New section for Microcystin-LR background document:

Treatment and control measures and technical achievability

Microcystins are largely cell-bound, with usually more than 95% of the toxin contained within healthy cells. Dying and decaying cyanobacteria may release microcystins into the water, but the data available indicate that usually biodegradation will be sufficiently effective to preclude the build-up of high concentrations of extracellular microcystin dissolved in water, unless cell lysis is induced artificially (for such a case, see Jones & Orr 1994). A very effective way to deal with high microcystin concentrations therefore is to remove the cells, intact and without damage (Drikas et al. 2001; Hart et al. 1998). Any damage, such as that caused by preoxidation, may lead to cell leakage, and consequently in an increase of the dissolved toxin concentration entering the treatment plant. This may be critical, as dissolved toxin is not removed by conventional treatment technologies.

Conventional treatment using coagulation will remove cyanobacteria cells; however, sludge containing toxic cyanobacteria should be isolated from the treatment process as cells contained in sludge can break down rapidly and release dissolved toxin (Chow et al. 1999). Experimental and full-scale studies for the removal of cyanobacteria using membranes are scarce. In general, micro- and ultra-filtration membranes could be expected to remove cyanobacterial cells effectively. Membrane filtration of toxic cyanobacteria should be carried out with frequent backwashing, and isolation of the backwash water from the plant due to the risk of the cells releasing dissolved toxin (Chow et al. 1997; Gijsbertsen-Abrahamse et al. 2006). The treatments mentioned above will not remove extracellular, or dissolved toxin to a significant extent.

Most of the common microcystin variants are well removed by activated carbon (Hart et al 1998; UKWIR 1996; Cook and Newcombe 2002). The exception is microcystin LA which is not readily removed and other processes are recommended (Cook and Newcombe 2002). For other microcystins wood-based, chemically activated carbon is the most effective, or a carbon with similar physical properties. Doses of powdered activated carbon required for removal to below the guideline value will depend on water quality, and site specific tests are recommended. Granular activated carbon filtration displays a limited lifetime for all toxins. This can vary between 2 months to more than one year depending on the type of toxin and the water quality (Newcombe 2002; UKWIR, 1996)

Dissolved microcystins have been shown to be removed by some reverse osmosis and nanofiltration membranes. As removal will depend of membrane pore size distribution and water quality, site specific tests are recommended (Smith et al 2002; Gijsbertsen-Abrahamse et al. 2006; Muntisov and Trimboli 1996; Neumann and Weckesser 1998).

Chlorination and ozonation are effective for the removal of microcystins. A residual of at least 0.3 mg L^{-1} of ozone for 5 minutes will be sufficient for all of the most common microcystins. For chlorine a dose of 3 mg L^{-1} applied to obtain a residual of 0.5 mg L^{-1} for at least 30 minutes will be effective (Nicholson et al. 1994; Newcombe 2002; Rositano et al. 1998: Rositano et al. 2001; Ho et al. 2006a; Acero et al. 2005).

Microcystin LA may require a higher residual, as it is slightly less susceptible to oxidation by chlorine (Ho et al. 2006). Potassium permanganate is effective for microcystins, and chlorine dioxide and chloramine are ineffective (Rositano et al. 1998).

Riverbank filtration and slow sand filtration have proven very effective in removing microcystins, as cyanobacterial cells are retained and dissolved toxin is degraded in the uppermost substrate layers. Grützmacher et al. (2006) show that a travel time of several days is likely to suffice, particularly if the underground consists of fine- to middle-grained sand and conditions are aerobic, not below 10°C, and some clogging layer (i.e. biofilm) is present.

Biological filtration can be very effective for the removal of most toxins. However, factors affecting the removal such as biofilm mass and composition, acclimation periods, temperature and water quality cannot be easily controlled (Ho et al., 2006b).

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Analytical methods for Microcystins:

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Although the provisional WHO GV is specifically for microcystin-LR, most samples contain several of microcystin variants, and for hazard analysis it is important to know the concentrations of all of them. Methods for determining microcystins include

1. Physico-chemical analysis by chromatographic separation (HPLC, GC, LC) and detection either by UV absorbance (photodiode array detector) or mass spectrometry.
2. Immunoassay (ELISA) for which several kits are commercially available
3. Enzyme assay using protein phosphatase inhibition.

An ISO-method for microcystin analysis by HPLC is available (ISO 20179 2005).

While chemical analysis differentiates between the structural variants of microcystin, immuno- and enzyme assays detect the sum of all microcystins in a sample. The errors associated with these assays are due to differences in reactivity between variants, but they are usually quicker to perform, require less elaborate equipment and may be cheaper when analyzing large numbers of samples.

Sampling:

For sampling raw water, it is important to take into account that some cyanobacterial taxa such as *Microcystis* and *Anabaena* may accumulate as scums, therefore, sampling needs to consider the purpose of determination. To determine the maximum concentration of microcystin in water bodies, it may be adequate to directly sample the scum or the surface water, taking a grab sample. To determine the mean concentration of microcystins in water bodies, a composite sample mixed from samples taken at different depths from the surface to the bottom of the water-body may be most representative. To determine concentrations in raw water, the choice may be the site and depth of the drinking-water offtake.

Sample Pretreatment:

The major share of microcystins in a raw water sample is usually cell-bound, i.e. occurs in the cyanobacterial biomass, however, particularly for assessing removal and/or breakthrough in drinking-water treatment, analysis of dissolved microcystins is important. Furthermore, the provisional WHO Guideline value is for the sum of cell-bound and dissolved microcystin-LR, sometimes termed “total microcystin” (caution is required here because “total microcystin” is also used by some authors for the sum of all structural variants). While analytical methods are the same for both fractions, they do require different sample pre-treatment.

For separating cells from dissolved microcystins, the sample is filtered (pore size 0.45 µm, at most 1 µm; the recommended volume to filter will depend on the cell density, frequently 50 ml to 100 ml will suffice).

Microcystins bound in biomass need to be extracted. This may be achieved through sequential extraction of the cells on the filter with aqueous methanol (to improve extraction efficiency freeze-thawing cycles prior to extraction can be added or the use of sonication).

Dissolved microcystins can be detected directly in the filtrate with immuno- and enzyme assays as well as with highly sensitive mass spectrometry usually down to 0.1 µg/L. For detection of lower concentrations as well as for UV-detection following chromatography, a concentration step is usually needed. This can be achieved through solid phase extraction (e.g. with ODS-C18

(see ISO 20179:2005).

Chemical analysis:

Determination of microcystins by UV

HPLC coupled with UV-detection, standardized by the above-mentioned ISO-method, is the most frequently used approach. It involves separation of the microcystins with a reversed phase column using a gradient elution. Absorption spectra are acquired between 200 nm and 300 nm (photodiode array detector), and microcystins are identified both by their characteristic absorption spectrum (see ISO TC 147/SC 2 N 0689) and retention time (when the standards are available). In absence of access to standards, verification of a peak as microcystin may be achieved through analyzing individual peaks from characteristic samples with the protein phosphatase assay or mass spectrometry, with the latter also providing information on the structural variant. Quantification is achieved by measuring the chromatographic signals of each microcystin against calibration curves produced from standards. Concentrations of further microcystins for which no quantitative standards are available may be inferred by relating their signal against that of MCYST-LR and then be reported as MCYST-LR concentration equivalents (As response factors of microcystins in UV detection appear to vary by a factor of maximally two, the uncertainty of this approach is negligible for hazard characterization).

The detection limit of this method is somewhat variable, depending on the matrix, but 0.1 µg/l may be achieved in most cases.

Determination of microcystins by mass spectrometry:

Unambiguous identification of microcystins may be achieved with a mass spectrometry (MS) detector, ideally with tandem mass spectrometry (MS-MS) in order to obtain information both on the molecular signal and fragments. Detection limits of 0.1 µg/l and less can be achieved. The drawback in relation to UV-detection is the potentially much higher variability of response factors, so that estimates of quantification are not possible without standards for the specific variant.

Determination of total microcystins by LC/MS or GC/MS:

Determination of microcystins is also possible through GC/MS and LC/MS after oxidation of microcystin to 2-methyl-3-methoxy-4-phenyl butyric acid (MMPB), ionization with ESI (atmospheric pressure ionization) for LC or chemical ionization for GC, and subsequent mass spectrometric detection. This method is highly sensitive as it detects MMPB as surrogate, achieving a detection limit of 0.1 µg/L.

Determination of total microcystins with immunoassay (ELISA):

Several ELISA kits are commercially available which have plates or vials prepared with microcystin antibodies attached to their walls. These determine total microcystins and do not differentiate between structural variants. Most of the ELISA kits can produce results within 2 hrs in a routine laboratory. Their detection limit is in the range of 0.1 - 0.2 µg/l.

Determination of total microcystins with enzyme assay (Protein Phosphatase Assay; PPA):

Colorimetric assays using the ability of microcystins to specifically inhibit type-1 protein phosphatase are available. They allow visual confirmation of the presence or absence of microcystin in a water sample by comparing the colour reaction against a control sample as well as to roughly estimate the amount of toxin present in the sample (positive control is 1.5 µg/L microcystin-LR). False positives may be possible due to other PPA inhibitors in the sample, but in practice have rarely proved problematic, particularly if information about potentially occurring cyanobacterial species is available and indicates a likelihood of microcystins to

occur.

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