Hazard Identification - Microcystins in Fish

One of the most common microcystins is microcystin-LR (MC-LR), though over 250 microcystins have been identified (WHO, 2020). MC-LR is also the microcystin that has been most studied toxicologically and is amongst the most potent.

The main target organ for toxicity of the microcystins is the liver, though other organs may also be affected (WHO, 2020). MC-LR did not induce gene mutations in bacterial cells or chromosome aberrations in mammalian cells *in vitro*, although an increased frequency of polyploid cells was observed in mammalian cells which indicated that it may be aneugenic (IARC, 2010). However, neither MC-LR nor cyanobacterial extracts increased micronucleus formation in cultured human lymphocytes, indicating neither clastogenic nor aneugenic effects (Abramsson-Zeterberg et al., 2010). Evidence suggests that microcystin-LR may act as a tumour promotor in the liver and possibly other tissues (WHO, 2020; IARC, 2010). MC-LR is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenicity to humans (Group 2B) based on studies in rats and mice in which it promoted the development of pre-neoplastic lesions (IARC, 2010). Developmental toxicity studies in mice did not identify adverse effects. A number of studies reported adverse effects on male and female reproductive organs. However, these studies mainly used intraperitoneal dosing which can lead to much higher internal exposures than would be achieved by oral dosing, and several more recent studies which used oral dosing had methodological and reporting deficiencies (WHO, 2020). Therefore, further reproductive toxicity data would be required to confirm the adverse effects and identify dose-response relationships. Only limited data are available on potential neurological, immune and haematological effects.

The mode of action is inhibition of protein phosphatases, resulting in destabilisation of the cytoskeleton and microtubules (WHO, 2020). This results in altered cellular function, followed by apoptosis and necrosis. At low doses, inhibition of the protein phosphatases results in cellular proliferation, hepatic hypertrophy and tumour promotion activity.

Key studies in laboratory animals

For full reviews of the toxicology data on microcystins see Testai et al. (2016) or WHO (2020). The following summarises key studies of value for risk characterisation.

Fawell et al. (1999) administered microcystin-LR by oral gavage to groups of 15 male and 15 female mice at dose levels of 0, 40, 200 and 1000 µg/kg body weight (bw)/day for 90 days. All mice were examined daily for signs of clinical toxicity. Bodyweights and food consumption were measured weekly, and eye examinations were conducted at the start and end of the study. Blood samples were taken during the final week for haematological and clinical chemistry analyses.

Histopathological analyses were conducted on all tissues from control and high dose group animals, and on the lungs, liver and kidneys from the other dose groups, with particular focus on any gross lesions observed at necroscopy. Histopathological changes were only observed in the liver, and were reported to be multifocal minimal/slight chronic inflammation with deposits of haemosiderin and multifocal single hepatocyte degeneration throughout the liver lobule. These were mainly observed in the high dose group with less marked lesions in smaller numbers of animals in the mid-dose group. There were no changes observed in the low dose or control groups. Haematological changes were limited to small but significant increases in mean haemoglobin concentration, red cell counts and packed cell volume in females in the high dose

group.

A number of changes in blood chemistry parameters were observed in the mid and high dose groups, including high plasma alkaline phosphatase levels in both sexes at the top dose, raised transaminases in both sexes at the top dose and in males at the mid-dose, and reductions in plasma albumin and total protein levels in males only at the mid and high doses. The no-observed-adverse-effect-level (NOAEL) was concluded to be 40 µg/kg bw/day.

Heinze (1999) administered doses of 0, 50 and 150g/kg bw/day microcystin-LR to groups of 10 male rats via their drinking water for 28 days. Relative liver weights were increased by 17% and 26% in the low and high dose groups, and absolute liver weights were also reported to be increased. Liver lesions were observed in both treatment groups, with slightly greater severity in the high dose group. Levels of alkaline phosphates (ALP) and lactate dehydrogenase (LDH) were increased in both treatment groups, while there were no changes in alanine aminotransferase (ALT) or aspartate aminotransferase (AST). No NOAEL was identified from this study. The low dose level of 50µg/kg bw/day is therefore the lowest observed adverse effect level (LOAEL).

Ueno et al. (1999) conducted a chronic toxicity study in which groups of 20 six-week-old female BALB/c mice were administered microcystin-LR in their drinking water at concentrations of 0 and 20µg/L for 18 months. There were no clinical signs of toxicity or treatment-related effects observed on survival, body weight, food or water consumption, haematology or histopathology. A statistically significant increase in serum cholesterol (22%) was observed at month 18, but not months 3, 6 or 12. The toxicological significance of this was considered uncertain as it was a single finding and not associated with any other treatment-related changes. In addition, immunohistochemical analysis did not indicate hepatic MC-LR accumulation. The 20µg/L group was stated to have received a total dose over the 18 months of 35.5µg/mouse. Based on the adult mean body weight reported of 26.68 g and the 567 days of dosing, this is approximately equivalent to a dose level of 2.3µg/kg bw/day (WHO, 2020).

Fawell et al. (1999) conducted a developmental toxicity study of microcystin-LR in mice. The mice were dosed by oral gavage at 0, 200, 600 or 2000µg/kg bw/day on days 6-15 of gestation. The dose level of 2000µg/kg bw/day was selected as it had been shown to cause maternal toxicity in a small dose range finding study, and the dose level of 200µg/kg bw/day was selected as being the likely NOAEL for maternal toxicity. Substantial maternal toxicity was observed at the top dose, including deaths of 7/26 dams and two being humanely euthanised. These dams also showed macroscopic changes to the livers. Surviving dams showed no clinical signs or effects on food consumption or body weight. Fetal body weight was reduced compared to controls and delayed skeletal ossification was observed. However, there was no evidence of embryolethality, and the numbers of implantations and live fetuses were unaffected. No treatment-related increases in the incidence of major or minor external, visceral or skeletal fetal abnormalities were apparent. There were no maternal or developmental effects apparent in the low or mid-dose groups and therefore the NOAEL was 600µg/kg bw/day for both maternal and developmental toxicity.

Key human data

An outbreak of acute liver failure occurred at a dialysis clinic in Caruaru, Brazil in 1996. The dialysis water was found to be contaminated with microcystins and cylindrospermopsin, and the microcystins were considered likely to be the major factor, specifically microcystin-YR, microcystin-LR and microcystin-AR (Carmichael et al., 2001). Out of 131 patients treated, 116 experienced visual disturbances, nausea and vomiting, and subsequently 100 developed acute liver failure, of which 76 died. Analyses of liver samples from 39 of the patients who died identified the presence of microcystin-YR, microcystin-LR and microcystin-AR, and the mean concentration of total microcystins was 223 ng/g. This was compared with a concentration of 125 ng/g microcystin-LR measured in the livers of mice dosed with a lethal dose of microcystin-LR by

intraperitoneal injection.

A second event occurred at a Dialysis clinic in Rio de Janeiro, Brazil in 2001. A survey identified a microcystin concentration 0.32 ug/L in the activated carbon filter used as an intermediate treatment step to prepare dialysate, and a concentration of 0.4 ug/L was measured in the source water (Hilborn et al., 2013). Out of 44 dialysis patients potentially exposed, 12 were followed up for a period of 8 weeks as they were found to have detectable serum concentrations of microcystins. The median serum concentration in these patients was 0.33 ng/mL. Levels of AST, ALT, gamma-glutamyltransferase (GGT), ALP and bilirubin exceeding their reference ranges were frequently observed throughout the 8 weeks. These were considered consistent with mild to moderate liver injury. In addition, decreased prothrombin time was statistically significantly associated with increased serum microcystin concentration.

During the early part of 1981, the reservoir supplying water to the Armidale region of Australia was affected by a large bloom of *Microcystis aeruginosa*, a cyanobacteria which produces microcystins, which was treated by the addition of copper sulphate to the water supply. Clinical and plasma enzyme data were collected from all patients treated at the regional hospital for three time periods: a five week period before the first signs of the bloom appeared, the two week period after copper sulphate treatment and a final five week period. The results were also compared between residents of Armidale receiving water from the affected source and those outside the city and in neighbouring towns who had independent water sources.

Analysis of variance showed a statistically significantly higher GGT level during the two-week period of the bloom, only in Armidale residents. ALT also appeared to show an increase in activity in samples from Armidale residents compared with residents with other water sources, but this did not reach statistical significance. The authors concluded that the evidence indicated an increase in liver damage among the population of Armidale during the period of a bloom (Falconer et al., 1993). No exposure data are available from this study.