

## NOTE

# MS-222 (tricaine methane sulfonate) does not kill the amphibian chytrid fungus *Batrachochytrium dendrobatidis*

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**ABSTRACT:** MS-222 (tricaine methane sulfonate) is an agent commonly used to anaesthetise or euthanize amphibians used in experiments. It is administered by immersing the animal to allow absorption through the skin. Chytridiomycosis is an important disease of amphibians and research involves experiments with live animals. *Batrachochytrium dendrobatidis*, the fungus which causes chytridiomycosis, is located in the skin and therefore the organism should come into contact with MS-222 when it is used. *B. dendrobatidis* is a sensitive organism which could possibly be killed by MS-222. Hence, results of chytridiomycosis studies in which MS-222 is used could be unreliable. A concentration of 2 g l<sup>-1</sup> and an exposure duration of 1 h is at the high end of the range at which MS-222 would be most commonly used. Exposure to 2 g l<sup>-1</sup> MS-222 for 1 h does not kill *B. dendrobatidis* cultures, suggesting that MS-222 is safe to use in chytridiomycosis studies.

**KEY WORDS:** MS-222 · Tricaine methane sulfonate · *Batrachochytrium dendrobatidis* · Chytridiomycosis · Anaesthetic

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Chytridiomycosis is infection with the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Berger et al. 1998, Longcore et al. 1999). In susceptible amphibian species, the mortality rate can approach 100% (Berger et al. 2004). *In vitro* experimental infections have played an important role in understanding chytridiomycosis (Nichols et al. 2001). To minimise pain and distress due to handling and sampling of amphibians used for experimental studies, MS-222 (tricaine methane sulfonate) is recommended as an anaesthetic. Concentrations of 1 to 1.5 g l<sup>-1</sup> with an exposure time of up to 30 min is sufficient for adult anuran anaesthesia (Kaplan 1969, Poole 1987, Arena & Richardson 1990, Wright 2001). A higher concentration (2 g l<sup>-1</sup>) for longer periods of time can be used for euthanasia (Poole 1987). Lower concentrations are required for urodele anaesthesia and euthanasia (Arena & Richardson 1990). MS-222 is an aqueous solution in which amphibians are

totally immersed and the duration of exposure is modified depending on the response of the amphibian and effect desired. During absorption through the skin, MS-222 must come into contact with the zoosporengia of *B. dendrobatidis* which are located only in the stratum corneum and stratum granulosum (Berger et al. 1998).

There is a range of chemicals that can cause 100% mortality of *Batrachochytrium dendrobatidis* (Johnson et al. 2003). There are no data available on the antifungal properties of MS-222. We were concerned that if MS-222 had a killing effect on *B. dendrobatidis*, the results obtained in amphibians with experimental chytridiomycosis either anaesthetised or killed by MS-222 would be adversely affected. We therefore evaluated the killing effect of MS-222 on *B. dendrobatidis*.

MS-222 was tested using 4 d old cultures of *Batrachochytrium dendrobatidis* in 96-well flat bottom plates, each well having initially contained 8 × 10<sup>4</sup> zoo-

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spores, as per Johnson et al. (2003). The MS-222 was buffered to neutral with  $\text{Na}_2\text{HPO}_4$  and the cultures were exposed to a range of concentrations and durations, the highest being  $2 \text{ g l}^{-1}$  for 1 h. None of the treatments caused 100% mortality of the *B. dendrobatidis* cultures. In the case of the  $2 \text{ g l}^{-1}$  for 1 h treatment, all 8 replicate wells contained active cultures by 5 d after exposure.

This result proves that, at the concentrations most commonly used, MS-222 will not kill *Batrachochytrium dendrobatidis*. This result is significant as it allows the results of past and future experiments on chytridiomycosis in which MS-222 has been used to be viewed with confidence, and encourages the humane treatment of experiment amphibians.

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