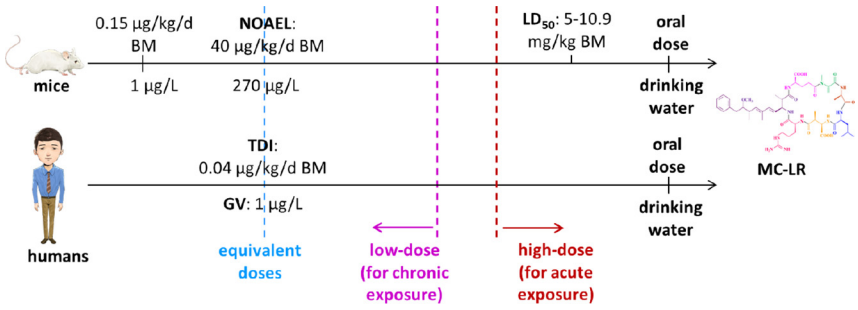
HIGHLIGHTS

- A critical review of a previous study was performed.
- Exposure to the same concentrations do not result in equivalent doses among species.
- Inter-species variations should be considered in experimental design.
- Ranges of low-dose for effects of microcystins in animals and humans should be defined.
- Chronic toxicity and especially carcinogenicities of microcystins need further studies.

GRAPHICAL ABSTRACT



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ABSTRACT

Some microcystins (MCs) might cause hepatotoxicity in animals and humans. MC-LR is also a tumor promoter and a suspect carcinogen. In 2010, the International Agency for Research on Cancer (IARC) classified MC-LR as a possible human carcinogen (Group 2B). Recently, an article entitled "Long-term, low-dose exposure to microcystin toxin does not increase the risk of liver tumor development or growth in mice" was published in *Hepatology Research* by Meaghan Labine and Gerald Y. Minuk. However, the experimental design was flawed and the conclusion is misleading. 1 μg/L MC-LR in drinking water is the provisional guideline value established by the World Health Organization (WHO) for humans in 1998, based on a tolerable daily intake (TDI) of 0.04 μg/kg body mass (BM). Assuming the mice drink 1.5 mL/10 g BM of water per day, the exposure dose would be 0.15 μg/kg/d BM, about 270-fold less than 40 μg/kg/d, the no-observed-adverse-effect level (NOAEL). Thus, the dose of MC-LR was too small and "unlikely to result in liver tumor development or enhance existing tumor growth", even with a long-term (28 weeks) exposure. Presumably, they didn't consider inter-species variations between mice and humans, including toxicokinetics and toxicodynamics. Ranges of "low-dose" MCs for animals and humans should be defined. Also, the authors misunderstood or misrepresented several previous studies. Before drawing final conclusions on the carcinogenicity of MCs, further well-designed experiments are warranted.

Recently, an article entitled "Long-term, low-dose exposure to microcystin toxin does not increase the risk of liver tumor development or growth in mice" was published in *Hepatology Research*, by Labine and Minuk (2015). Since we have been doing work in the area of algal toxins, we read with interest the paper regarding the carcinogenicity of long-term, low-dose oral exposure to microcystins (MCs). As

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interested yet critical readers of the article, we would like to share some understanding and comments on the paper. First, we would like to acknowledge Drs. Labine and Minuk for their effort to report on safety issues of MCs. However, in that paper the authors concluded that MCs are not complete carcinogens (acting as both initiator and promoter) or tumor promoters, which is contrary to results published by other authors who have found that microcystin-LR (MC-LR) is a tumor promoter and a suspect carcinogen (Žegura et al., 2011; Žegura, 2016). Their conclusion is also inconsistent with conclusions drawn by international agencies. For instance, in 2010, the International Agency for Research on Cancer (IARC) classified MC-LR as a possible human carcinogen (Group 2B) (IARC, 2010). We were surprised by the conclusions of Labine and Minuk (2015). Upon studying their article, we have concluded that the experimental design applied was flawed and resulted in data insufficient to support the conclusions drawn and thus making the conclusions made misleading. Specifically, the dose applied to the mice was not relevant for making comparisons with or extrapolating to effects in other species.

Toxicity is a function of both exposure and effect. That is, responses are a function of duration and intensity of exposure and potency, which is an inherent property of the toxicant in a specific species. While the statement "The dose makes the poison" (Latin: "S*ola dosis facit venenum*"), which is attributed to the Swiss physician Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim) in 1538 (Anon, 1965), is a toxicological maxim that means: "All things are poison and nothing is without poison; only the dose makes a thing not a poison". However, it can also mean that, if a threshold for effects was not reached, there will not be an observed response or adverse effect. If the dose in the body or at a critical site of action does not reach a sufficient concentration or internal dose, which is sometimes referred to as the critical body burden", then no toxicity would be observed. With this concept as background, we evaluated the doses applied in their study and then drew different conclusions than did Labine and Minuk (2015).

In their study, Labine and Minuk (2015) exposed mice to 1 μg/L MC-LR in drinking water, which is the provisional guideline, established by the World Health Organization (WHO) for protection of humans (WHO, 1998). In their study, male CD-1 mice were exposed to either drinking water alone (water group), drinking water containing 1 μg/L MC-LR (MC-LR group), MC-LR plus thioacetamide (MC-LR/TAA group) or thioacetamide alone (TAA group) (n = 20/group). After 28 weeks of exposure, no tumors were detected in the water or MC-LR alone groups, while 4 mice in the TAA group and 5 in the MC-LR/TAA group developed liver tumors. The mean size of the tumors in the MC-LR/TAA and TAA alone groups were similar as were the results of Ki-67 staining, number of atypical mitoses and liver cancer gene expression profiles. The authors concluded that long-term, low-dose exposure to MCs is unlikely to result in development of tumors in liver, or enhance existing tumor growth.

Assuming mice drink 1.5 mL/10 g body mass (BM) of water per day, the dose, normalized to body mass would be 0.15 μg/kg/d BM, which would be about 270-fold less than the no-observed-adverse-effect level (NOAEL) of 40 μg/kg BM/d (Fawell et al., 1994, 1999; WHO, 1998). Thus, the internal dose delivered to a mouse would have been less than the dose of MC-LR that would have been able to result in development of tumors in the liver or enhance growth of existing tumors, even during longer-term (28 weeks) exposure. Presumably, the authors of that paper didn't consider inter-species, allometric differences between mice and humans, including toxicokinetics and toxicodynamics. Ranges of "low-dose" for effects of MCs on animals and humans should be defined. Also, the authors misunderstood or misrepresented results and/or conclusions of several previous studies. Before drawing their conclusions on carcinogenicities of MCs, further, well designed experiments are warranted. Here, we present some background and analyses that might be useful when designing studies that are more appropriate and rigorous.

1. Background

Cyanotoxins, such as microcystins (MCs), are released by blooms of cyanobacteria that result from eutrophication where excess quantities of nutrients, generally phosphorus (P), are present, but higher temperatures also favor occurrence of blooms. Thus, frequencies, durations and intensities of blooms are expected to worsen with increasing loading of nutrients and warming of the climate, reported worldwide (Carmichael and Boyer, 2016). MCs are the largest and most diverse group of cyanotoxins, with more than 100 structural variants, with molecular masses between 895 and 1115 Da (Niedermeyer, 2013; Chen et al., 2016). Their general structure is cyclo(-D-Ala^1-L-X^2-D-erythro-β-methylAsp^3-L-Z^4-Adda^5-D-Glu^6-N-methyldehydro-Ala^7). Although modifications have been reported for all of the amino acids, two L-amino acid residues X and Z are responsible for most of the congeners (Niedermeyer, 2013; Chen et al., 2016). Concentrations of dissolved MCs in a range of 0.1–10 μg/L have been observed in surface waters, while cell-bound concentrations were several orders of magnitude greater (Žegura et al., 2011; Chen et al., 2017). MC-LR is one of the most common and potent variants and it is also the most widely studied MC (Chen and Xie, 2016).

Due to reports of intoxications of both humans and animals and evidence that MCs cause toxicities, such as hepatotoxicity, reproductive toxicity, neurotoxicity, immunotoxicity and disrupt endocrine systems of animals, MCs have received increasing attention, especially as a public health threat (Chen et al., 2016; Hu et al., 2016; Valério et al., 2016; Buratti et al., 2017; Svirčev et al., 2017). MC-LR has been shown to be a promoter of growth of tumors and is suspected to be a carcinogen (Žegura et al., 2011; Žegura, 2016). Exposures of humans to MCs can occur by ingestion of contaminated drinking water, inhalation and dermal contact with toxins during recreation, consumption of cultivated plants, aquatic products including fish and blue-green algae supplements, and via the intravenous route during haemodialysis with contaminated water (Svirčev et al., 2017).

2. In exposures, the same concentrations do not result in equivalent doses among species

Interpretation of results of studies of carcinogenicity is profoundly affected by conditions during exposures, especially by inappropriately selected doses. This is particularly important for interpreting results of studies, where, due to insufficiently large doses, exposures do not result in significant carcinogenicity (USEPA, 2005). In fact, 1 μg/L MC-LR, the dose used in the study by Labine and Minuk (2015), is the provisional guideline value established by the World Health Organization (WHO, 1998) in drinking water for protection of humans. That value was based on a tolerable daily intake (TDI) 0.04 μg/kg BM (Fig. 1) (WHO, 1998; Chorus and Bartram, 1999). Due to limited information from epidemiological studies of humans exposed to MCs, assessments of hazard and risk have relied on extrapolation from toxicological data for animal models, generally mice and rats. However, data on toxicity to animal models is limited, especially for chronic, adverse effects at lesser doses. Results of a 13-week study in which mice were dosed orally with MC-LR are considered the most suitable for derivation of a guideline value (Fawell et al., 1994, 1999; WHO, 1998). In that study, a NOAEL of 40 μg MC-LR/kg BM per day (by gavage), based on pathology of the liver, was determined for both male and female Cr1:CD-1(ICR)BR mice. A fairly conservative (protective) TDI of 0.04 μg/kg/d BM for humans can be calculated by applying an uncertainty factor (UF) of 1000 (10 for inter-species variation, 10 for intra-species (individual) variation, and 10 for limitations in the database, in particular, lack of data on chronic toxicity and carcinogenicity) to the NOAEL (Eq. (1), WHO, 1998; Chorus and Bartram, 1999; Codd et al., 2005; Dietrich and Hoeger, 2005; Falconer and Humpage, 2005). This TDI is supported by a 44-day study, in which pigs were given extracts of *Microcystis aeruginosa* in their drinking water (Fig. 1, Falconer et al., 1994; WHO,