Charge to External Reviewers for the
Toxicological Reviews of Cyanobacterial Toxins: Anatoxin-a, Cylindrospermopsin and Microcystins LR, RR, YR and LA

Introduction

The U.S. Environmental Protection Agency (EPA) is conducting a peer review of the scientific basis for the human health assessment of three cyanobacterial toxins: anatoxin-a, cylindrospermopsin and microcystins (LR, RR, YR and LA).

Feedback on the Toxicological Reviews of Cyanobacterial Toxins is currently being sought in three general areas: (1) general clarity and thoroughness of the documents, (2) issues concerning the derivation of reference values specific to these toxins, and (3) characterization of the carcinogenic potential of these toxins.

General Questions

1. Are the Toxicological Reviews logical, clear and concise? Has EPA accurately, transparently and objectively represented and synthesized the scientific evidence for noncancer and cancer hazards?

2. Are you aware of additional studies that should be considered in the assessment of the noncancer and cancer health effects of these toxins?

3. Please discuss research that you think would be likely to reduce uncertainties in the reference values for future assessments.

Questions Related to the Derivation of Reference Values for Anatoxin-a, Cylindrospermopsin and Microcystins (LR, RR, YR and LA)

Oral Reference Dose (RfD) Values

Anatoxin-a

1. The conclusion was reached that the available oral toxicity information was inadequate to support derivation of oral RfD values for acute and chronic exposure durations. Do you agree with this conclusion? Is the rationale for not developing acute or chronic oral RfDs transparent and objective? If you do not agree with the conclusion that the data are inadequate to support derivation of oral RfD values for acute and chronic durations, and hence you conclude that derivation for acute and/or chronic derivations is appropriate, then please describe how you would recommend such derivation(s) be completed and the rationale for such derivation(s).

2. The 28-day gavage study in mice (Fawell and James, 1994; Fawell et al., 1999) was selected as the basis for the short-term oral RfD. Is the selection of this study as the principal study appropriate? Is the rationale for selecting this study transparent and objective?

3. In the 28-day gavage study selected as the basis for the short-term RfD, two potentially treatment-related deaths were reported. The original study authors concluded that the NOAEL for this study was 0.1 mg/kg-day based on these two deaths. This conclusion was based on their inability to determine the cause of death (i.e., to completely rule out a relationship with treatment), and they indicated that the true NOAEL may actually be 2.5 mg/kg-day.

Due to the low incidences of mortality (that showed no dose-response or gender consistency), the lack of characteristic clinical signs of acute neurotoxicity in the two animals that died, and the absence of toxicologically significant effects in the surviving mice, as well as the lack of effects at 2.5 mg/kg-day in mice reported in 5-day and developmental toxicity studies (Fawell and James, 1994; Fawell et al., 1999), EPA concluded that the deaths are likely to be incidental and that the actual NOAEL is 2.5 mg/kg-day. Is the designation of 2.5 mg/kg-day as the NOAEL for this study scientifically justified? Has the rationale for this designation been transparently and objectively described?

4. The 7-week drinking water study in rats (Astrachan and Archer, 1981; Astrachan et al., 1980) was selected as the basis for the subchronic oral RfD. Is the selection of this study as the principal study appropriate? Is the rationale for selecting this study transparent and objective?

5. Are the uncertainty factors applied to the points of departure (PODs) for the derivation of the short-term and subchronic RfD values scientifically justified and transparently and objectively described?

Cylindrospermopsin

1. The conclusion was reached that the available oral toxicity information was inadequate to support derivation of oral RfD values for acute or short-term exposure durations. Do you agree with this conclusion? Is the rationale for not developing acute or short-term oral RfDs transparent and objective? If you do not agree with the conclusion that the data are inadequate to support derivation of oral RfD values for acute and chronic durations, and hence you conclude that derivation for acute and/or chronic derivations is appropriate, then please describe how you would recommend such derivation(s) be completed and the rationale for such derivation(s).

2. The 11-week gavage study in mice (Humpage and Falconer, 2003) was selected as the basis for the subchronic oral RfD. Is the selection of this study as the principal study appropriate? Is the rationale for selecting this study transparent and objective?

3. The critical effect identified in Humpage and Falconer (2003) was increased relative kidney weight. Is selection of this finding as a critical effect scientifically justified? Is the rationale for selecting this effect transparent and objective?

4. Benchmark dose (BMD) modeling was utilized to estimate a BMD and BMDL for increased mean relative kidney weight using 1 standard deviation above the control mean as the benchmark response level (BMR). After dropping the high dose group, the linear model adequately fit the data and produced an estimated BMD of 43.1 µg/kg-day and BMDL of 33.1 µg/kg-day. Was the modeling appropriately conducted and interpreted? The BMDL of 33.1 µg/kg-day was chosen as the POD for RfD derivation. Is the rationale for selecting this as the POD transparent and objective?

5. The conclusion was reached that the 11-week gavage study in mice (Humpage and Falconer, 2003) selected as the basis for the subchronic oral RfD study could not be utilized for the derivation of a chronic oral RfD due to the excessive uncertainty inherent in doing so. Do you agree with this conclusion? Is the rationale for not developing a chronic oral RfD transparent and objective? If you do not agree with the conclusion that the data are too uncertain to support derivation of oral RfD values for chronic durations, and hence you conclude that derivation for chronic derivations is appropriate, then please describe how you would recommend such derivation be completed and the rationale for such derivation.

6. Are the uncertainty factors applied to the point of departure for the derivation of the subchronic oral RfD scientifically justified and transparently and objectively described?

Microcystins (LR, RR, YR and LA)

1. The conclusion was reached that the available toxicity information was inadequate to support derivation of oral RfD values for microcystin-RR, -YR or LA. Do you agree with this conclusion? Is the rationale for not developing oral RfD values transparent and objective? If you do not agree with the conclusion that the data are inadequate to support derivation of oral RfD values for microcystin-RR, -YR or LA, and hence you conclude that derivation is appropriate, then please describe how you would recommend such derivation(s) be completed and the rationale for such derivation(s).

Microcystin-LR

1. The conclusion was reached that the available oral toxicity information was inadequate to support derivation of an acute oral RfD value. Do you agree with this conclusion? Is the rationale for not developing an acute oral RfD transparent and objective?

2. The 28-day drinking water study in rats (Heinze, 1999) was selected as the basis for the short-term and subchronic oral RfDs. Is the selection of this study as the principal study appropriate? Is the rationale for selecting this study transparent and objective?

3. Liver toxicity observed by Heinze (1999) included liver lesions, serum enzyme changes and changes in relative liver weight. All three of these endpoints were considered for determining the point of departure for RfD derivation. BMD modeling was utilized to estimate a BMD and BMDL for each of these endpoints. Was the modeling appropriately conducted and transparently and objectively presented?

For the purposes of BMD modeling, the moderate and severe liver lesion categories reported by Heinze (1999) were combined and the BMDs and BMDLs for these lesions estimated by the log probit model were 11.0 and 6.4 µg/kg-day, respectively. The BMDL of 6.4 µg/kg-day was chosen as the POD for RfD derivation. Is the rationale for selecting this as the POD transparent and objective? Is selection of liver lesions as a critical effect scientifically justified? Is combining the moderate and severe lesions scientifically justified? Is the rationale for selecting this effect transparent and objective?

4. Data from the 90-day gavage study in mice conducted by Fawell et al. (1999) was considered for the derivation of the subchronic RfD. The BMDL from the Heinze (1999) study (6 µg/kg-day) is approximately an order of magnitude lower than any of the BMDL values derived from endpoints from the 90-day gavage study (57-66 µg/kg-day) therefore, the BMDL of 6 µg/kg-day from Heinze (1999) was chosen as the POD for subchronic RfD derivation. Do you agree with this decision? Is the rationale for selecting 6 µg/kg-day as the POD transparent and objective?

5. The 18-month drinking water study in mice (Ueno et al., 1999) was selected as the basis for the chronic oral RfD. This study used only a single dose level and identified a freestanding NOAEL but was chosen for RfD derivation because it was a well-conducted study of chronic duration and employed a relevant exposure route (drinking water). Is the selection of this study as the principal study appropriate? Is the rationale for selecting this study transparent and objective?

6. Are the uncertainty factors applied to the points of departure for the derivation of the short-term, subchronic and chronic oral RfDs scientifically justified and transparently and objectively described?

Questions Related to the Cancer Assessments for Anatoxin-a, Cylindrospermopsin and Microcystins (LR, RR, YR and LA)

1. Do the available data support the conclusion that the database for each of these toxins provides inadequate information to assess carcinogenic potential based on the weight-of-evidence categories in the EPA 2005 *Guidelines for Carcinogen Risk Assessment*? Please describe the basis for your view.