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To the Minister of Agriculture, Nature and Food Quality

Advice from the Director of the Office for Risk Assessment and Research

Advice on cyanotoxins in surface water for agricultural use

Background

Cyanobacteria (blue-green algae) can grow explosively in water during warm periods, such as in the summer. There are many different types of cyanobacteria that produce various toxins. The toxins of cyanobacteria (cyanotoxins) can be harmful to health. If water contaminated with cyanobacteria is used for irrigation of crops or for drenching of livestock, this can result in a health hazard for humans and/or animals.

In 2006, the Office for Risk Assessment and Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA) derived a maximum total cyanotoxin concentration of 1 μg/L for spray irrigation of food crops and 40 μg/L for drenching of livestock. This risk assessment was based on the Acceptable Daily Intake (ADI) for microcystine-LR (MC-LR) of 0.04 μg/kg body weight per day derived by the World Health Organization (WHO). ADI is an estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health. In 2018, on the basis of a literature study, BuRO confirmed that the conclusions from the advice issued in 2006 were still relevant and current.

In 2019, the French Agence Nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) published new health-based guidance values for two cyanotoxins: cylindrospermopsin (CYN) and MC-LR. For MC-LR, ANSES derived a Toxicological Reference Value (TRV) of 0.0008 μg/kg body weight per day. TRV is a toxicological index that, compared with the exposure, is used to qualify or quantify the risk to human health. This is far lower than the ADI derived by WHO. The ANSES publication led BuRO to evaluate and if necessary update its previous BuRO advice.

Research question

What is the health risk to humans of consuming food contaminated with cyanotoxins?
The research question is divided into the following questions.

1. What is the maximum concentration of cyanotoxins in surface water at which no food safety risk occurs if food crops are spray irrigated with the water and subsequently consumed?
2. What is the maximum concentration of cyanotoxins in surface water that can be used for drenching of livestock, at which no food safety risk occurs through consumption of animal products?

Scope and approach

This advice is focused on food safety risks that can occur due to agricultural use of surface water contaminated with cyanotoxins. Surface water contaminated with cyanobacteria can result in a health risk if for example used for swimming or an animal health risk if drunk by pets or other animals. However, this is not included in the scope of this advice.

The substantiation describes how the advice was arrived at. Comments were added to a draft version of the advice by an external expert.

Findings

Hazard identification

- An overview supplied by Wageningen Food Safety Research (WFSR) shows that of all cyanotoxins, microcystins (MCs) are most commonly observed in Dutch surface water, followed by anatoxins (ATX). CYN, saxitoxins (STX) and β-N-methylamino-L-alanine (BMAA) have been incidentally observed.

Hazard characterisation

- MC-LR is one of the MCs viewed as a hepatoxic and reprotoxic substance. Based on a subchronic study (13 weeks) with mice, a No Observed Adverse Effect Level (NOAEL) of 40 µg/kg body weight per day was derived on the basis of liver damage. NOAEL is the greatest concentration of a substance at which no detectable adverse effects occur in an exposed population. For chronic exposure, the RIVM/WFSR Front Office Food and Product Safety (FO) derived a Benchmark Dose Lower Confidence Limit (BMDL) of 0.02 µg/kg body weight per day on the basis of a 6-month study with mice, in which reduced sperm quality was the critical effect. BMDL is the exposure in respect of which it can be stated with 95% confidence that the actual dose that causes an adverse effect is higher.
- CYN is cytotoxic and under chronic exposure affects the liver and kidneys. The FO derived an Acute Reference Dose (ARfD) on the basis of fat infiltration in the liver in mice of 0.5 µg/kg body weight. ARfD is an estimate of the amount of a substance in food or drinking water that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer. For (sub)chronic effects, the FO derived a BMDL for CYN of 9.4 µg/kg body weight per day, on the basis of increased liver and kidney weight and increased serum concentrations of liver enzymes in mice in a 90-day study. On the basis of a literature study, the FO concluded that CYN must be viewed as a genotoxic substance.
- Anatoxin-a (ATX) is a neurotoxin. An NOAEL of 98 µg/kg body weight per day was derived on the basis of a subacute study (28 days) in mice.
- Saxitoxin (STX) is a neurotoxin that can cause paralytic shellfish poisoning (PSP). In mild cases of PSP, the clinical symptoms are a tingling feeling or loss of feeling around the lips. Based on reported poisonings of more than 500 people, the European Food Safety Authority (EFSA) determined a Lowest Observed Adverse Effect Level (LOAEL) for STX in humans of 1.5 µg/kg body weight per day. LOAEL is the lowest level of a substance that has been observed to cause harm in an exposed population. EFSA derived an ARfD for STX of 0.5 µg/kg body weight per day.
- BMAA is a neurotoxin for which no published health-based guidance value has been found.
- BuRO has adopted the ARfD and BMDLs derived by the FO, and the NOAEL and ARfD values published by the EFSA. For MC-LR, CYN and ATX, the Margin of Exposure (MOE) approach is applied. MOE is the ratio between the reference point from the animal study (NOAEL or BMDL) and human exposure. For non-genotoxic substances (MC-LR and ATX), an MOE of 100 is employed. As CYN is viewed as genotoxic, for this substance an MOE of 10,000 is applied.

Legislation and supervision

- There are no legal limits for cyanotoxins in food.
- The NVWA does not supervise the agricultural use of surface water (irrigation and drenching of livestock). The NVWA is however responsible for supervising food safety. The Ministry of Health, Welfare and Sport (VWS) is responsible for food safety; the Ministry of Agriculture, Nature and Food Quality (LNV) is responsible for agriculture.
- The quality of Dutch surface water falls within the responsibility of the Ministry of Infrastructure and Water Management (I&W). The Water authorities are tasked with enforcing legislation for regional waters. For bathing water there are legal requirements for blue-green algae and cyanotoxins, but not for other surface water. Central government (I&W) does offer advice to growers not to irrigate with water contaminated with blue-green algae in periods of drought. The recommended guidance value for cyanotoxins is 1 µg/L.
- The Netherlands has no structural or representative monitoring programme for cyanotoxins in surface water, with the exception of bathing water.

Exposure estimate

- There are no NVWA data for cyanotoxins in food crops, milk or meat.
- Blue-green algal bloom occurs in warm periods, in particular in July and August. Open crops grown in the Netherlands and harvested in this period are lettuce, tomato, carrot, French bean and Chinese cabbage. The most probable scenario is incidental intake of food crops containing these cyanotoxins. This also applies to milk and meat originating from production animals exposed to cyanotoxins. For that reason, an acute scenario has been selected: acute extreme (P95) consumption on consumption days and acute health-based reference values of cyanotoxins. For the selected food crops, milk and meat, the maximum permitted cyanotoxin concentration has been calculated, without exceeding the health-based guidance value (see Table 18 in substantiation).
- A literature search was conducted into the accumulation of cyanotoxins in food crops due to irrigation and the so-called uptake factor. The uptake factor is the ratio between the concentration in the food crop and in the irrigation water. The highest uptake factor of 20 was found for MC-LR in lettuce and carrots. For other food crops and cyanotoxins, the uptake factor was lower, sometimes lower than 1. For CYN, the highest uptake factor found was lower than 1. For STX and ATX, no studies were found in literature and 20 was taken as the worst case uptake factor. The maximum permitted concentration in water at which no food safety risk occurs was calculated (see Table 19 in the substantiation).
- For a subchronic scenario, in which consumers consume food crops over a period of 1-2 months originating from a single grower, the maximum permitted concentration of cyanotoxins in food crops and subsequently in the water was calculated. For CYN, the calculated guidance value of 1 offers insufficient protection; for the subchronic scenario, a guidance value of 0.1 µg/L was calculated. For the other cyanotoxins, the calculated guidance value in Table 1 offers sufficient protection.
- With regard to transfer in production animals, the only studies found related to MC-LR in cattle. These studies revealed no detectable transfer to milk and meat. The maximum exposure of cattle in these studies is maintained as the safe guidance value. This has been converted to a maximum concentration for drenching of livestock, whereby account is also taken of consumption of grass spray irrigated with the same water (Table 1).

Table 1: Overview of calculated maximum concentrations for cyanotoxins in surface water for irrigation of food crops and drenching of livestock.

	MC-LR	CYN	ATX	STX
Irrigation (µg/L)	1	30	3	2
Drenching of dairy cow (µg/L)	45			
Drenching of beef cattle (µg/L)	5			

- The WFSR overview shows that MCs were detectable in 92% of the water samples analysed for agricultural use (>0.01 µg/L). The median was 0.39 µg/L, the maximum concentration 7.8 µg/L. MC is a group designation; concentrations found are for total MC and may represent an overestimate of the MC-LR concentration. For other cyanotoxins, data is only available for other surface water (not specifically for agricultural use). CYN was detected in 3% of the analysed water samples, whereby the highest concentration found was 0.27 µg/L. ATX was detected in 27% of the water samples analysed for this substance, whereby the majority of samples had a concentration of less than 0.1 µg/L. STX was detected in 3% of the analysed samples, the highest concentration was 15 µg/L.

Risk characterisation

- In respect of MC-LR, half of the analysed samples for agricultural use are below the guidance value for irrigation of food crops. 4% exceeded the calculated guidance value for MC-LR for the drenching of dairy cattle.
- The maximum concentration of CYN and ATX found is lower than the calculated maximum value. For STX, one exceedance was found.
- There are many uncertainties due to the lack of data. This relates both to data about health-based guidance values of cyanotoxins and data about accumulation in various food crops, intake by beef cattle in respect of surface water and fresh grass, transfer in production animals and structural monitoring data of cyanotoxins in agricultural surface water.

Answers to the research question

What is the maximum concentration of cyanotoxins in surface water at which no food safety risk occurs if food crops are spray irrigated with the water and subsequently consumed?
For (spray) irrigation of crops, the current recommendation value (1 µg/L) offers sufficient protection. This value is based on MC-LR, the most commonly occurring cyanotoxin in Dutch surface water.

What is the maximum concentration of cyanotoxins in surface water that can be used for drenching of livestock, at which no food safety risk occurs through consumption of animal products?
For dairy cattle, 45 µg MC-LR/L is a safe value, for beef cattle 5 µg MC-LR/L. Due to a lack of data, no calculation could be made for the other cyanotoxins.

Advice from BuRO

To the Minister of Agriculture, Nature and Food Quality

- Establish a system for monitoring cyanotoxins in surface water for agricultural use, comparable to that for bathing water. In that system employ the guideline values calculated by BuRO as reference value for agricultural use of surface water. Do this in close consultation with the ministries of Health, Welfare and Sport and Infrastructure and Water Management.
- Ensure that this advice is published on the internet site of central government, drought dossier.

Yours sincerely,

Prof. Antoon Opperhuizen
Office for Risk Assessment & Research

Substantiation

Introduction

Cyanobacteria (blue-green algae) can occur in surface water. Water rich in nutrients, such as nitrogen and phosphorus, is an excellent breeding ground for cyanobacteria. These algae can bloom particularly in stagnant water and at warmer temperatures of between 20 and 30 °C (Lürling et al., 2013). Cyanobacteria can occur in freshwater, brackish water and saltwater. Cyanobacteria can secrete toxins, so-called cyanotoxins, which can have both acute and chronic health effects.

By (spray) irrigating food crops with surface water, consumers could be exposed to cyanotoxins that end up in or on the food crops from the water. Moreover, surface water can be used for the drenching of farm animals. Via transfer to milk and meat, consumers could ingest cyanotoxins by consuming these products.

This advice is an update on previous published BuRO advisory reports (BuRO, 2006;2018). In response to publication of new health-based guidance values of cyanotoxins by the French Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (ANSES, 2019a;2019b), the Office for Risk Assessment and Research(BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA) conducted new research into the possible health risks for consumers due to the irrigation of food crops and the drenching of livestock with water contaminated with cyanotoxins.

Approach

After studying the ANSES publications, BuRO decided to submit four requests for assessment to RIVM/WFSR Front Office Food and Product Safety (FO). The first request for assessment in 2019 concerns answers to the following questions.

1. Taking account of the health-based guidance values derived by ANSES for microcystine-LR (MC-LR) and cylindrospermopsin (CYN), does the limit for toxins in irrigation water of 1 μg/L, as proposed by BuRO, still offer sufficient safety for humans, following consumption of crops spray irrigated with water in which these toxins are observed?
2. Taking account of the health-based guidance values derived by ANSES for MC-LR and CYN, does the limit for toxins (cyanobacteria, MC-LR and CYN) in water for the drenching of livestock at 40 μg/L as proposed by BuRO still offer sufficient safety for farm animals, dogs and cats?
3. Are there any expected effects on food safety of animal products from animals that have drunk surface water containing MC-LR and CYN?
4. Are there further considerations in the framework of risk assessment on this subject that you wish to bring to the attention of BuRO?

The second request for assessment in 2020 addressed to the FO related to an assessment of the method used in the ANSES publications for determining a point of departure for deriving the health-based guidance values for CYN and MC-LR. To derive the Toxicological Reference Value (TRV), ANSES selected two toxicity studies, namely that of Chen et al., 2011 and Chernoff et al., 2018. TRV is a toxicological index that, compared with the exposure, is used to qualify or quantify the risk to human health. The FO answered the following questions:

1. Using Benchmark Dose (BMD) modelling, is it possible to derive a point of departure on the basis of data from the studies of Chen et al., 2011 and Chernoff et al., 2018?
2. Based on these studies, what point of departure does the FO derive?

After studying all information received, a third assessment question was put to the FO in 2020, namely: should CYN be viewed as a genotoxic substance? For a different risk assessment, a fourth request was submitted to FO, namely to derive an Acute Reference Dose (ARfD) for CYN (FO, 2020a). ARfD is an estimate of the amount of a substance in food or drinking water that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer.

Wageningen Food Safety Research (WFSR) analyses the water quality, including cyanotoxins, for various organisations. In 2020, WFSR was asked to prepare an overview of which cyanotoxins they have observed in surface freshwater in the Netherlands, and in what concentrations.

In April 2021, via the Focal Point network of the European Food Safety Authority (EFSA), BuRO consulted other European Member States and other European countries about whether they have specific legislation or reference values in respect of cyanotoxins in drinking water and surface water.

BuRO additionally conducted a literature study into the accumulation of cyanotoxins in food crops and the transfer of cyanotoxins in farm animals to animal products (see Annex 1 for the search strategy). BuRO took the FO assessments (FO, 2020b;2020c;2020d) and the WFSR report (Faassen et al., 2021) as the starting point for this advisory report. Based on the health-based guidance values for cyanotoxins, data about uptake in food crops and transfer to animal products, BuRO calculated the maximum concentrations of cyanotoxins in surface water for (spray) irrigation of food crops and the drenching of livestock.

Hazard identification

The composition of so-called blue-green algae bloom is very diverse. Various types of cyanobacteria can be present in the bloom, capable of producing many different cyanotoxins. The following groups of cyanotoxins are identified: neurotoxins (substances toxic for the nervous system), cytotoxins (substances toxic for cells), hepatotoxins (substances toxic for the liver) and dermatoxins (skin irritants) (WUR, 2019).

Based on measurement data from the period 2009-2020 for each water type (bathing water, urban water, water playgrounds and recreation areas, water for agricultural use, shellfish production areas and other waters), WFSR indicated which cyanotoxins were measured and what the concentrations found were (Faassen et al., 2021). These measurements considered both blue-green algae in the water column and on the water surface (pelagic blue-green algae) and blue-green algae growing on the bottom or other substate (benthic blue-green algae).

The monitoring data in this WFSR report (Faassen et al., 2021) are the result of targeted sampling. The analysis is often also restricted to known cyanotoxins or cyanotoxins for which a legal requirement applies (for example microcystins in bathing water). Except for bathing water, the Netherlands operates no structural and representative monitoring programme for cyanotoxins. There are few measurement data for cyanotoxins in surface water for agricultural use. In the summer period of 2018, the concentration of microcystins (MCs) was monitored over a two-month period, in a single control area. Other data were gathered as a consequence of incidents or with a view to testing in advance whether the water is suitable for drenching of livestock.

The WFSR report reveals that MCs are observed in the vast majority (84%) of Dutch pelagic blue-green algae samples (Faassen et al., 2021). The MC concentration can be very high specifically in floating layers. The highest concentration found is 21,000 µg MC/L. The next most commonly observed cyanotoxins are the anatoxins. Cylindrospermopsins, saxitoxins and β-N-methylamino-L-alanine (BMAA) were observed incidentally. Nodularin was observed very incidentally. This toxin occurs mainly in brackish waters and is not included in this risk assessment because it is unlikely that brackish water will be used for agricultural purposes. In benthic samples, cyanotoxins are observed less frequently, but in almost every measured toxin group, high concentrations were found (incidentally).

For this risk assessment, the following cyanotoxins that occur in Dutch surface water have been assessed:

- Microcystin (MC)
- Cylindrospermopsin (CYN)
- Anatoxin (ATX)
- Saxitoxin (STX)
- β-N-methylamino-L-alanine (BMAA)

Hazard characterisation

Microcystine-LR (MC-LR) (CAS number 1043-37-2)

Toxicity
Not all MCs have the same structure and are equally toxic. Of all MCs, MC-LR is the most commonly observed, and is considered the most toxic MC (Chernoff et al., 2021).

In the report by the World Health Organization (WHO) about MC-LR, the toxicokinetics are described as follows (WHO, 2020d): MC-LR is absorbed in the gastrointestinal tract, probably via the organic-anion-transporting polypeptides (OATP). Following oral exposure, this substance is found in the liver, lungs, kidneys, brain and reproductive tissue. Conjugation with thiol compounds (glutathione and cysteine) is the most important biotransformation reaction for MC-LR. Primary elimination of both MC-LR and metabolites MCs takes place via urine and faeces. Due to low passive membrane permeability of MC-LR in the gastrointestinal tract, the oral biological availability of MCs is limited. The non-absorbed MCs are removed via faeces. The follow-up of dialysis patients exposed to MCs in dialysis water revealed that total clearance is a protracted process because MCs were still detected in the serum of patients more than 50 days following exposure (Soares et al., 2006; Hilborn et al., 2007).

MCs are absorbed into the cell via OATP. In the cell, MCs inhibit the functioning of protein phosphatases, which can lead to cell death. Acute symptoms of MC poisoning include bleeding in the liver. MC-LR was formerly characterised as a hepatotoxin. Numerous studies over the past years have also revealed other harmful effects: reprotoxicity, neurotoxicity and pulmonal toxicity. In one incident in a dialysis clinic in Caruaru (Brazil) in 1996, 116 of the 130 patients demonstrated symptoms including eye problems, nausea and vomiting. At least 26 deaths due to acute liver failure are ascribed to kidney dialysis with water containing MC-LR (Jochimsen et al., 1998).

Health-based guidance value
Table 2 contains an overview of the health-based guidance values for MC-LR found in literature.

Table 2: Overview of available toxicological guidance values for MC-LR

	Guidance value	Critical effect	Description of animal test
LD_{50}	10 mg/kg bw	Death/Mortality	Single dose via gavage in mice (Yoshida et al., 1997)
LD_{50}	5 mg/kg bw	Death/Mortality	Single dose via gavage in mice and rats (Fawell et al., 1999a)

	Guidance value	Critical effect	Description of animal test
LOAEL	50 µg/kg bw/day	Liver damage	Rats exposed daily over a 28-day period via drinking water (Heinze, 1999)
NOAEL	40 µg/kg bw/day	Increased liver weight, liver damage	Mice exposed via gavage over a 13-week period (Fawell et al., 1999a)
NOAEL	1 µg/kg bw/day	Reduced sperm quality	Male mice exposed via drinking water over a 6-month period (Chen et al., 2011)
BMDL	0.02 µg/kg bw/day	Reduced sperm quality	Male mice exposed via drinking water over a 6-month period (Chen et al., 2011; FO, 2020c)

For MC-LR, Yochida et al. derived an oral lethal dose median (LD_{50}) of 10 mg/kg body weight (Yoshida et al., 1997). LD_{50} is the amount of a chemical that is lethal to one-half (50%) of the experimental animals exposed to it. Mice were administered a single dose of MC-LR via a gavage. Liver and kidney damage were observed in the mice exposed to a dose higher than 10 mg/kg. Fawell et al. derived an LD_{50} of 5 mg/kg body weight for mice and rats (Fawell et al., 1999a). ANSES, WHO, United States Environmental Protection Agency (US EPA) and Health Canada derived no ARfD.

Heinze conducted a subacute study by exposing rats to MC-LR via their drinking water at a dose of 50 and 150 µg/kg body weight, over a 28-day period (Heinze, 1999). Following this period of exposure, rats in both dose groups showed increased liver weight, changed serum enzyme activities and histological damage to the liver. The Lowest Observed Adverse Effect Level (LOAEL) was determined at 50 µg/kg body weight per day. LOAEL is the lowest level of a substance that has been observed to cause harm in an exposed population.

In a chronic study, Fawell et al. administered MC-LR to mice via a gavage over a 13-week period in doses of 0, 40, 200 ad 1000 µg/kg body weight per day (15 mice per gender and dose) (Fawell et al., 1999a). At a dose of 200 µg/kg body weight/day, minor liver damage was observed in some male and female mice. The No Observed Adverse Effect Level (NOAEL) of this study amounted to 40 µg/kg body weight per day. NOAEL is the greatest concentration or amount of a substance at which no detectable adverse effects occur in an exposed population.

In 2003, the World Health Organization (WHO) derived an Acceptable Daily Intake (ADI) for MC-LR in drinking water of 0.04 µg/kg body weight per day (WHO, 2003). ADI is an estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health. This ADI is based on the NOAEL of 40 µg/kg body weight per day from the study by Fawell et al. (Fawell et al., 1999a). A safety factor of 1000 was then applied: 100 for intra and interspecies and 10 for the lack of data on chronic toxicity and carcinogenicity. In 2021, WHO published a second edition of the book on toxicity of cyanotoxins in water (Chorus & Welker, 2021). In this book, the same health-based guidance value (ADI) was maintained for MC-LR as derived by WHO in 2003. The previously published BuRO advisory reports (BuRO, 2006; 2018) are based on this ADI (0.04 µg/kg body weight per day).

Based on the LOAEL from the study by Heinze (Heinze, 1999), the US EPA derived a Reference Dose (RfD) of 0.05 µg/kg body weight per day (US EPA, 2015c). RfD is an estimate of the daily exposure of the human population (including sensitive target groups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. They applied a safety factor of 1000 to the LOAEL; this is based on a factor of 100 for intra and interspecies variation, a factor of 3 ($10^{0.5}$) for the use of an LOAEL instead of an NOAEL, and a factor of 3 ($10^{0.5}$) for uncertainties in the database.

For MC-LR in drinking water, Health Canada derived a Tolerable Daily Intake (TDI) of 0.056 µg/kg body weight per day (Health Canada, 2021), on the basis of a study by Heinze (Heinze, 1999). TDI is an estimate of the amount of a substance in food or drinking water which is not added deliberately and which can be consumed over a lifetime without presenting an appreciable risk to health. Health Canada selected this study because the blooming of cyanobacteria in water is seasonal and takes place during a short period (less than 30 days); there is therefore no evidence of chronic exposure via drinking water. Health Canada applied a safety factor of 900 to convert the LOAEL into a TDI. This consists of a factor of 100 for intra and interspecies variation, a factor of 3 for the use of a LOAEL instead of a NOAEL and a factor of 3 for uncertainties in the database.

In 2019, ANSES derived a TRV for MC-LR for humans of 0.0008 µg/kg body weight per day (ANSES, 2019a). The starting point for deriving this TRV was a study by Chen at al. (Chen et al., 2011). In this study, over a period of three and six months, MC-LR was administered to male mice via drinking water, at a dose of 0, 1, 3.2 and 10 µg/L. Each dose group consisted of 20 mice. At a dose of 3.2 µg/L and higher, after three months, sperm quality had declined. At the highest dosage, the concentration of testosterone had decreased, while the concentration of luteinising hormone and follicle-stimulating hormone had increased. Leydig cells demonstrated apoptosis. After six months, these effects were comparable, but more clearly present in the exposed group. ANSES selected reduced sperm quality as the critical effect, and on the basis of the NOAEL of 1 µg/L derived a subchronic TRV. In this calculation, the NOAEL was converted from 1 µg/L drinking water to 0.15 µg/kg body weight per day. ANSES then applied allometric scaling to convert the NOAEL from mouse to human. This NOAEL was then converted by ANSES to a TRV by applying a safety factor of 25. The study by Chen et al. (Chen et al., 2011) is not reported in the second edition of the WHO book on cyanotoxins in water (Chorus & Welker, 2021), although the study by Chen et al. was published well before the publication of the WHO book.

By preference the Benchmark Dose (BMD) approach is used for deriving a health-based guidance value (i.e. BMDL (Benchmark Dose Lower Confidence Limit)) (EFSA, 2017). $BMDL_x$ is the exposure in respect of which it can be stated with 95% confidence that the actual dose that causes an adverse effect is higher. The subscript x is the specification of the response level, an increase or reduction of x% in response. ANSES concluded that the BMD approach could not be applied to the above study, because the BMD/BMDL ratio was greater than 10, and the resultant BMD was 14 times lower than the first tested experimental dose. BuRO questioned whether the rejection of the BMD approach was justified. This question was submitted to the FO, which concluded that the BMD approach may be applied (FO, 2020c). BuRO follows the FO in this conclusion. On the basis of the critical study (Chen et al., 2011), FO derived a BMDL for MC-LR of 0.13 µg/L in the drinking water of the mice. This BMD value in drinking water was subsequently converted by BuRO to a BMDL per kg body weight using a conversion factor of 0.15 (EFSA, 2012). This results in a BMDL of 0.13 * 0.15 = 0.02 µg/kg body weight per day.

Choice of health-based guidance value
No ARfD has been found for MC-LR, only LD_{50} values. Two subchronic studies were found, on the basis of which WHO, Health Canada and US EPA derived their guidance values: a NOAEL of 40 µg/kg body weight per day (Fawell et al., 1999a) and an LOAEL of 50 µg/kg body weight per day (Heinze, 1999). Both studies took liver damage as their critical end point. For the acute (or subacute) health-based

guidance value, BuRO has opted for the NOAEL from Fawell et al. at 40 µg/kg body weight per day, because a NOAEL is preferable to a LOAEL. For a LOAEL, an additional safety factor would have to be applied. By using this subchronic NOAEL as the acute health-based guidance value, the risk assessment will probably be more conservative.

The BMDL, derived by the FO, is at a far lower level: 0.02 µg/kg body weight per day. This BMDL is based on a critical end point other than liver damage, namely reprotoxicity. This BDML is viewed as a chronic health-based guidance value. Study by Soares et al. and Hilborn et al. revealed that MC remains in the body for an extended period following exposure (Soares et al., 2006; Hilborn et al., 2007).

Cylindrospermopsin (CYN) (CAS number 143545-90-8)

Toxicity
ANSES and WHO conducted literature studies into the toxicity of CYN (ANSES, 2019b; WHO, 2020c). No studies involving CYN were found that describe absorption in the body. However, as systemic effects (including in the liver and kidneys) were observed following oral ingestion in mice, intake via the gastrointestinal tract is likely (Shaw et al., 2000; Humpage & Falconer, 2003). Systemic effects are toxic effects caused as a consequence of absorption and distribution of a substance in the body. As CYN is a small hydrophilic molecule, absorption in the intestines can take place through a combination of active transport and passive diffusion. Experiments in which ^{14}C labelled CYN was administered to mice via intraperitoneal injection, revealed that 50% of the ^{14}C labelled CYN was secreted in the urine unchanged (Norris et al., 2001). This suggests that half of the administered CYN undergoes biotransformation. CYN appears to be metabolised by the enzyme system cytochrome P450. The secretion of CYN and metabolites is in particular via urine and faeces. Following intraperitoneal injection of a sublethal dose 0.1 mg/kg body weight) in mice, 73% had been secreted after 12 hours, mainly via urine (Norris et al., 2001). No elimination studies were found involving humans.

CYN is cytotoxic. Acute effects observed following oral intake of CYN are effects on the liver (cytolysis, infiltration of inflammatory cells and proteins), kidneys (tubular necrosis, changes to the proximal tubules or glomeruli) and intestines (bleeding) (ANSES, 2019b). On Palm Island, 140 people became ill after drinking water contaminated with algae (Byth, 1980). The following symptoms emerged within a week: vomiting, enlarged liver and kidney failure. Recovery took between one and three weeks. It only became clear years later that CYN was responsible for this sickness, which was named the Palm Island mystery disease (Ohtani et al., 1992).

Various *in vitro* studies reveal that CYN may be genotoxic. Studies by Humpage et al. suggest that CYN induces DNA damage (Humpage et al., 2000). ANSES argues that the results of *in vitro* studies support the existence of a genotoxic effect. ANSES considers the *in vivo* results to be too fragmented and therefore does not classify CYN as genotoxic. BuRO asked the FO to determine whether CYN is genotoxic, based on a literature study. The FO concluded that CYN should be considered a genotoxic substance (FO, 2020d). Based on the results of *in vitro* micronucleus tests in rats, the FO concluded that CYN is a clastogenic substance. This means that CYN can cause structural chromosome defects. CYN probably has to be converted in the body into active metabolites before it can cause these clastogenic effects. The available *in vitro* studies on gene mutations (mutagenicity) provide no evidence for mutagenic characteristics of CYN. However, the concentration used in these studies were probably too low (FO, 2020d). In combination with the positive results of the *in vivo* comet assays, which are capable of detecting both clastogenic and mutagenic characteristics of a substance, mutagenicity of CYN cannot be excluded (FO, 2020d). BuRO adopts this conclusion, and assumes that CYN must be considered a genotoxic substance.

Health-based guidance value
Table 3 contains a list of the health-based guidance values found for CYN.

Table 3: Overview of available toxicological guidance values of CYN

	Guidance value	Critical effect	Description of animal test
LOAEL	1 mg/kg bw	Liver and kidneys	Single dose in mice (Shaw et al., 2000)
NOAEL	50 µg/kg bw per day	Fat infiltration in the liver	Oral administration to mice over a 2-week period (Shaw et al., 2000)
ARfD	0.5 µg/kg bw	Fat infiltration in the liver	Oral administration to mice over a 2-week period (Shaw et al., 2000; FO, 2020a)
NOAEL	30 µg/kg bw per day	Increased kidney weight	Exposure in mice via gavage over an 11-week period (Humpage & Falconer, 2003)
LOAEL	75 µg/kg bw per day	Increased liver and kidney weight	90-day study with mice, (Chernoff et al., 2018)
BMDL	9.4 µg/kg bw per day	Increased liver and kidney weight	90-day study with mice, (Chernoff et al., 2018; FO, 2020c)

No health-based guidance value for acute (single) or chronic exposure to CYN is available in literature. For that reason, BuRO asked the FO to derive an ARfD for CYN (FO, 2020a). Initially, the FO studied two acute toxicity studies in which following oral administration of single high dosages, harmful effects were observed. The FO concludes that the uncertainties in these studies are too great to use the results to derive an ARfD. For that reason, the FO also considered studies in which multiple lower dosages were administered (subacute toxicity studies). Starting at an oral dosage of 8 µg/kg body weight per day, effects were observed that were not harmful. With an oral dosage of 150 µg/kg body weight per day, fat infiltration in the liver was demonstrated, in mice. This effect was also observed in the studies into acute toxicity. The FO considers fat infiltration to be a critical, harmful effect. With an oral dosage of 50 µg/kg body weight per day in mice, following two weeks exposure, no harmful effects were observed. The FO identifies this dosage as a No Observed Adverse Effect Level (NOAEL). The FO then applies a standard safety factor of 100 (intra and interspecies variation), which results in an ARfD of 0.5 µg/kg body weight (FO, 2020a).

In 2020, the WHO derived a limit for drinking water and recreational water. This limit is based on a NOAEL of 30 µg/kg body weight per day from a study with mice over a period of 11 weeks (Humpage & Falconer, 2003; WHO, 2020c). The critical effect in this study was the increase in relative kidney weight.

In 2015, the US EPA derived an RfD for CYN on the basis of the same NOAEL of 30 µg/kg body weight (Humpage & Falconer, 2003), of 0.1 µg/kg body weight per day (US EPA, 2015b). A safety factor of 300 was applied: factor 10 for interspecies variation; factor 10 for intraspecies variation; and a factor of 3 for uncertainties in the database.

ANSES derived a subchronic TRV for humans of 0.14 µg/kg body weight per day. ANSES took as its critical effect the increase in liver and kidney weight and increased serum levels of liver enzymes in the mouse. The study by Chernoff et al. was selected by ANSES as critical study (Chernoff et al., 2018). This study was conducted in accordance with OECD guideline no. 408. In a subchronic 90-day study, mice (male and female) were administered CYN orally, in dosages of 0, 75, 150 and 300 µg/kg body weight per day. Ease dose group consisted of 18 to 20 mice. The researchers concluded that in all dose groups, both clinical and histopathological effects occurred in the liver and kidney. No NOAEL could be derived. A LOAEL was however derived from this study of 75 µg/kg body weight per day, which was the lowest dose administered in this study.

ANSES converted this LOAEL for mice into a subchronic TRV for humans of 0.14 µg/kg body weight per day (ANSES, 2019b). ANSES first applied the BMD approach to the abovementioned study. The values obtained from this modelling could not be retained because the first exposure dose was too far from the control dose, making the model uncertain around the BMD values. In addition, these BMD values were within the linear extrapolation range of the model, making it even more uncertain. Therefore ANSES concluded that the uncertainty in the derived BMD values was too high for the BMD approach to be used to derive a health-based guidance value. At the request of BuRO, the FO investigated whether the BMD approach may be applied to the study by Chernoff et al. (see approach). The FO concluded that the BMD approach may in fact be applied and, on the basis of this study (Chernoff et al., 2018) calculated a BMDL for CYN of 9.4 µg/kg body weight per day (FO, 2020c).

Choice of health-based guidance value
For acute exposure, the ARfD of 0.5 µg/kg body weight as derived by the FO is selected (FO, 2020a).

For subchronic exposure, a NOAEL of 30 µg/kg body weight per day is available (Humpage & Falconer, 2003) and a BMDL based on the study by of 9.4 µg/kg body weight per day (Chernoff et al., 2018; FO, 2020a). According to the EFSA guideline, a BMDL is preferable to a NOAEL or LOAEL (EFSA, 2017). For subchronic exposure, the BMDL of 9.4 µg/kg body weight per day, as derived by FO, is selected.

Anatoxin (ATX), (CAS number 64285-06-9)

Toxicity
Anatoxin-a (ATX) (CAS number 64285-06-9) and its analogues are alkaloids produced by strains of various types of blue-green algae that occur primarily in freshwater environments (WHO, 2020b). Many of these species are benthic. ATX has been connected to the death of dogs and wild animals.

The ATX enantiomer binds with high affinity to nicotinic acetylcholine receptors of nerve cells and causes chronic overstimulation of muscle cells. This can result in increased heart rate and blood pressure, fatigue and eventual muscle paralysis. This latter symptom can cause death if occurring in the respiratory muscles. Although ATX is the best studied analogue, there are indications based on limited evidence that homoanatoxin-a (HTX) and the dihydro derivatives of ATX and HTX bond to the same receptor, and if administered orally can have a similar potential as ATX (WHO, 2020b).

In acute toxicity studies in animals, signs of neurotoxicity occur within a few minutes following oral exposure, including loss of coordination, muscle spasms and death due to respiratory paralysis. This suggests that following oral exposure, ATX is rapidly absorbed. Because of these rapid effects following administration, which occur in both the central and peripheral nervous system, it is assumed that this substance is distributed throughout the body (WHO, 2020b). WHO was unable to find any studies in which the metabolism in mammals has been investigated.

Health-based guidance value
Table 4 contains a list of the toxicological guidance values for ATX found in literature.

Table 4: Overview of available toxicological guidance values for ATX

	Guidance value	Critical effect	Description of animal test
LD_{50}	13.3 mg/kg bw	Death/Mortality	Single dose via gavage in mice (Stevens & Krieger, 1991)
NOAEL	98 µg/kg bw/day	Above the dose a mouse died. Cause of death unknown.	28 days mice via gavage (Fawell et al., 1999b)

Stevens and Krieger determined an oral LD_{50} value of 13.3 mg/kg body weight in mice (Stevens & Krieger, 1991). ATX was administered via a gavage. The US EPA concluded that there are insufficient data to derive an ARfD (US EPA, 2015a).

In a subacute study, Fawell et al. administered ATX to mice over a period of 28 days via a gavage, in doses of 0.098, 0.49 and 2.46 mg/kg body weight per day (Fawell et al., 1999b). In the two highest dose groups, one mouse died in each group. The cause of death could not be determined, but ATX toxicity could not be excluded. Based on this study, a NOAEL of 0.098 mg/kg body weight per day was determined (Fawell et al., 1999b). WHO derived a health-based guidance value for ATX for drinking water of 30 µg/L (WHO, 2020b), on the basis of the NOAEL from this study by Fawell at al. (Fawell et al., 1999b).

No chronic toxicity studies for anatoxin were found.

The NOAEL of Fawell et al. of 98 µg/kg body weight per day is the only health-based guidance value found that can be used for a risk assessment (Fawell et al., 1999b).

Saxitoxin-A (STX), (CAS number 35523-89-8)

Toxicity
Saxitoxins are natural alkaloids also known as paralytic shellfish poisons (PSP) because they were originally observed in molluscs, the consumption of which led to human poisoning (WHO, 2020a). STX can bond to sodium channels in neurons, which are consequently blocked. This blocks the transmission of a nerve impulse by the axon. In mild cases of PSP, the clinical symptoms are a tingling feeling or loss of feeling around the lips, which generally occurs within 30 minutes and which gradually spreads to the face and neck. These effects are probably due to the local absorption of STX via the mucous membranes of the mouth. These are generally followed by a tingling feeling in the fingertips and toes, headache, dizziness, nausea, vomiting and diarrhoea. Temporary blindness can sometimes occur. The majority of symptoms occur within several hours following intake but can then persist for days.

STX is efficiently absorbed from the gastrointestinal tract, with symptoms that persist between minutes and hours following oral exposure to STX (WHO, 2020a). Specific studies into the systemic distribution of STX are restricted to a small number of animal studies following intravenous or intraperitoneal administration. These studies reveal rapid distribution to a number of tissues, including the central nervous system. In patients recovering from PSP outbreaks in Alaska in 1994, clearance of the serum was clear within 24 hour; urine was identified as an important route of toxin secretion in humans (Gessner et al., 1997).

Health-based guidance value
Table 5 contains a list of the health-based guidance values for STX found in literature.

Table 5: Overview of available toxicological guidance values for STX

	Guidance value	Critical effect	Description of animal test
LD_{50}	356 µg/kg bw	Death/Mortality	Single dose via gavage in mice (Munday et al., 2013)
ARfD	0.5 µg/kg bw		Based on reported poisonings of more than 500 people (EFSA, 2009)
NOAEL	163 µg/kg bw	abdominal breathing, lethargy, reduced exploratory behaviour.	Single dose via gavage in mice (Munday et al., 2013)

In a study on mice, in which STX was administered in a single dose via a gavage, Munday et al. determined an LD_{50} value of 356 µg/kg body weight (Munday et al., 2013). In the same study, STX was also administered in a single sublethal dose, via a gavage. The grip strength of the mice was measured. In addition, abdominal breathing, lethargy and exploratory behaviour were studied. Based on these results, a NOAEL of 163 µg/kg body weight was determined (Munday et al., 2013).

Based on series of publications about PSP poisoning in humans after eating shellfish, it can be concluded that STX is acutely toxic. In 2009, EFSA published an opinion on STX in shellfish (EFSA, 2009). In the absence of chronic data, EFSA was unable to derive a TDI. Based on reported poisonings of more than 500 people, a LOAEL in humans of 1.5 µg/kg body weight was determined for STX. On this basis, EFSA derived an ARfD of 0.5 µg/kg body weight (EFSA, 2009).

In 2020, ANSES derived an acute TRV of 0.1 µg/kg body weight per day (ANSES, 2020), a factor of 5 lower than the ARfD derived by EFSA. ANSES based this TRV on the NOAEL of 163 µg/kg from the study by Munday et al. (Munday et al., 2013). Due to the absence of a clear dose-response relationship in this study, BMD modelling was not applied.

For its health-based guidance value, BuRO selected the ARfD value derived by EFSA because this value is based on human data and because in the study by Munday et al., there was no clear dose-response relationship. No subacute, subchronic or chronic toxicity studies were found. It is therefore unknown whether STX also has chronic effects.

β-N-methylamino-L-alanine (BMAA), (CAS number 15920-93-1)

Toxicity
BMAA is a neurotoxic amino acid (Chorus & Welker, 2021). BMAA was discovered in 1976 on the island of Guam, where it was quickly related to the locally occurring neurological disease ALS/PDC (Amyotrophic Lateral Sclerosis/Parkinson Dementia Complex) (Chorus & Welker, 2021). BMAA can occur in food and research has mainly been conducted into aquatic systems (water plants, fish, shellfish). BMAA is neurotoxic at cell level, but many scientists believe that the evidence for a possible relationship with the occurrence of ALS, Parkinson’s disease and dementia to be very weak (Chorus & Welker, 2021).

Health-based guidance values
In documents from WHO, EFSA and ECHA, BuRO was unable to find any health-based guidance value for BMAA (see Annex 1 for search strategy).

List of health-based guidance values cyanotoxins

Table 6 provides a list of the selected cyanotoxins and the accompanying health-based guidance values. Wherever present, a health-based guidance value is given for acute or subchronic exposure, and a health-based guidance value for chronic exposure.

Table 6: Health-based guidance values for cyanotoxins selected by BuRO

Cyanotoxin	Health-based guidance value	value	Duration of study	Reference
MC-LR	NOAEL	40 µg/kg bw per day	13 weeks	(Fawell et al., 1999a)
MC-LR	BMDL	0.02 µg/kg bw per day	6 months	(Chen et al., 2011; FO, 2020c)
CYN	ARfD	0.5 µg/kg bw		(FO, 2020)
CYN	BMDL	9.4 µg/kg bw per day	90 days	(Chernoff et al., 2018; FO, 2020c)
ATX	NOAEL	98 µg/kg bw per day	28 days	(Fawell et al., 1999b)
STX	ARfD	0.5 µg/kg bw		(EFSA, 2009)

The health-based guidance value used for acute exposure is an ARfD. However, this is not available for MC-LR and ATX. In that case, the subacute or subchronic NOAEL is used.

For subchronic exposure, preference is given to a study of up to 3 months. For MC-LR, the NOAEL from the study by Fawell et al. is used (Fawell et al., 1999a). For CYN, the BMDL originating from the study by Chernoff et al. is selected (Chernoff et al., 2018; FO, 2020c). For STX, only an ARfD is available. The ECHA guideline specifies no assessment factor for acute to subchronic (ECHA, 2012). For ATX, only a NOAEL is available from a subacute study (Fawell et al., 1999b). According to the ECHA guideline, for extrapolation from subacute to subchronic, an assessment factor of 3 must be applied (ECHA, 2012).

For chronic exposure, for MC-LR, the BMD is taken, derived from the study by Chen et al. (Chen et al., 2011; FO, 2020c). For CYN, the BMDL originating from the study by Chernoff et al is used (Chernoff et al., 2018; FO, 2020c). For ATX, for extrapolation from subacute to chronic, an assessment factor of 6 is applied (ECHA, 2012).

For the risk assessment of cyanotoxins based on a BMDL or NOAEL, the Margin of Exposure (MOE) approach is applied. MOE is the ratio between the reference point (BMDL, LOAEL or NOAEL) and exposure. Intraspecies and interspecies variation in toxicokinetics and toxicodynamics are present whenever data from animal studies are used for human risk assessment. To take account of these uncertainties in the risk assessment of non-genotoxic substances, an MOE of 100 is normally sufficient (EFSA, 2005). For genotoxic and carcinogenic substances, an additional safety factor is required because of interspecies variation in the control of the cell cycle and the DNA recovery, the processes that influence the carcinogenicity process. For a carcinogenic and genotoxic substance, an MOE of 10,000 or higher is considered to be of low concern from a public health point of view (EFSA, 2005). For MC-LR and ATX, an MOE of 100 is applied; for CYN an MOE of 10,000 because this substance is possibly genotoxic (FO, 2020d).

Legal aspects

For cyanotoxins in foodstuffs, such as food crops, milk and meat, no maximum concentrations are specified in legislation.

For water, a distinction can be made between drinking water and surface water. Microbiological and chemical requirements are included for drinking water in the Drinking Water Decree (Staatsblad (Official Journal) 2011, 313). However, this decree contains no specific requirements for cyanobacteria or cyanotoxins.

Surface water can be classified as bathing water, recreational water (including urban water, water playgrounds and recreation areas), water for agricultural use, shellfish production areas and other waters. In Dutch waters officially designated as swimming location, the water quality is regularly analysed during the bathing season, between 1 May and 1 October. The European Bathing Water Directive 2006/7/EC (European Parliament and the Council, 2006) for monitoring risks related to blue-green algae is included in the blue-green algae protocol (Schets et al., 2020). Water managers use this protocol to inspect swimming locations. The risk of the presence of pelagic blue-green algae is estimated according to the chlorophyl-a concentration. For MCs, at a concentration of between 10 and 20 µg/L, risk level 1 applies (negative bathing advice). Above 20 µg/L level, the water falls in risk category 2 (bathing ban). The province is responsible for imposing negative bathing advice or a bathing ban.

For water playgrounds, urban waters, water for agricultural use, shellfish production areas and other waters, there are no legal requirements or reference values in the Netherlands, for cyanobacteria and cyanotoxins.

The WHO has drawn up reference values. The reference values for drinking water and recreational water are published in the second addition of the WHO guide for cyanotoxins in water (Chorus & Welker, 2021), see Table 7. In addition, in April 2021, via the EFSA Focal Point network, BuRO consulted other European Member States and other European countries whether they operate specific legislation or reference values for cyanotoxins. Fifteen countries responded that they have no specific legislation for surface waters, other than specified in the Bathing Water Directive 2006/7/EC. France indicated that it is currently preparing national regulations for both drinking water and recreational water, including guidance values for MC-LR, CYN, ATX and STX, see Table 7. For ATX, France employs as its guidance value the detection limit (LOD). The level of this LOD is unknown, but it is probably far lower than the reference values for ATX released by WHO. For MC-LR and STX (drinking water), the French guidance values are also considerably lower than the WHO guideline (Chorus & Welker, 2021). For CYN, higher guidance values have been identified by France.

Table 7: WHO reference values for cyanotoxins in drinking water and bathing and recreational water (µg/L) (Chorus & Welker, 2021) and draft French guidance values

	MC-LR	CYN	STX	ATX
WHO drinking water	1	0.7	3	30
WHO recreational water	24	6	30	60
France: draft drinking water	0.2	1	0.8	< LOD[1]
France: draft recreation water	0.3	42	30	< LOD[1]

Supervision

In the Netherlands, supervision regarding the quality of surface water is a responsibility of the Ministry of Infrastructure and Water Management. Rijkswaterstaat, as implementing organisation, is responsible for the water quality of open swimming locations and the safety of this bathing water in respect of blue-green algae and cyanotoxins. The inspections in regional waters are carried out by the Water authorities. The WFSR overview report shows that structural monitoring for cyanotoxins in Dutch waters, other than bathing water, is not carried out, partly due to the absence of a legal framework (Faassen et al., 2021).

The management of national waters is the responsibility of Rijkswaterstaat. The management of regional waters is the responsibility of the Water authorities. They supervise the quality of the surface water and whether there is sufficient water. They conduct measurements at the water inlet points in their control area. Normally speaking, this does not include measurements of blue-green algae or cyanotoxins.

The Dutch government internet site includes an information sheet that contains an overview of questions and answers about the consequences of prolonged drought and the water shortages for agriculture. During periods of drought, the rules on the use of water for agriculture and horticulture can differ from region to region. This is because these matters are the responsibility of the Water authorities (surface water) and the provinces (groundwater). This information sheet was written for the media and government authorities concerned, such as Water authorities, provinces, municipalities and Rijkswaterstaat. This information sheet states that growers must prevent water contaminated with blue-green algae from being used for spray irrigating crops in order to avoid risks to public health. The following measures are listed:

- Use flowing surface water;
- Do not use water that appears green with algae;
- Test water for toxins (guidance value of 1 μg/L);
- Use deeper water layers.

No mention is made of the use of water for drenching livestock.

The NVWA does not carry out supervision on the agricultural use of surface water, such as (spray) irrigation of crops and the drenching of livestock. This is the responsibility of the farmers themselves. The safety of the eventual food is subject to supervision by the NVWA. Food safety is the responsibility of the Ministry of Health, Welfare and Sport (VSW); agriculture is the responsibility of the Ministry of Agriculture, Nature and Food Quality (LNV)

Exposure estimate

Cyanotoxins in surface water

In the period 2009-2020, WFSR analysed 826 water samples in various projects (Faassen et al., 2021). The water samples were taken from Dutch fresh surface water. The samples originated from larger monitoring projects and incidental sampling.

- In 84% of the 826 samples analysed for microcystins (MC) these toxins were found. This makes MC the most frequently identified toxin in the analysed water samples. The MC concentration in water samples varied. In 50% of the samples in which MC was identified, the concentration was lower than 1 μg/L; 4% had a concentration higher than 50 μg/L, but concentrations were found of up to 2900 μg/L.
- 242 of the analysed water samples (29%) were analysed for ATX; ATX was shown to be present in 65 samples (27%). 82% of these samples had a concentration lower than 0.1 μg/L; a concentration higher than 1 μg/L was observed in only one sample, namely 130 μg/L.
- CYN was found to be present in 7 of the 242 water samples analysed for CYN. The highest concentration found for this toxin group was 0.27 μg/L.
- STX was shown to be present in 3 of the 100 water samples, the highest concentration found was 15 μg/L.
- These water samples were not analysed for BMAA.

In water for agricultural use, measurements were conducted on the presence of MC (Faassen et al., 2021). In the period of extreme drought in August and September 2018, a total of 119 water samples were taken in a single control area, at 29 points where water could be let into the polder. MC was found in 92% of the samples. The median concentration was 0.39 µg/L and the maximum concentration measured was 7.8 µg/L. In addition, 3 further floating layer samples were taken at 3 different locations, and analysed. In these samples, a far higher concentration of MC was observed, namely up to 110 µg/L.

Following incidents, analyses were conducted (Faassen et al., 2021). In September 2012, samples were taken in the floodplains at Wageningen. The background to these measurements was the death of horses grazing in this area, which had drunk water in which a Microcystis bloom was present. The floating layer was sampled, revealing MC at a concentration of 13,000 µg/L.

In June 2019, four samples from a peat moorland polder were analysed for MC, nodularin, CYN, ATX and STX (Faassen et al., 2021). The background to this analysis was a blue-green algae bloom in the ditches, from which cows had drunk. MC was shown to be present in all four samples; the highest MC concentration found was 0.73 µg/L. ATX was also present in all four samples. The highest measured ATX concentration was 21 ng/L. Nodularin, CYN and STX were not found.

Irrigation of food crops

If surface water that contains cyanobacteria is used for (spray) irrigating food crops, the toxins can end up on and in the crops. To estimate the concentration of toxins in the crop, a number of factors are important: how much toxin is present in the water, how much toxin is absorbed by or is present on the crop and are these toxins degraded?

When food crops are spray irrigated, cyanotoxins can end up on the outer surface of the plant (leaves and stems). These toxins can also be absorbed into the plant via the roots, shoots and leaves. Accumulation depends on a number of factors, including the toxin itself, the dose of the toxin, the duration of the irrigation and the type of food crop. Toxins can be rinsed off the plant. In addition, toxins present in and on food crops can be degraded, for example under the influence of sunlight and temperature fluctuations. The eventual concentration of cyanotoxins on or in food crops is influenced by uptake, degradation and removal. For each cyanotoxin, a literature search was conducted into studies in accumulation in food crops (for search strategy, see Annex 1). BuRO calculated the uptake factor by dividing the concentration of cyanotoxin in the crop by the concentration of cyanotoxin in the water.

MC-LR

Table 8 contains a list of uptake found in literature for MC-LR in food crops. Bell peppers were measured on the basis of dry weight.

Table 8: Literature overview accumulation MC-LR in food crops.

Concentration in water (µg/L)	Crop	Uptake factor	Concentration in crop (µg/kg)	Reference
10	broccoli	0.003	0.026	(Järvenpää et al., 2007)
12.5	lettuce	14	178	(Hereman & Bittencourt-Oliveira, 2012)
5	lettuce	20	103	(do Carmo Bittencourt-

Concentration in water (µg/L)	Crop	Uptake factor	Concentration in crop (µg/kg)	Reference
				Oliveira et al., 2016)
10	lettuce and rocket	10	100	(Cordeiro-Araújo et al., 2016)
100	tomato	0.1	11	(Gutiérrez-Praena et al., 2014)
245	bell pepper	0.5	118	(Drobac et al., 2017)
10	lettuce carrots French beans	7 20 2.5	70 200 25	(Lee et al., 2017)

Järvenpää et al. conducted a study on the accumulation of MC in broccoli and mustard plants (Järvenpää et al., 2007). 47-day-old broccoli seedlings were watered over a 29-day period with 0, 1 and 10 µg/L MC. The MC-LR concentration in broccoli amounted to up to 2.6 ng/kg.

A Brazilian study on the accumulation of MC-LR in lettuce revealed that the MC-LR concentration in lettuce was up to a factor of 20 compared with the concentration in the water (Hereman & Bittencourt-Oliveira, 2012; do Carmo Bittencourt-Oliveira et al., 2016). These lettuce plants were irrigated over a 15-day period with water in which MC-LR was present. The uptake of MC-LR in lettuce was linear proportional to the exposure concentration of the toxin and increased over time (Cordeiro-Araújo et al., 2016). Estimates suggest that the lettuce becomes saturated after 30 days uninterrupted exposure (Cordeiro-Araújo et al., 2016). If clean water is subsequently used for irrigation, the concentration of MC-LR falls. The calculated half-life was 2.9 and 3.7 days respectively for lettuce spray irrigated with 5 and 10 µg/L MC-LR (Cordeiro-Araújo et al., 2016). These Brazilian studies are experiments into the uptake of MC-LR in lettuce and rocket. The normal growing time for lettuce up to harvest is between 50 and 70 days and for rocket between 40 and 60 days (Cordeiro-Araújo et al., 2016).

Spanish studies on tomatoes in which a high dose of MC-LR was present in the irrigation water (100 µg/L), showed that MC-LR was absorbed in the roots, flesh and leaves of the tomato plant (Gutiérrez-Praena et al., 2014). Gutiérrez-Praena et al. watered mature tomato plants in a greenhouse with water containing MC-LR over a 2-week period. MC-LR was transported throughout the plant. The uptake factor in the tomato flesh was 0.1.

In Finland, studies were conducted on the accumulation of MC-LR in bell peppers (Drobac et al., 2017). Water with a high concentration (245 µg/L) of MC-LR was used for irrigation for a three-month period. The leaves of the plant contained no MC-LR. The fruit did contain MC-LR, determined in the dried weight of the bell pepper. The uptake factor was 0.5 for the dry weight.

American studies on the accumulation of MC-LR in lettuce, carrots and French beans demonstrated dose-dependency (Lee et al., 2017). Six-week-old plants were exposed 3 times a week over a 4-week period to water containing MC-LR (1, 5 and 10 µg/L). The water was added both by drip and spray irrigation. The concentration of MC-LR varied between food crops, between the parts of the plant and between irrigation methods. The highest uptake was found for carrots, followed by lettuce and French beans.

Literature shows that the uptake of MC-LR varies according to the crop. For tomatoes and bell peppers, values of less than 1 were found. The highest uptake factor was found in lettuce and carrots, at 20.

CYN
Table 9 shows the literature overview for the uptake of CYN in food crops.

Table 9: Literature overview accumulation of CYN in food crops.

Concentration in water (µg/L)	Crop	Uptake factor	Concentration in crop (µg/kg)	Reference
18	cabbage	0.15	2.7	(Kittler et al., 2012)
10	lettuce rocket	0.38 0.95	3.8 9.5	(Cordeiro-Araújo et al., 2017)

German studies on CYN accumulation in cabbage varieties revealed that 15% was taken up by the plant (Kittler et al., 2012). A Brazilian study on CYN accumulation in lettuce and rocket showed that CYN was absorbed in these crops (Cordeiro-Araújo et al., 2017).

A limited number of studies have been published for CYN. The highest uptake factor found was 0.95 for rocket. In this advisory report, BuRO assumes 1 as the worst-case value for uptake of CYN.

BMAA
Table 10 contains an overview of accumulation of BMAA in food crops. BMAA is absorbed by Chinese cabbage via contaminated soil and not via irrigation water. The figures for wheat refer to seedlings. The bottom row (lettuce and spring onion) was measured on the basis of dry weight.

Table 10: Literature overview accumulation of BMAA in food crops.

Concentration of BMAA in water	Crop	Uptake factor	Concentration in crop (µg/kg)	Reference
4 µg/g soil	Chinese cabbage	3.5	13,820	(Li et al., 2019)
213 mg/l	Alfalfa	5 10^{-6} shoots 5 10^{-5} roots	0.1 10	(Samardzic et al., 2021)
1000 µg/L (4d) 100 µg/L (28 d)	Wheat Wheat	0.55 1	550 100	(Contardo-Jara et al. 2014)
10 µg/L (205 d)	Wheat	2.2	22	(Contardo-Jara et al. 2018)
50 µg/L (weekly)	Lettuce Lettuce Spring onion Spring onion	0.008 roots 0.0 shoots 0.06 roots 0.008 shoots	0.4 0 3.2 0.4	(Esterhuizen-Londt Pflugmacher, 2019)

A Chinese study on the accumulation of BMAA in Chinese cabbage revealed that BMAA was absorbed from the soil into root, stem and leaf of Chinese cabbage during the growth cycle (Li et al., 2019). A concentration of 13.8 mg/kg was observed in the edible leaves.

Samardzic et al. studied the accumulation of BMAA in alfalfa (Samardzic et al., 2021). Over a period of four days, alfalfa was irrigated with an 1800 µM solution of BMAA (212 mg/L). BMAA was absorbed in the roots of this plant.

In Germany, studies were conducted into the accumulation of BMAA in wheat (Contardo-Jara et al., 2014). Seven-day-old seedlings were irrigated over a four-day period with water with a BMAA concentration of 100 µg/L and 1000 µg/L. Only in the seedlings spray irrigated with 1000 µg/L BMAA was found, at a concentration of 50 µg/kg. The highest measured concentration in the shoots was 100 µg/kg. In another study wheat seedlings were raised to full growth and seed-bearing (205 days) (Contardo-Jara et al., 2018). Irrigation was carried out with 100 µg BMAA/L. In the roots and shoots, 25 and 22 µg/kg BMAA were found, respectively. In the wheat grains, up to 360 µg/kg BMAA was found.

Esterhuizen-Londt and Pflugmacher conducted a study on the accumulation of BMAA by lettuce and spring onion, both under laboratory conditions and through irrigation with water containing a BMAA-producing cyanobacterial bloom (Esterhuizen-Londt & Pflugmacher, 2019). Under laboratory conditions, BMAA was observed in the edible ripe part of the lettuce and spring onion. The crops were sprayed weekly with 10 mL water (50 µg BMAA/L). BMAA was found in the roots of both crops. BMAA was only detectable in the shoots of the spring onion, at a concentration of 0.5 µg/kg. When the crops were irrigated with water containing cyanobacterial bloom (40 µg/L BMAA), no detectable BMAA was found to be present in the edible part of the crops.

A limited amount of research was found into the accumulation of BMAA in food crops. For lettuce, spring onion and alfalfa, low values were found for uptake. The highest uptake factor found of BMAA in edible parts of the food crop was 3.5, in Chinese cabbage. However, this factor cannot applied to irrigation water, because BMAA was added via the soil. In wheat this factor is slightly higher, namely 2.2. This wheat was a fully mature food crop.

ATX and STX
Publications have only been found in literature about accumulation of these toxins in aquatic systems (water plants, fish, shellfish) and not in food crops. In the WHO guide, accumulation is only reported in fish and shellfish (Chorus & Welker, 2021). The EFSA opinion about STX relates to concentrations in shellfish (EFSA, 2009). No uptake factors are known for these cyanotoxins in food crops.

Consumption of food crops
A search was conducted for food crops grown on the land, in the Netherlands, during the summer period. Annex 3, Table 23, contains a list obtained from the internet site of Statistics Netherlands (CBS) for crops grown in 2021 on open land in the Netherlands. This relates partially to crops grown under glass and partially to crops grown in the field. The search related to the normal time for sowing or planting out seedlings, the period of harvesting and the growing time of the crop. This table shows that the following food crops could be relevant for (spray) irrigation with water contaminated with cyanotoxins in the period prior to harvest:

- Lettuce
- Tomato
- Carrot
- French bean
- Chinese cabbage

RIVM conducts food consumption surveys (VCP) amongst the Dutch population, on behalf of the Ministry of VWS. These food consumption surveys consist of two 24-hour food surveys on non-consecutive days amongst a representative sample of the Dutch population (N=4313; VCP 2012-2016) (Van Rossum et al., 2020). High consumption is shown by the P95 of the consumption distribution curve (95th percentile); in other words, amongst the study group, 5% of the consumers eat more and 95% eat the same amount or less. For the risk assessment, BuRO selected the P95 consumption for the age groups: infants (1 to 4 years) and adults (18 to 80 years). For an infant, a body weight of 12 kg was used and for an adult (18 to 80 years), the standard body weight of 60 kg was used. Table 11 contains an overview of the consumption of these food crops by Dutch consumers, both chronic and acute, on all days and on only consumption days.

Table 11: P95 consumption of various food crops by infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg) in the Netherlands according to VCP 2012-2016 (Van Rossum et al, 2020).

	Crop	P95 acute all days (g/day)	P95 acute consumption days (g/day)	P95 chronic all days (g/day)	P95 chronic consumption days (g/day)
Infants	Tomatoes	50	125	45	65
Infants	Chinese cabbage	0	69	0	35
Infants	Lettuce	76	198	78	108
Infants	French beans	8	111	30	56
Infants	Carrots	56	112	40	68
Adults	Tomatoes	124	221	103	140
Adults	Chinese cabbage	0	173	0	86
Adults	Lettuce	0	100	14	50
Adults	French beans	27	230	56	116
Adults	Carrots	76	198	78	108

For lettuce and Chinese cabbage, the number of observations was below 5, which means that no reliable calculation can be carried out using these data. For lettuce, the decision was made to take the data from FoodEx level 3: lettuce and lettuce plants. This probably results in an overestimation of the intake. For Chinese cabbage, no reliable calculation can be carried out; for that reason, this food crop is not further included in the calculations.

As cyanobacteria bloom over a restricted and short period, it is unrealistic to assume that consumers will consume food crops over a long period of time or on a chronic basis that have been (spray) irrigated with water contaminated with cyanotoxins. Consumers primarily purchase their vegetables in retail: supermarkets, greengrocers or at markets. These vegetables originate from multiple growers. It is also to likely that consumers vary the vegetables they eat, and do not consume the selected vegetables on a daily basis (see Table 11). It is therefore unlikely that all the vegetables a consumer purchases and consumes contain cyanotoxins. In other words, there will be a situation of incidental consumption of food crops contaminated with cyanotoxins. For that reason an acute scenario has been selected: P95 acute consumption on consumption days. This scenario is associated with an acute health-based guidance value.

Based on the consumption of these food crops (Table 11), the health-based guidance values (Table 6) and the body weight, a calculation has been made of the maximum permitted concentration in these food crops, at which the health-based guidance value is not exceeded. For BMAA, no health-based guidance value was found, so no maximum concentration in food crops can be calculated. Based on the following formula, this maximum concentration is calculated. Table 12 lists the results for the acute scenario. For MC-LR, this calculation was carried out on the basis of a NOAEL of 40 µg/kg body weight per day and an MOE of 100. For CYN and STX, the calculation is based on an ARfD of 0.5 µg/kg body weight. For ATX, the calculation was carried out on the basis of a NOAEL of 89 µg/kg body weight per day and an MOE of 100. The table shows that for MC-LR and CYN the calculated maximum concentrations in food crops are the lowest. These are the cyanotoxins with the lowest health-based guidance values.

$$G_{max} = \frac{HBGV*BW}{MOE*I}$$

Where:

G_{max} : Maximum concentration of toxin in food crop (µg/kg)
HBGV : Health-based guidance value (µg/kg body weight)
MOE : Margin of Exposure
BW : Body weight (kg)
I : Daily consumption (kg food/day)

Table 12: Maximum concentration of cyanotoxins in food crops (µg/kg) whereby the health-based guidance value is not exceeded at acute P95 consumption for infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg)

	Food crop	MC-LR (µg/kg)	CYN (µg/kg)	ATX (µg/kg)	STX (µg/kg)
Infants	Tomatoes	38	48	94	48
Infants	Lettuce	24	30	59	30
Infants	French beans	43	54	106	54
Infants	Carrots	43	54	105	54
Adults	Tomatoes	109	136	266	136
Adults	Lettuce	240	300	588	300
Adults	French beans	104	130	255	130
Adults	Carrots	121	152	297	152

Another scenario is that consumers purchase all their vegetables locally, for example from a local grower, or grow their own, which could result in a situation of subchronic exposure to cyanotoxins in vegetables. This scenario will be less common than the incidental, acute scenario reproduced in Table 12. For comparison, this scenario has been calculated. This is shown in Annex 2. The calculated maximum concentrations for CYN in the subchronic scenario are lower than for the acute scenario. This is caused by application of a different health-based guidance value (BMDL) in combination with a high MOE for CYN (10,000) because of possible genotoxicity. For the other cyanotoxins, the maximum concentration in the subchronic scenario is higher compared with the acute scenario. This is caused by the lower chronic consumption of these food crops.

Calculation of maximum concentration of cyanotoxins in water for irrigation
The following uptake factors for food crops have been selected from the literature studies accumulation of cyanotoxins in food crops (Table 13). No studies were found in literature for ATX and STX. For these cyanotoxins the highest uptake factor found for MC-LR is taken as worst case. The effect of preparation on the concentration of cyanotoxins in food crops, such as washing, cooking or stir-frying is unknown. Part of the cyanotoxins may be removed by these steps. Research in fish has shown that cooking can reduce the MC concentration by 25 to 50% (Gutiérrez-Praena et al., 2013). No research was found in literature for food crops. On the other hand, food crops can also be eaten raw. Worst case assumption is that these cyanotoxins remain present during the preparation of the food crops.

Table 13: Selected uptake factors for cyanotoxins in food crops

Cyanotoxin	Uptake factor	Reference
MC-LR	20 lettuce 0.1 tomato 20 carrot 2.5 French bean	(do Carmo Bittencourt-Oliveira et al., 2016; Lee et al., 2017) (Gutiérrez-Praena et al., 2014) (Lee et al., 2017) (Lee et al., 2017)
CYN	1	(Cordeiro-Araújo et al., 2017)
ATX	20	worst case assumption
STX	20	worst case assumption

For irrigation of crops, BuRO has calculated the maximum concentration of cyanotoxins that may be present in irrigation water at which the health-based guidance value is not exceeded, according to the following formula where:

$$C_{max} = \frac{G_{max}}{UF} = \frac{HBGV*BW}{MOE*UF*I}$$

Where:

C_{max} : Maximum concentration of toxin in irrigation water (µg/L)
G_{max} : Maximum concentration of toxin in food crop (µg/kg)
UF : Uptake factor
HBGV : Health-based guidance value (µg/kg body weight)
MOE : Margin of Exposure
BW : Body weight (kg)
I : Daily consumption (kg food/day)

With this formula the maximum concentration of cyanotoxins is calculated in irrigation water, at which the health-based guidance value is not exceeded (see Table 14). The lowest concentration of cyanotoxins is calculated for MC-LR and amounts to 1 µg/L; this is also the current recommended value (BuRO, 2018).

Table 14: Calculated maximum concentration of cyanotoxins in irrigation water for food crops for acute P95 consumption by infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg)

	MC-LR (µg/L)	CYN (µg/L)	ATX (µg/L)	STX (µg/L)
Tomatoes				
Infant	384	48	5	2
Adult	1085	136	13	7
Lettuce				
Infant	1	30	3	2
Adult	12	300	29	15
French beans				
Infant	17	54	5	3
Adult	42	130	13	7
Carrots				
Infant	2	54	5	3
Adult	6	152	15	8

The maximum concentration of cyanotoxins in irrigation water is also calculated for the subchronic scenario (see Annex 2). The lowest calculated value for MC-LR in the subchronic scenario is 3 µg/L. For CYN, the lowest value is considerably lower than in the acute scenario, namely 0.1 µg/L.

Drenching of livestock

Consumption of milk and beef
Farm animals can be exposed to cyanotoxins by drinking water and/or by eating grass spray irrigated by surface water contaminated with cyanotoxins. This can possibly be transferred into the milk and meat. Table 15 contains an overview of high consumption (P95) of milk and beef by Dutch consumers, both acute and chronic, according to the most recent Dutch food consumption survey (Van Rossum et al., 2020).

Table 15: P95 consumption of milk and beef by infants (1-4 years) and adults (18-80 years) in the Netherlands according to VCP 2012-2016 (Van Rossum et al, 2020).

		P95 acute all days (g/day)	P95 acute consumption days (g/day)	P95 chronic all days (g/day)	P95 chronic consumption days (g/day)
Infant	Beef	0	119	13	60
Infant	Milk	515	560	489	518
Adult	Beef	71	346	72	173
Adult	Milk	567	773	524	628

As cyanobacteria bloom over a restricted and short period, BuRO considers it unrealistic to assume that consumers will consume milk and beef over a long period of time or on a chronic basis that originates from animals that have drunk water in which cyanotoxins were present, or have consumed grass spray irrigated with this water. For that reason an acute scenario has been selected: P95 acute consumption on consumption days. This scenario comes with an acute health-based guidance value.

Based on the acute P95 consumption of milk and beef on consumption days (Table 15), the acute health-based guidance values (Table 6) and the body weight, a calculation has been made of the maximum permitted concentration in milk and beef, at which the health-based guidance value is not exceeded. The results are shown in Table 16. For MC-LR, this calculation was carried out on the basis of a NOAEL of 40 µg/kg body weight per day and an MOE of 100. For CYN and STX, the calculation is based on an ARfD of 0.5 µg/kg body weight. For ATX, the calculation was carried out on the basis of a NOAEL of 89 µg/kg body weight per day and an MOE of 100.

$$G_{max} = \frac{HBGV*BW}{MOE*I}$$

Where:

G_{max} : Maximum concentration of toxin in food (µg/kg)
HBGV : Health-based guidance value (µg/kg body weight)
MOE : Margin of Exposure
BW : Body weight (kg)
I : Daily consumption (kg food/day)

Table 16: Maximum concentration of cyanotoxins in milk and beef at which the health-based guidance value is not exceeded for acute P95 consumption for infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg).

	MC-LR (µg/kg)	CYN (µg/kg)	ATX (µg/kg)	STX (µg/kg)
Infant				
Beef	40	51	99	51
Milk	9	11	21	11
Adult				
Beef	14	17	34	17
Milk	6	8	15	8

A different scenario applies if consumers consume milk or meat from a single source. For example by purchasing meat from a single animal and consuming it over a specified period. This results in a subchronic scenario rather than an acute scenario. This scenario is further elaborated in Annex 2.
For CYN, the maximum concentration of cyanotoxins is considerably lower in the subchronic scenario compared with the acute scenario. This is caused by application of a high MOE for CYN (10,000) because of its possible genotoxicity. For the other cyanotoxins, in particular the maximum concentration in beef is higher in the subchronic scenario. This is caused by the large difference in consumption (acute versus chronic).

Transfer studies
In 2019, the FO searched literature for transfer studies of cyanotoxins to animal products (FO, 2020b). No transfer data for farm animals were found in literature for CYN. For MC-LR, limited data are available concerning the transfer of MC-LR to animal products. A single study studied the transfer to the liver and blood in cattle, and two studies studied transfer to milk in cattle. In addition to the FO results, BuRO searched the literature for transfer studies, also about the other selected cyanotoxins (for the search strategy see Annex 1). No additional studies were found on transfer of cyanotoxins in farm animals.

In none of these three transfer studies was any detectable transfer to milk or beef. In all three transfer studies, the analysis method for MC-LR in milk or meat was sufficiently sensitive. In other words the, detection or quantification limit was lower than the calculated maximum concentration in milk or meat, at which the health-based guidance value is not exceeded (Table 16). Table 17 contains an overview of the studies found. As no transfer to milk or meat was detected, this table shows the limit of quantification (LOQ) for milk and the limit of detection (LOD) for meat.

Table 17: Consumption of MC-LR and transfer in cattle to animal products (milk and liver) obtained from available transfer studies.

Animal	MC-LR intake (µg/kg bw/day)	MC-LR milk (µg/kg)	MC-LR liver (µg/kg)	Reference
Dairy cow	1.21	< 0.002		(Orr et al., 2001)
Dairy cow	13	< 0.2		(Feitz et al., 2002)
Beef bull	1.42		< 2	(Orr et al., 2003)

Orr et al. conducted a transfer study with four one-year-old beef bulls (Orr et al., 2003). Over a period of four weeks, live *Microcystis aeruginosa* cells (1×10^5 cells/mL) were added daily to their drinking water. Of the MCs present, 93% consisted of MC-LR, the remaining 7% consisted of an unknown other MC, which was not included by the authors. Based on the quantity of MC-LR in the *Microcystis aeruginosa* cells, the water intake and the average weight of the animal measured during the exposure period, it was possible to calculate that on average, the cattle ingested 1.42 µg MC-LR/kg body weight per day via their drinking water. No measurable quantity of MC-LR was found in the blood and the liver of the beef bulls. The detection limit in meat was 2 µg/kg.

In a transfer study into cow's milk, conducted by the same study group, over a three-week period, three dairy cows (Holstein-Friesian) were exposed to MC-LR via drinking wate, in the same way (Orr et al., 2001). Live *Microcystis aeruginosa* cells (1×10^5 cells/mL) were added to their drinking water. Based on the quantity of MC-LR in the *Microcystis aeruginosa* cells, the water intake and the average weight of the animal measured during the exposure periods, it was possible to calculate that on average, the cattle ingested 1.21 µg MC-LR/kg body weight per day via their drinking water. The concentration of MC-LR in the drinking water of these cows was 9.8 µg/L. No measurable quantity of unbound MC-LR was observed in the skimmed milk originating from the dairy cows prior to and during the exposure (limit of quantification was 2 ng/L (0.002 µg/L). In tests conducted in advance, Orr et al. determined that MC-LR is evenly distributed in both the aqueous and the fatty phase, so that the skimming of milk has no effect on the MC-LR concentration in the milk (Orr et al., 2001).

Feitz et al. conducted a study on the transfer of MC-LR to cow's milk. Four dairy cows (Holstein-Friesian) were administered a dried blue-green algae material containing MCs, including MC-LR, via gelatine capsules, over a period of four weeks (Feitz et al., 2002; BuRO, 2006). In this experiment, cows were exposed to increased dosages up to a maximum of 13 µg MC-LR/kg body weight per day. There were no physiological effects (effects on liver parameters measured in plasma) and no detectable transfer of MC-LR to the milk took place (measured concentrations <0.2 µg/L).

Calculation of maximum concentration of MC-LR in water for drenching livestock
For the transfer of MC-LR to milk, on the basis of the study by Feitz et al., (Feitz et al., 2002), a guidance value for exposure for cows of 13 µg/kg body weight per day can be maintained. At this level of exposure to MC-LR in dairy cows, no detectable transfer takes place to milk. To calculate the maximum concentration of MC-LR in water for drenching livestock, BuRO assumes the average high production Dutch dairy cow with a body weight of 600 kg (Van Raamsdonk et al., 2007). The daily intake by dairy cows amounts to 50 L of water and 25 kg of grass. For this risk assessment, it is assumed that the grass was spray irrigated with water contaminated with the same concentration of cyanotoxins. The BuRO advisory report from 2006 is based on an uptake factor of 5 for MC-LR on grass (BuRO, 2006). This factor 5 is an assumption based on the volume of water absorbed by plants. Table 8, Table 9 and Table 10 reveal that the uptake in food crops varies, but a factor of 5 is a reasonable assumption. The maximum volume of MC-LR in surface water is then calculated as follows:

Maximum exposure dairy cow = 13 * 600 = 7800 µg MC-LR/day

$$\text{Maximum concentration in water} = \frac{\text{maximum exposure MC-LR per day}}{\text{number L water} + (5 \text{ x number kg grass})}$$

$$\text{Maximum concentration in water} = \frac{7800}{50+(25*5)} = 45\ \mu\text{g MC-LR/L}$$

The study by Orr et al. reveals that no transfer to meat takes place when cattle are exposed to 1.42 µg/kg body weight per day (Orr et al., 2003). For beef cattle, an intake of 38 L of water per day is assumed, and a body weight of 500 kg, of which 330 kg is production meat (66%) (Van Raamsdonk et al., 2007). According to Van Raamsdonk et al., the feed for beef cattle consists mainly of corn, pellets and concentrate, and not fresh grass. This would mean that the beef cattle are kept in sheds, which makes it unlikely that they would drink surface water. Therefore, for the transfer to meat, beef cattle are assumed to be kept in pasture, with a daily intake of 38 L of surface water and 20 kg of grass. The maximum concentration of MC-LR in water for drenching livestock is calculated as follows:

Maximum exposure beef cattle = 1.42 * 500 = 710 µg MC-LR/day

$$\text{Maximum concentration MC-LR in water} = \frac{710}{(38+(5*20))} = 5.1\ (\mu g/L)$$

In the previous BuRO advisory report (BuRO, 2018), a guidance value for cyanotoxins of 40 µg/L was calculated, for drenching beef cattle. In this calculation, no account was taken of beef cattle eating grass.

Maximum concentration CYN, ATX and STX in water for drenching livestock
For CYN, ATX and STX, no transfer studies have been found in literature. This makes it impossible to carry out calculations for these cyanotoxins.

Risk characterisation

Table 18 contains an overview of all calculated maximum concentrations of cyanotoxins in food crops, milk and beef, at which the health-based guidance value is not exceeded. This is based on acute P95 consumption (Van Rossum et al., 2020) and the acute health-based guidance values for cyanotoxins. This is a summary of Table 12 and Table 16. Consultation of WFSR revealed that there are no analysis data for cyanotoxins in food crops, milk and meat. Concentrations in food crops found in literature (see Table 8 and Table 9) are often well above the maximum concentrations in food crops. However, these are concentrations found under experimental conditions, often with high concentrations of cyanotoxins in the water.

Table 18: Maximum concentration of cyanotoxins in food crops and in milk and beef at which the health-based guidance value is not exceeded based on acute P95 consumption by an infant (1-4 years, 12 kg) and an adult (60 kg, 18-80 years).

	MC-LR (µg/kg)	CYN (µg/kg)	ATX (µg/kg)	STX (µg/kg)
Infant				
Tomatoes	38	48	94	48
Lettuce	24	30	59	30
French beans	43	54	106	54
Carrots	43	54	105	54
Beef	40	51	99	51
Milk	9	11	21	11
Adult				
Tomatoes	109	136	266	136
Lettuce	240	300	588	300
French beans	104	130	255	130
Carrots	121	152	297	152
Beef	14	17	34	17
Milk	6	8	15	8

The maximum permitted concentration in water for irrigation of food crops (Table 14) and drenching livestock has been calculated. Table 19 contains an overview of the calculated maximum concentrations of cyanotoxins in surface water for both (spray) irrigation of food crops and for drenching livestock. The lowest calculated guidance value was taken.

Table 19: Calculated guidance values for cyanotoxins in water for (spray) irrigation of food crops and drenching of livestock

	MC-LR (µg/L)	CYN (µg/L)	ATX (µg/L)	STX (µg/L)
Food crops	1	30	3	2
Milk	45			
Beef	5			

The study by WFSR shows that MC are the most frequently observed cyanotoxins in all analysed water samples (Faassen et al., 2021). MCs were observed in 84% of the analysed water samples. In 50% of the samples in which MC was identified, the concentration was lower than 1 µg/L; 4% had a concentration higher than 50 µg/L, but concentrations were found of up to 2900 µg/L.

In the study by WFSR, no analysis data for agricultural surface water are available for the other cyanotoxins. Therefore, analysis data for other surface waters were considered. CYN was found in 3% of the analysed water samples; the highest observed concentration was 0.27 µg/L (Faassen et al., 2021). This is far lower than the calculated guidance values for (spray) irrigation.

ATX was observed in 27% of the water samples analysed for this substance, whereby the majority of samples revealed a concentration of lower than 0.1 µg/L (Faassen et al., 2021). There was one water sample with an ATX concentration higher than 1 µg/l, namely 130 µg/L. The calculated guidance values for (spray) irrigation are not exceeded, for this outlier.

STX was observed in 3% of the analysed samples, whereby the highest concentration was 15 µg/L (Faassen et al., 2021). This is higher than the calculated guidance value for (spray) irrigation) of food crops.

Annex 2 shows the calculation for the subchronic scenario. The subchronic scenario will occur far less often than the acute scenario. In the subchronic scenario, the lowest calculated concentrations for (spray) irrigation of food crops are slightly higher for MC-LR (3 µg/L), ATX (4 µg/L) and STX (4 µg/L) compared with the acute scenario. The calculated guidance value of 1 µg/L offers sufficient protection for these cyanotoxins. However, for CYN, the maximum guidance value in the subchronic scenario is 0.1 µg/L. Although CYN is not commonly found, in the WFSR measurements, higher concentrations than 0.1 µg/L were measured.

Uncertainties

In the calculation of guidance values for irrigation of food crops and the drenching of livestock, there is a major uncertainty. This uncertainty is caused by the absence of the following information:

- Health-based guidance values for BMAA.
- Acute health-based guidance values for MC-LR and ATX.
- Subchronic health-based guidance values for ATX and STX.
- Uptake of cyanotoxins in the selected food crops. These are only available to a limited extent for MC-LR and CYN. For ATX and STX, no studies were found on uptake on or in food crops.
- Transfer studies of MC-LR in other production animals than cattle.
- Transfer studies of CYN, ATX, STX and BMAA in farm animals.
- Intake scenarios for beef cattle of surface water and fresh grass are uncertain. Uptake of cyanotoxins in grass is unknown.
- There are no monitoring data for cyanotoxins in water for agricultural use.
- Information about the possible combination toxicology of cyanotoxins.

Conclusions

MC is the most commonly occurring cyanotoxin in Dutch surface water. MC-LR and CYN are the cyanotoxins with the lowest health-based guidance values. There are no analysis data for cyanotoxins in Dutch food crops, milk and beef. As a consequence, it is not possible to determine whether there is a possible health risk due to the consumption of food crops, meat and milk.

For the (spray) irrigation of food crops, 1 µg/L cyanotoxins is a safe guidance value. This guidance value is calculated on the basis of MC-LR. The available data reveals that 50% of the analysed water samples comply with this guidance value. With just one exception, no violations of the calculated maximum concentration in water for CYN and ATX were found. STX was observed in few water samples, but could exceed the maximum calculated value.

Literature has revealed that there is no detectable transfer of MC-LR to milk and beef. This equates to a guidance value in water for drenching of dairy cattle of 45 µg/L and for beef cattle of 5 µg/L. More than 50% of the analysed water samples comply with this level. No data are known in literature for the transfer of CYN, ATX, STX and BMAA from farm animals to meat and milk. As a consequence, no calculation can be carried out.

For the subchronic scenario, consumers who consume their food crops from a single source (grower), the guidance value of 1 µg/L offers sufficient protection for MC-LR, ATX and STX. For CYN, the guidance value of 0.1 µg/L must be maintained. CYN occurs seldom in Dutch surface water, but higher concentrations than 0.1 µg/L have been found. For the drenching of livestock, there is no difference in calculated guidance value between the acute and the subchronic scenario, because these values are based on transfer studies.

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Overview of Annexes

Annex 1: Literature search strategy

Annex 2: Subchronic scenario

Annex 3: Planting and harvesting periods for food crops

Annex 1: Literature search strategy

For health-based guidance values of cyanotoxins, in addition to the ANSES documents, a search was conducted in documents from ECHA, EFSA, WHO, EPA and Health Canada. The substance name and CAS number was searched for on the internet site of ECHA and EFSA.

For MC-LR and ATX, a search was conducted in PubMed for an acute reference value with the search term ‘microsystin’ and ‘anatoxin’ and ‘acute reference dose’, respectively. For BMAA, a specific search was conducted in PubMed with the search term ‘BMAA’, β-N-methylamino-L-alanine’ or the CAS no. in combination with ‘ARfD’, ‘TDI’ and ‘ADI. This only resulted in publications about BMAA in food and about the analytical technique. However, no NOAEL, ADI or TDI were reported.

For the accumulation of cyanotoxins in food crops, a search was conducted in PubMed with the search terms ‘bioaccumulation’ in combination with ‘microsystin’, ‘cylindrospermopsin’, ‘anatoxin’, ‘saxitoxin’, ‘BMAA’ and β-N-methylamino-L-alanine’. This produced 13 results for food crops for MC-LR, cylindrospermopsin and BMAA For anatoxin and saxitoxin, publications were only found about accumulation in aquatic systems (water plants, fish, shellfish).

For the transfer of cyanotoxins to animal products, a search was conducted in PubMed with the search terms ‘cyanotoxin’, ‘microsystin’, ‘anatoxin’, ‘cylindrospermopsine’, ‘β-N-methylamino-L-alanine’, ‘BMAA’, ‘milk’, ‘beef’ and combinations. No new studies were found compared with the BuRO advisory report from 2018.

Annex 2: Subchronic scenario

Food crops

One scenario is that consumers purchase all their vegetables locally or grow their own, which could result in a situation of subchronic exposure to cyanotoxins in vegetables. This scenario will occur far less often than the scenario of incidental acute exposure as calculated in Table 12. In the subchronic scenario, chronic P95 consumption on all days is selected. In this scenario, comparison is made with a chronic (or subchronic) health-based guidance value (see Table 6). If not available, the acute health-based guidance value will used. Table 20 shows the calculated maximum concentration of cyanotoxins in food crops in the case of subchronic exposure, at which the health-based guidance value is not exceeded. For MC-LR, the calculation was carried out on the basis of a NOAEL of 40 µg/kg body weight per day and an MOE of 100. For CYN, the calculation is based on a BMDL of 9.4 µg/kg body weight per day and an MOE of 10,000. For ATX, the calculation was carried out on the basis of a NOAEL of 98 µg/kg body weight per day, an MOE of 100 and an assessment factor of 3. For STX, the calculation is based on an ARfD of 0.5 µg/kg body weight.

Table 20: Maximum concentration of cyanotoxins in food crops (µg/kg) whereby the health-based guidance value is not exceeded at subchronic P95 consumption for infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg)

	Food crop	MC-LR (µg/kg)	CYN (µg/kg)	ATX (µg/kg)	STX (µg/kg)
Infants	Tomatoes	106	0.2	86	132
Infants	Lettuce	61	0.1	50	77
Infants	French beans	162	0.4	133	203
Infants	Carrots	119	0.3	97	149
Adults	Tomatoes	234	0.5	191	292
Adults	Lettuce	1702	4.0	1390	2127
Adults	French beans	431	1.0	352	539
Adults	Carrots	307	0.7	251	383

These calculated maximum concentrations for CYN are lower than the calculated maximum concentrations for acute exposure. This is caused by the lower subchronic health-based guidance value in combination with an MOE of 10,000 for CYN compared with the acute health-based guidance value. For the other cyanotoxins, the maximum concentration in the subchronic scenario is higher compared with the acute scenario. This is caused by the lower chronic consumption of these food crops.

Subsequently, the maximum permitted concentration of cyanotoxins in the water for (spray) irrigation was calculated (Table 21).

Table 21: Calculated maximum concentration of cyanotoxins in (spray) irrigation water for food crops in the event of subchronic P95 consumption by infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg)

	MC-LR (µg/L)	CYN (µg/L)	ATX (µg/L)	STX (µg/L)
Tomatoes				
Infant	1056	0.2	4	7
Adult	2338	0.5	10	15
Lettuce				
Infant	3	0.1	3	4
Adult	85	4	69	106
French beans				
Infant	65	0.4	7	10

	MC-LR (µg/L)	CYN (µg/L)	ATX (µg/L)	STX (µg/L)
Adult	172	1	18	27
Carrots				
Infant	6	0.3	5	7
Adult	15	0.7	13	19

For MC-LR, ATX and STX, the calculated maximum concentrations in the subchronic scenario are higher than in the acute scenario. The calculated guidance value of 1 µg/L for the acute scenario offers sufficient protection. For CYN, the lowest calculated value in the subchronic scenario however is 0.1 µg/L. For CYN, 1 µg/L offers insufficient protection in the subchronic scenario. CYN was only observed in 3% of the water samples analysed by WFSR, but sometimes in higher concentrations.

Beef and milk

Consumers can purchase their beef and milk locally, for example meat from a single bovine animal. In that case, this too is a subchronic scenario. In the subchronic scenario, chronic P95 consumption on all days is selected. In this scenario, comparison is made with a chronic (or subchronic) health-based guidance value (see Table 6). If not available, the acute health-based guidance value will be taken. Table 22 shows the calculated maximum concentration of cyanotoxins in the case of subchronic exposure, at which the health-based guidance value is not exceeded. For MC-LR, the calculation was carried out on the basis of a NOAEL of 40 µg/kg body weight per day and an MOE of 100. For CYN, the calculation is based on a BMDL of 9.4 µg/kg body weight per day and an MOE of 10,000. For ATX, the calculation was carried out on the basis of a NOAEL of 98 µg/kg body weight per day, an MOE of 100 and an assessment factor of 3. For STX, the calculation is based on an ARfD of 0.5 µg/kg body weight.

Table 22: Maximum concentration of cyanotoxins in food crops (µg/kg) at which the health-based guidance value is not exceeded in case of subchronic P95 consumption for infants (1-4 years, 12 kg) and adults (18-80 years, 60 kg)

		MC-LR (µg/kg)	CYN (µg/kg)	ATX (µg/kg)	STX (µg/kg)
Infants	Beef	368	0.9	301	460
Infants	Milk	10	0.02	8	12
Adults	Beef	333	0.8	272	416
Adults	Milk	46	0.1	37	57

For CYN the maximum concentration of cyanotoxins is considerably lower than in the acute scenario (see Table 16). This is caused by the lower subchronic health-based guidance value in combination with an MOE of 10,000 for CYN compared with the acute health-based guidance value. For the other cyanotoxins, in particular the maximum concentration in beef is higher in the subchronic scenario compared with the acute scenario. This is caused by the major discrepancy in consumption (acute versus chronic).

The guidance value for MC-LR in water for drenching livestock is calculated on the basis of transfer studies. The exposure of bovine animals to MC-LR at which no detectable transfer takes place to milk and meat is taken as the safe guidance value. This is independent of the acute or subchronic scenario.

Annex 3: Planting and harvesting periods for food crops

Table 23 shows a list of the food crops that according to CBS were grown in 2021 in the Netherlands on open land or under glass. Use is also made of the sowing calendar to obtain an impression of the periods normal for the sowing of seeds or the planting of seedlings and harvesting of the food crops and the normal growing time for the food crop.

Table 23: Food crops grown in the Netherlands in 2021 on open land

Food crop	**Harvest (million kg)**	**Sowing period outdoor/planting on**	**Harvest month**	**Growing period (days)**	**Comment**
Mushrooms	260				Mushrooms are not grown on open land or under glass but in dark spaces (so-called cells).
Endive	9.3	April-August	July-November	90	In the Netherlands, endive is grown both under glass and on open land.
Asparagus	17.5	February-June	February-June	365	In the Netherlands, asparagus is mainly grown on open land but also to a limited extent under glass (approx. 3 percent)
Fennel	3.5				
Leek	105	March	July-October	170	Leek is mainly harvested in the winter.
Celery, leaf/green	10.8	April–May	August-November	160	Leaf celery (green celery) and celery
Lettuce, head and other	18.5	April-August	May-October	30	The ordinary head lettuce (= butter lettuce) and other lettuce varieties such as oakleaf lettuce, curly leafed lettuce and rocket. These lettuce varieties are grown in the Netherlands both under glass and on open land.
Lettuce, iceberg	90.6	March-July	June-September	65	In the Netherlands, iceberg lettuce is only grown on open land.
Spinach	74.7	January-August	April-November	35	In the Netherlands, spinach is mainly grown on open land, but also to a limited extent under glass (around 3 percent).
Chicory	58.9	May-June	September-December	280	
Carrot and washed carrot	157.9	April-June	June-September	240	Carrots are sold in bound bunches with leaf still attached. Washed carrots are sold washed, with the leaf removed.
Celeriac	82.2	April–May	September-December	260	
Beetroot	44.7	February-March	April-June	85	
Radish	25	April-September	April-October	40	In the Netherlands, radish is mainly grown under glass.
Salsify	16.2	April–May	October-December	200	Salsify are primarily harvested in the winter. The harvest in the first months of the new year is included in the harvest for the year prior to the new year.

Food crop	Harvest (million kg)	Sowing period outdoor/planting on	Harvest month	Growing period (days)	Comment
Onions	1916.4	April	August	220	
Winter carrot	485.3	May-July	August-December	240	
Cauliflower	51.2	March-May	August-September	175	Period applies to summer crop.
Kale	7.3	July	August-December	160	Kale is primarily harvested in the winter. The harvest in the first months of the new year is included in the harvest for the year prior to the new year.
Broccoli	24.8	June	August	100	Period applies to summer crop.
Chinese cabbage	6.9	April-August	June-October	90	
Green cabbage	0.9	May	July-August	160	Period applies to summer crop. Green cabbage is also known as savoy cabbage.
Red cabbage	40.5	May	July-August	160	Period applies to summer crop.
Pointed cabbage.	14.7	May	July-August	120	Period applies to summer crop.
Brussels sprouts	62.8	May-June	September-December	250	Brussels sprouts are mainly harvested in the winter. The harvest in the first months of the new year is included in the harvest for the year prior to the new year.
White cabbage	96.5				
Peas	28.6	April–May	June-July	70	
French beans	59.8	May-July	August-September	70	
Broad beans	5	March–April	July-September	70	
Aubergines	63				Aubergines are grown in the Netherlands under glass.

Food crop	Harvest (million kg)	Sowing period outdoor/planting on	Harvest month	Growing period (days)	Comment
Courgette	17.1	May-July	September-November	195	In the Netherlands, courgettes are grown both under glass and in open land.
Cucumbers	440				Cucumbers are grown in the Netherlands under glass.
Bell peppers	440				Bell peppers are grown in the Netherlands under glass.
Tomatoes	880				Tomatoes are grown in the Netherlands under glass.
Other vegetables	79.6				Other including kohlrabi, mange-tout, pumpkin, rhubarb, sweetcorn and garden herbs.