ORD CLEARANCE FORM

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Middle Initial:		EPA Publication #:	
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Product Title

Evaluation of Microcystin Toxicity in 2D and 3D Primary Human Hepatocyte Cultures

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Impact / Purpose Statement

Note: The Impact / Purpose Statement information for this work product will be displayed on the additional pages.

Product Description / Abstract

Note: All Product Description / Abstract information for this work product will be displayed on the additional pages.

Tracking and Planning

Note: All Tracking and Planning Field data for this work product will be displayed on the additional pages.

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☐ EPA Only ☑ Public	☐ Yes (Permission Attached) ☐ No ☑ Not Applicable

Quality Assurance	Accepted Date	Available Online Date	Published Date
Is there an approved QAPP (or QAPPs) supporting this product?			
☑ Yes ☐ No ☐ Not Applicable			

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E-ISTD-0031318-QP-1-0

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1. Climate Change	4. Microcystin
2. Hepatocytes	5. Cytotoxicity
3. Cyanotoxin	6.

Comments
Note: All Comments for this work product will be displayed on the additional pages.

Digital Signatures (As applicable)	
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Pre-2019 Tracking and Planning Field Set(s)
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Task: Health, Ecosystem, and Economic Effects

Product Title: N/A - Not Applicable

Product Description: N/A - Not Applicable

Project: Harmful Algal Blooms

Topic: Nutrients

Research Program Area: Safe and Sustainable Water Resources

Impact / Purpose Statement
Presentation to the Society of Toxicology 61st Annual Meeting and ToxExpo March 2022. This study investigates the cytotoxic effects of hydrophobic and hydrophilic microcystin congeners on primary human hepatocytes in 2D or 3D cultures. The data show that hydrophobic microcystin congeners are more toxic than hydrophillic congeners and the congeners are more toxic in hepatocytes cultured in 3D. These results show the utility of 3D hepatic cultures when determining the toxicity of microcystin congeners.

Product Description / Abstract
Microcystin (MC) are cyanobacterial hepatotoxins and known inhibitors of serine/threonine protein phosphatases. Considered one of the most common cyanobacterial toxins, MC are commonly found in surface and drinking water and their presence poses a risk to public health. Over 200 MC congeners have been identified with variable amino acid compositions. The amino acid composition determines physical/chemical properties including hydrophilicity and is also thought to determine the chemicals' kinetics that will in part determine the toxicity of each congener. While Microcystin LR (MCLR) is the most widely researched congener, toxicity data for other MC are limited. Previous studies show that the more hydrophobic congeners cause a greater decrease in hepatocyte viability, compared to the more hydrophilic congeners. Considered the "gold-standard", primary human hepatocytes (PHH) are often used to evaluate hepatic metabolism and toxicity of other xenobiotic compounds in vitro. The conventional 2D culture of PHH is easily attained and thus represents the most common culture strategy; however, 3D cultures have been shown to better recapitulate in vivo liver physiology and maintain a stable expression of metabolic enzymes. This study aims to characterize the viability of PHH in 2D and 3D culture in response to microcystin exposure. PHH were cultured in 96-well collagen-coated plates in a 2D monolayer or in 96-well ultra-low attachment plates to support 3D spheroid development. Cells were treated for 24 hours with microcystin-LR (MCLR) or the more hydrophilic microcystin-WR (MCWR) in concentrations ranging from 0 to 0.6 µM. EC50s from cell viability studies were used to determine the cytotoxic potency of each MC congener. In both methods of cultivation, MCLR was more toxic than MCWR; however, spheroids showed a greater sensitivity to MCLR, as indicated by a 56% lower EC50 relative to the monolayer ($p<0.01$). A similar but more pronounced result was observed in cultures treated with MCWR, whereas the EC50 for the spheroid model was found to be 89% less than the monolayer culture ($p<0.01$). This study shows that the more hydrophobic congener (MCLR) caused a greater decrease in cell viability, compared to the more hydrophilic congener (MCWR) in both culture methods. The spheroid cultures were significantly more sensitive to microcystin than the 2D cultures. Our results show the utility of using spheroid cultures for understanding and predicting the toxic effects produced by MC congeners in vivo. This abstract does not represent EPA policy.

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Comments
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