Cylindrospermopsin
[CASRN 143545-90-8]

Review of Toxicological Literature

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EXECUTIVE SUMMARY

The nomination of the cyanobacterial toxin cylindrospermopsin is based on its presence in surface waters that are used as drinking water supplies for humans and domestic animals and its severe acute hepatotoxicity at low concentrations and possible liver damage or other toxicity resulting from chronic exposure.

Properties

Cylindrospermopsin is a tricyclic alkaloid consisting of a tricyclic guanidine moiety combined with hydroxymethyluracil. It is a naturally produced toxin of certain cyanobacterial strains. Certain strains of *Cylindrospermopsis raciborskii* (in Australia, Hungary, and the United States), *Umezakia natans* (in Japan), and *Aphanizomenon ovalisporum* (in Australia and Israel) have been found to produce cylindrospermopsin. The production of cylindrospermopsin is strain-specific and not species specific.

Cylindrospermopsin is a glassy solid that is zwitterionic and highly water-soluble. Chemical property information for cylindrospermopsin is scarce, but the stability of cylindrospermopsin to extreme conditions has been studied. Cylindrospermopsin is relatively stable at extreme temperatures (no degradation at 100 ¡C for 15 minutes) and pH (~25% degraded at pH of 4, 7, and 10 for 8 weeks). Cylindrospermopsin is not commercially produced and there is no known use for the compound.

Analysis

The analytical determination of cylindrospermopsin involves the purification of cylindrospermopsin from purified and sonicated extracts of cyanobacterial isolates with HPLC or over silica gel and characterization with mass spectrometry (MS) or nuclear magnetic resonance (NMR). Methods for the sensitive and rapid analysis of water samples are currently being investigated using enzyme-linked immunosorbent assays (ELISAs), polymerase chain reaction (PCR), and characterization of DNA and RNA unique to cylindrospermopsin-producing cyanobacteria.

Environmental Occurrence

As more bodies of water are analyzed for the presence of the cyanobacteria that produce cylindrospermopsin, these bacteria are being found on a global scale. Since the outbreak of hepatoenteritis on an Australian island in 1979 that was attributed to C. *raciborskii*, this cyanobacterium has been the most extensively studied. It was once believed that *C. raciborskii* was only found in tropical and subtropical regions, such as regions of Australia, but now it is apparent that cyanobacteria that produce cylindrospermopsin can thrive in temperate regions of the United States, Israel, Japan, and even Hungary. *C. raciborskii* is reported to have an optimal growth temperature of 25 ¡C or greater. Although most studies indicate that the appearance of *C. raciborskii* is seasonal and occurs during the warmer summer months, a study of five lakes in

Florida revealed that *C. raciborskii* dominated in one lake all year round, and that *C. raciborskii* may have entered the Florida Lakes around 30 years ago.

Cyanobacteria are found in freshwater ponds, rivers, reservoirs, and eutrophic lakes. Usually the moderate concentrations of cyanobacteria co-exist with other organisms in a body of water; but when conditions are favorable, the cyanobacteria proliferate and form blooms. Cyanobacteria can be composed of many filamentous cells arranged into chains or strands called trichomes. When the body of water is visibly colored by the cyanobacteria, then it is considered a bloom and the concentration of cyanobacterial cells can number more than 10,000 cells/mL. Some cyanobacterial species (*A. ovalisporum* and *C. raciborskii*) contain gas vacuoles that aid in orienting the bacteria in the water column by regulating buoyancy. Some blooms may result in floating cyanobacterial masses on the surface of the water depending on the buoyancy of the cells. These floating cells are called scum and usually contain about 1,000,000 cells/mL.

Cylindrospermopsin is believed to be derived from a polyketide that uses an amino acid starter unit such as glycocyamine or 4-guanidino-3-oxybutyric acid. Generally toxins are retained in the cyanobacterial cells when conditions are favorable. In studies of *A. ovalisporum*, the concentration of cylindrospermopsin within the cell increased during the growth phase and decreased during the stationary (S) phase. The cytoplasmic decrease during S phase was attributed to the release of cylindrospermopsin into the culture medium so that the amount of toxin in the culture medium was positively correlated with the age of the culture. This same correlation has also been observed in *C. raciborskii*. The increased amount of toxin in the medium is most likely a result of cell lysis. A single *C. raciborskii* cell has been found to contain between 0.0041 and 0.026 pg of cylindrospermopsin in several studies which is equal to 1.5 to 5.5 mg cylindrospermopsin/g dried cells. In one study, four batches from the same strain (*C. raciborskii* AWT 205) grown at different times resulted in varying concentrations of cylindrospermopsin (1.3-5.4 mg/g extract). One gram of *A. ovalisporum* biomass contained approximately 2 mg of cylindrospermopsin. During blooms, it is not uncommon for 70-98% of the total cylindrospermopsin produced by cells to be dissolved in the water column. Various environmental conditions such as nutrient availability, temperature, and light conditions affect the formation of cyanobacterial blooms and the resulting production of cylindrospermopsin.

As mentioned above, cylindrospermopsin is very stable when exposed to extreme conditions and environmental factors. Unlike microcystin-LR, another hepatotoxin produced by cyanobacteria, which had a short half-life (10 minutes) under shortwave (400 mW/m^2) UV light, cylindrospermopsin s half-life was much longer (18 hours). The degradation of cylindrospermopsin was more rapid when exposed to sunlight (29% remained after exposure for 3 hours) when compared to exposure to UV light alone. A higher concentration of cylindrospermopsin resulted in a more rapid decrease. Degradation of cylindrospermopsin was more rapid when plant pigments were also present, probably proceeding by free radical mechanisms.

Treating lakes with algicides may kill the cyanobacteria, but it also will result in an increased concentration of cylindrospermopsin in the water due to cell lysis. In two cases of toxin poisoning in Australia, the toxic symptoms occurred after the application of copper sulfate to dense blooms. It is suggested that another source for drinking water be made available after treatment of a municipal water supply. The most effective method of algae control in reservoirs is though the use of aeration and destratification.

Current drinking water treatment methods are inadequate for the removal of highly water soluble cyanobacterial toxins, and in fact, may promote higher concentrations of the toxin by lysing cyanobacterial cells. New methods of water treatment are currently being developed to eliminate the risk of toxin exposure by the removal of cyanobacterial cells and/or by the adsorption and destruction of toxins.

Human Exposure

Human exposure to cylindrospermopsin may occur by ingesting toxin contaminated water during recreational activities or by the ingestion of food or water contaminated with the toxin. Dermal contact with cylindrospermopsin may occur during showering or bathing, or during recreational activities such as wading, swimming, boating, or water skiing. No quantitative estimates of human exposure based on occurrence of cylindrospermopsin in surface waters or finished drinking water were located.

The occurrence of cyanobacterial blooms has not been a major concern in the United States, but with emerging information about the occurrence of blooms in water supplies in the United Sates this may be changing. In the United Sates, many surface reservoirs are at the age when they start to turn eutrophic (40-50 years) as a result of excess nutrient availability. Almost 80% of 50 surface drinking water reservoirs tested in the United States contained detectable amounts of toxins (unidentified in article); however, the toxins were at low concentrations (not provided) and most treatment processes were capable of removing the toxins. The determination of cyanobacteria and cyanobacterial toxins in Florida water supplies indicates that there is potential for human exposure in temperate regions of the United States. Significant concentrations of toxigenic cyanobacterial species (*Microcystis*, *Cylindrospermopsis*, and *Anabaena* spp.) were found in 88 of 167 samples representing 75 individual water bodies. Cylindrospermopsin was detected in 34 water bodies throughout the state of Florida. Many of these samples were lethal to mice in bioassays. In a study of five lakes in Florida, the concentration of *C. raciborskii* ranged from 88,000 to 176,000 units/mL when counts of all cyanobacterial species ranged from 95,000 to 209,500. *C. raciborskii* was found in past samples from several Minnesota lakes (1965) and one lake in Kansas (1955) and identified as *Anabaenopsis raciborskii*. This indicates that the entire United States may be suitable for the colonization of *C. raciborskii* in bodies of water when conditions are favorable for growth (temperate climate and excess nutrients).

Demands on groundwater supplies in Florida are currently exceeding or threatening to exceed sustainable yields, forcing water managers to rely more often on surface drinking-water supplies. Existing surface-water treatment plants in Florida are currently not required to monitor for cyanobacterial toxins and may not be adequately equipped to treat surface waters for taste, odor, and toxins associated with immense cyanotoxic blooms. The monitoring of reservoirs and drinking-water supplies for cyanobacteria can lead to the early initiation of treatment measures to prevent blooms from occurring.

A tolerable daily intake (TDI) of cylindrospermopsin along with guideline values (GV) for human exposure were calculated by the authors in a recent review based on acute toxicity studies in mice. The TDI for cylindrospermopsin was 0.02 g/kg body weight/day. It was estimated that GVs for adults, children, and infants were 0.48, 0.16, and 0.11 g/L, respectively, based on drinking water consumption of 2 L for a 60-kg adult, 1 L for a 10-kg child, and 0.75 L for a 5-kg

infant. The guideline values for the number of cyanobacterial cells (based on 0.026 pg cylindrospermopsin/cell) were 4231 in infants, 6154 in children, and 18,461 in adults.

Exposure to cylindrospermopsin may occur by the consumption of fish that have been exposed to cyanobacteria that produce the toxin. Eating fish from reservoirs containing blooms could result in exposure to the cyanobacteria and/or cylindrospermopsin. A study reporting the bioaccumulation of cylindrospermopsin in muscle tissue of the redclaw crayfish (*Cherax quadricarinatus*) and visceral tissues of rainbow fish (*Melanotaenia eachamensis*) show that exposure could occur from farm-raised freshwater aquatic foods. The study also showed that bioaccumulation could be greater in fish due to the longer exposure to cyanobacterial blooms in natural bodies of water, sometimes as long as 4 months, when compared to shorter acute exposure in the laboratory. Fish are generally more tolerant of algal toxins than mammals and tend to accumulate them over time.

Acute Toxicity

Human Data

In 1979, a major cyanobacterial bloom occurred in a reservoir on Palm Island off the coast of Australia. This reservoir was the source of chlorinated, unfiltered drinking water for it s indigenous residents. One week after the treatment of the reservoir with an algicide, 139 and 10 adults complained of hepatitis-like symptoms. Kidney malfunction along with bloody diarrhea and urine were reported. Kidney damage was severe in those with bloody urine (20% of patient), and when coupled with severe damage to the gastrointestinal lining caused fluid and electrolyte loss so severe that many experienced hypovolemic/acidotic shock and had to be given intravenous fluids. *C. raciborskii* extracts isolated from the reservoir caused the same pathology observed in humans when injected intraperitoneally (i.p.) to mice. It is believed that *C. raciborskii* may be the cause of a debilitating and sometimes fatal disease in northern and central outback Australia known as Barcoo spews.

Animal Data

When ingested or injected i.p into mice, cylindrospermopsin caused gastroenteritis though injury to the gut, hepatitis from injury to liver cells, kidney malfunction from injury to renal cells, and hemorrhage from damage to blood vessels.

Hepatotoxicity was shown after administration of purified cylindrospermopsin (0.2 mg /kg body weight) in 24 male ICR mice and was described as a four-step process:

- ribosome detachment from the surface of the rough endoplasmic reticulum and accumulation in the cytoplasm of hepatocytes
- a phase that occurs about 24 hours after cylindrospermopsin administration and is characterized by a considerable decrease in the amount of P450 enzymes and membrane proliferation
- accumulation of fat droplets in the central portion of the hepatic lobules, probably induced by free radicals
- terminal phase of severe hepatic necrosis

Low doses of cylindrospermopsin to mice typically result in injury or death of hepatocytes near central veins (centrilobular), pale livers, and fatty degeneration of hepatocytes, all occurring generally more than 24 hours after the initial dose. High doses lead to extensive hepatocyte necrosis (panlobular) and a reddened, swollen liver usually within six to nine hours after the initial dose. Extensive liver damage was observed in every toxicity study reviewed. Some mechanisms for the liver toxicity of cylindrospermopsin have been proposed. The inhibition of protein synthesis in the liver by cylindrospermopsin may account for triglyceride accumulation in the liver as a result of decreased lipoprotein synthesis and excretion. The toxicity of cylindrospermopsin may depend on metabolic transformation in the liver by cytochrome P450. Glutathione, which may be required to inactivate cylindrospermopsin, or possibly a metabolite, is inhibited in the hepatocytes themselves.

The main target of cylindrospermopsin toxicity is the liver, but other organs such as the thymus, kidneys, adrenal glands, lungs, intestinal tract, and heart may also be affected. Mice dosed by the oral route in many studies contained cyanobacteria in the digestive tract and experienced gastrointestinal hemorrhaging along with anorexia.

In mice dosed orally with cylindrospermopsin in culture suspension, the lowest lethal dose was 4.4 mg/kg body weight, but one animal survived a dose of 6.9 mg/kg body weight. Oral administration of 8 mg/kg body weight to mice resulted in 100% mortality while greater than 0.05 mg cylindrospermopsin/kg given i.p. killed all mice tested.

Injection of either pure cylindrospermopsin or an extract of cultured *C. raciborskii* in fish (*Rutilus rutilus* L.) at lethal and sublethal doses caused liver damage and inhibited respiration in fish.

A discrepancy between the toxicity of the cellular lysates to the kidney and liver of mice and the cylindrospermopsin content of the lysates was observed in two studies. The variability of the *in vivo* toxicity and the histological damage observed does not correlate with the known cylindrospermopsin content of the lysates. It was concluded from this evidence that possibly more than one toxic compound may be present in *C. raciborskii*.

Chemical Disposition, Metabolism and Toxicokinetics

Absorption and Distribution

No mammalian studies on the absorption and distribution of cylindrospermopsin were found. However, acute and subchronic toxicity studies in mice showed that absorption of cylindrospermopsin from the gastrointestinal tract does occur.

The absorption and distribution of cylindrospermopsin was assessed in the redclaw crayfish (*Cherax quadricarinatus*) and rainbow fish (*Melanotaenia eachamensis*) during a *C. raciborskii* bloom in an aquaculture pond. The highest concentration of cylindrospermopsin was found in the hepatopancreas tissue (4.3 g/g freeze-dried hepatopancreas) of crayfish collected from the pond while the concentration of cylindrospermopsin in muscle tissue was less (0.9 g/g freeze-dried muscle). No cylindrospermopsin was detected in the tissues of the rainbow fish but were evident in the viscera at concentrations similar to cylindrospermopsin in crayfish muscle. Trichomes *of C. raciborskii* were found in the gut contents of the crayfish, but not in the gut contents of the rainbow fish.

A group of crayfish were experimentally exposed to a pure culture isolated from the aquaculture pond described above for 14 days. The concentration of cylindrospermopsin in the hepatopancreas (0.54 g/g freeze-dried hepatopancreas) and muscle (0.12 g/g freeze-dried muscle) tissue of the laboratory-exposed crayfish was less than that observed in the pond-exposed crayfish. However, the same 5:1 ratio of cylindrospermopsin found in the hepatopancreas and muscle tissue of aquaculture pond-exposed crayfish was observed in the laboratory-exposed crayfish. As the concentration of cylindrospermopsin in the water increased so did the concentration in the hepatopancreas and muscle, but the ratio remained 5:1. No histopathologic abnormalities were observed in either the group of crayfish exposed in the pond or the group of crayfish exposed in the laboratory. It was proposed that the difference in the bioaccumulation of cylindrospermopsin between the laboratory- and pond-exposed groups of crayfish may be attributed to the longer length of exposure of the pond-exposed fish (months) when compared to the experimental group (14 days).

Metabolism

Two deoxy analogs of cylindrospermopsin were isolated from *C. raciborski* and represented 0.02% of the mass of the lyophilized cells. P450 inhibition provides partial protection from toxicity and that glutathione is depleted in hepatocytes of exposed animals points to the contribution of metabolites to the toxicity of cylindrospermopsin. Cylindrospermopsin was made toxic only after metabolic activation in cultured rat hepatocytes.

Subchronic Toxicity

No pathological changes were observed in the lungs, liver, kidney, or heart of mice administered cylindrospermopsin (0.02 mg/kg body weight/day) i.p. for 12 days. Deaths were observed at doses greater than 0.05 mg/kg/day. In another study mice were dosed either daily by gavage with a cellular suspension of *C. raciborskii* or given a cell-free extract *ad libitum* in drinking water. Lymphophagocytosis was observed in mice dosed by gavage. Periacinar coagulative necrosis was commonly observed in mice dosed by both routes. The toxicity of cylindrospermopsin was lower in mice exposed to *C. raciborski* extract ad libitum in drinking water for 90-day (NOAEL = 0.15 mg/kg/day) than in mice receiving *C. raciborski* by 14-day gavage administration (NOAEL = 0.01 mg/kg body weight/day). This difference could be due to increased absorption due to gastrointestinal tract damage after gavage administration.

Chronic Toxicity/Carcinogenicity

No studies were located on the chronic toxicity or carcinogenicity of cylindrospermopsin. The toxin was proposed for evaluation in future IARC Monographs and was deleted from consideration due to inadequate animal data.

Reproductive and Teratological Effects

No mammalian reproductive or teratogenicity studies were found for cylindrospermopsin. A study of alligators in Lake Griffin, Florida, showed that *C. raciborskii* had no effect on the

viability of alligator eggs even when the presence of *C. raciborskii* in the lake was in concentrations (not provided in the abstract) great enough to kill laboratory mice.

Neurotoxicity

The neurotoxicity of cylindrospermopsin was assessed in two snail species (*Helix pomatai* and *Lymanea stagnalis*). An extract of a *C. raciborskii* culture had a direct depolarizing or hyperpolarizing effect on neurons. The response of neurons to acetylcholine was inhibited. The hepatotoxin microcystin had no effect on snail neurons.

All of the alligators found in a Florida lake dominated by a *C. raciborskii* bloom demonstrated one or more signs of severe neurological impairment such as depressed clinical responses, reduced nerve conduction velocities, axonal degeneration, and necrosis of specific foci in the midbrain. These neurological symptoms could not be directly attributed to *C. raciborskii*. It is known that some strains of *C. raciborskii* can also produce the potent neurotoxin saxitoxin.

Genotoxicity

Genotoxicity of cylindrospermopsin was investigated in mice and was shown to bind to liver DNA and form single DNA adducts. The binding of cylindrospermopsin or metabolite(s) to DNA or possibly RNA, has been proposed as a possible mechanism for the inhibition of protein synthesis that occurs with cylindrospermopsin toxicity.

Immunotoxicity

Multiple studies found that mice dosed either by gavage or i.p. with purified cylindrospermopsin or *C. raciborskii* cell extracts showed lymphophagocytosis in the spleen and thymus; however, lymphocytes in the epithelial reticular cells in the cortical layer of the thymus and large lymphocytes in the medulla survived.

Effects on Enzyme or Protein Synthesis

Cylindospermopsin severely depleted glutathione (GSH) *in vivo* in mouse bioassays.

A dose and time dependent inhibition of GSH synthesis was observed in vitro in rat hepatocytes incubated with cylindrospermopsin (≥1.6 M). In all cases, the decrease in GSH preceded signs of toxicity in the cells as determined by lactate dehydrogenase release.

The decrease in the amount of total protein content of hepatic microsomes from ICR mice dosed i.p. with 0.2 mg/kg of cylindrospermopsin (11.2 ± 1.2 mg/g liver) extract was much greater than the decrease in phospholipid concentration (3.0 ± 1.1 mol/g liver) when compared to control mice (16.6 ± 1.3 mg/g liver and 3.8 ± 0.4 mol/g liver, respectively). There was also a significant decrease in hepatic P450 of mice dosed with cylindrospermopsin (4.6 ± 2.8 nmol/g liver) when compared to controls (14.4 ± 2.8 nmol/g liver).

Cylindrospermopsin (48 ng/mL) completely inhibited globin synthesis in a rabbit reticulocyte cell-free *in vitro* system.

Structure-Activity Relationships

Currently only two deoxy isomers of cylindrospermopsin have been isolated and characterized, but like microcystins, anatoxins, and paralytic shellfish poisons (PSPs), it is possible that more congeners of cylindrospermopsin exist (Hawkins et al., 1997). This may explain the varying toxic potencies from different blooms.

A relatively nontoxic deoxygenated analog of cylindrospermopsin, in which the hydroxyl group on the uracil bridge (C-7) has been removed, was isolated in all samples from *C. raciborskii* and *A. ovalisporum* (Moore et al., 2000). When the toxicity of cylindrospermopsin was compared to that of two deoxycylindrospermopsin analogs it was suggested that the presence of the hydroxyl on the uracil bridge or perhaps the keto-enol status of the uracil moiety is critical for the hepatotoxic action of cylindrospermopsin

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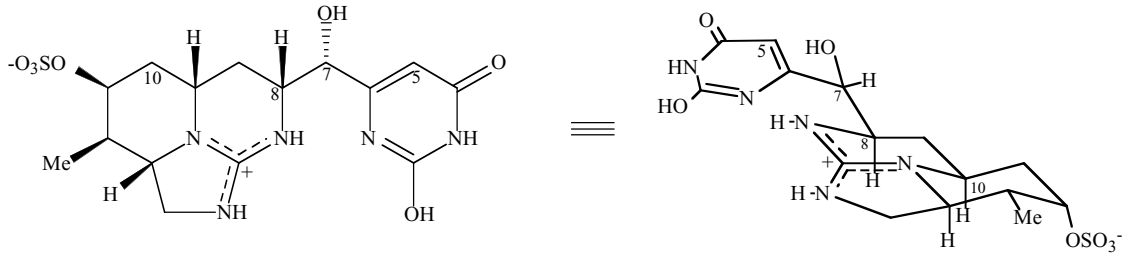
1.0 BASIS FOR NOMINATION

The nomination of the cyanobacterial toxin cylindrospermopsin is based on its presence in surface waters that are used as drinking water supplies for humans and domestic animals and its severe acute hepatotoxicity at low concentrations and possible liver damage or other toxicity resulting from chronic exposure.

2.0 INTRODUCTION

Cylindrospermopsin
[143545-90-8]



Due to tautermerization in the guanidinium substructure the positive charge is shared among the three nitrogens and not the carbon as depicted by the diagram. Figures taken from Heintzelman and Weinreb (1996).

2.1 Chemical Identification and Method of Analysis

2.1.1 Chemical Identification

Cylindrospermopsin ($[C_{15}H_{21}N_5O_7S]$; mol. wt. = 415.43) is also called:

- 2,4(1*H*,3*H*)-Pyrimidinedione, 6-[(*R*)-hydroxy[2a*R*,3*S*,4*R*,5a*R*,7*S*)-2,2a,3,4,5,5a,6,7-octahydro-3-methyl-4-(sulfooxy)-1*H*-1,8,8b-triazaacenaphthylen-7-yl]methyl]-, rel-(-)- (9CI)
- 1*H*-1,8,8b-Triazaacenaphthylene, 2,4(1*H*,3*H*)-pyrimidinedione deriv.
- 2,4(1*H*,3*H*)-Pyrimidinedione, 6-[hydroxy[2,2a,3,4,5,5a,6,7-octahydro-3-methyl-4-(sulfooxy)-1*H*-1,8,8b-triazaacenaphthylen-7-yl]methyl]-2aα,3α,4α,5aα,7β(*R**)]-(-)-
- (-)-Cylindrospermopsine
- Cylindrospermopsine

Source: Chemical Abstracts Service Registry File (2000)

Cylindrospermopsin is a tricyclic alkaloid consisting of a tricyclic guanidine moiety combined with hydroxymethyluracil (Ohtani et al, 1992). The compound is zwitterionic and highly water soluble (Moore et al., 1998 abstr.). Cylindrospermopsin is stable to extreme temperatures (no degradation at 100 ¡C for 15 minutes) and pH (Chiswell et al., 1999). Cylindrospermopsin has been isolated from the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju (in Australia, Hungary, and the U.S.), *Umezakia natans* Watanabe (in Japan),

and *Aphanizomenon ovalisporum* (in Australia and Israel) (Chiswell et al., 1997; Harada et al., 1994; Shaw et al., 1999; all cited by Duy et al., 2000; Hawkins et al., 1985; Banker et al., 1997; Ohtani et al, 1992).

The chemical structure of cylindrospermopsin was only elucidated in 1992 (Ohtani et al., 1992b). Cylindrospermopsin is believed to be derived from a polyketide that uses an amino acid starter unit such as glycocyamine or 4-guanidino-3-oxybutyric acid (Moore et al., 1993; cited by Duy et al., 2000). A more recent study proposed that cylindrospermopsin is derived from a guanidinoacetic acid starter unit. The pathway leading to the formation of guanidinoacetic acid from glycine and the origin of the NH-CO-NH segment in the uracil moiety are both unknown (Burgoyne et al., 2000).

2.1.2 Methods of Analysis

Chemical screening methods for the determination of cylindrospermopsin were initially developed by Harada et al. (1994; cited by Hawkins et al., 1997). A method using HPLC-MS/MS has recently been described for the analysis of cylindrospermopsin in water samples (Eaglesham et al., 1999). Methods used to isolate cylindrospermopsin rely on high-performance liquid chromatography (HPLC) separation of purified extracts (Harada, 1994).

Clean-up procedures involve the extraction of cylindrospermopsin from *U. natans* with methanol and separation over a silica gel column (Harada et al., 1994). Further purification of cylindrospermopsin was performed using HP-20 resin, which removed most of the ionic components from the fraction. Samples were analyzed using nuclear magnetic resonance (NMR) and HPLC (ODS column with 5% methanol-95% water as a mobile phase) with poor retention power on the ODS column and slight tailing under HPLC conditions. Using this method, the authors were able to analyze the bloom samples in about two hours. Several other compounds were found to elute in the toxic fraction along with cylindrospermopsin. They were the two aromatic amino acids phenylalanine and tyrosine, the two nucleosides cytidine and uridine, and uracil.

Two highly sensitive assays are currently being investigated for the detection of cylindrospermopsin (CYANOTOX, 1998). An enzyme-linked immunosorbent assay (ELISA) for the detection of microcystins is currently being developed and development of ELISA methods for the detection of cylindrospermopsin are planned. Development of ELISA methods for the detection of cylindrospermopsin will aid in the determination of other cyanobacterial species and strains that may produce the hepatotoxin. ELISAs can also provide a rapid means to determine the presence of harmful cyanobacterial toxins in bodies of water. A protein phosphatase inhibition assay is also being studied for the detection of cylindrospermopsin.

A recent technique was described that uses HPLC/mass spectrometry (MS) with an electrospray interface that can facilitate faster analysis by increased solvent flow rates (Eaglesham, 1997 abstr.). Sample preparation for this method was simple and involved lysing by freezing/thawing and concentration by rotary evaporation. The detection limit for cylindrospermopsin in water was 0.2 mg/L.

Polymerase chain reaction (PCR)-based methods are being studied for the detection of toxin

producers.

2.1 Physical-Chemical Properties

Property	Information	Reference
Physical State	glassy solid	Banker et al. (1997)
Odor	N/A	
Taste	N/A	
Boiling Point (^{o}C)	N/A	
Melting Point (^{o}C)	N/A	
Density (18 ^{o}C/4^{o}C)	N/A	
Soluble in:		
water	highly soluble	Moore et al. (1998 abstr.)

2.3 Commercial Availability

There is no reported commercial availability of cylindrospermopsin. Some studies obtained *C. raciborskii* cultures from Dr. Peter Hawkins of Australian Water Technologies Ensight in Sydney, Australia (Shaw et al., 2000; Chiswell et al., 1999). Cylindrospermopsin was purified from *C. raciborskii* cultures using high-performance liquid chromatography (Chiswell, 1999).

3.0 PRODUCTION PROCESSES

No commercial production of cylindrospermopsin has been reported; however, many groups are working on methods to synthesize cylindrospermopsin (Snider, 1998; Xie et al., 2000). A 20-step synthetic method for the preparation of (±)-cylindrospermopsin from 4-methoxy-3-methylpyridine, in 3.5% overall yield, was reported by Xie et al. (2000).

4.0 PRODUCTION AND IMPORT VOLUMES

No commercial production of cylindrospermopsin has been reported.

5.0 USES

There are currently no known uses for cylindrospermopsin.

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

The environmental conditions that foster the growth of the cyanobacteria that produce this potent toxin are important for understanding the environmental occurrence of cylindrospermopsin. The majority of information on cylindrospermopsin toxicity is by isolation from the organism or from bodies of water containing blooms of *C. raciborskii*, so this section and most sections of this report will deal mostly with *C. raciborskii*. The cylindrospermopsin-producing cyanobacterium *C. raciborskii* belongs to the genus *Cylindrospermopsis*. This genus was previously ascribed to *Anabaenopsis*, *Raphidiopsis*, and *Cylindrospermum* (Hawkins et al., 1997). *Cylindrospermopsis* is taxonomically more related to *Cylindrospermum* but can be differentiated by the presence of

gas vacuoles, the shape of the terminal heterocysts, and the true planktonic nature of *Cylindrospermopsis*. Cyanobacteria can be composed of many filamentous cells arranged into chains or strands called trichomes. *C. raciborskii* is assembled into either large, straight-chain trichomes or small, coiled trichomes (Fabbro and Duivenvoorden, 1996; Saker, 1996; both cited by Thomas et al., 1998). The gas vacuoles of *A. ovalisporum* and *C. raciborskii* act to regulate its position in the water column by regulating buoyancy and may contribute to its even spatial distribution during a bloom (Banker et al., 1997).

C. raciborskii is found in freshwater ponds, rivers, reservoirs, and eutrophic lakes. *C. raciborskii* probably occurs worldwide and has been well characterized in Australia due to blooms resulting in human exposure (Hawkins et al., 1997). Cylindrospermopsin-producing cyanobacteria occur in tropical or subtropical regions, whereas cyanobacterial genera (*Microcystis*, *Anabaena*, *Nodularia*, *Aphanizomenon*, *Nostoc*, and *Oscillatoria*) that produce toxic microcystins thrive in temperate regions (Duy et al., 2000, Hawkins et al., 1997; Carmichael and Falconer, 1993). Also, two species of cyanobacteria that are currently known to produce cylindrospermopsin (*Aphanizomenon* and *Cylindrospermopsis*) are filamentous cyanobacteria while *Microcystis* is unicellular (Shaw et al., 1999). Although *C. raciborskii* is mostly found in tropic regions, it has been found in warmer temperate regions of Australia, although blooms are not as common in temperate climates (Baker and Humpage, 1994; Thomas et al., 1998). In Lake Balaton, Hungary, *C. raciborskii* was the predominant algal species (Hiripi et al., 1997 abstr.). This strain was found to be lethal to insects and fish in *in vivo* assays. *C. raciborskii* has recently been found in several bodies of water in Florida, showing that it can thrive in temperate regions of the United States (Williams et al., 2000 abstr.; Burns, 2000 abstr.; Chapman and Schelske, 1997). Although most studies indicate that the appearance of *C. raciborskii* is seasonal and occurs during the warmer summer months, a study of five lakes in Florida revealed that *C. raciborskii* dominated in one lake all year round, and that *C. raciborskii* may have entered the Florida Lakes around 30 years ago (Chapman and Schelske, 1997). *C. raciborskii* has an optimal growth temperature of 25 ¡C or greater (Vinogradska, 1974; Hawkins, unpublished; both cited by Hawkins et al., 1997; Thomas et al., 1998).

A.ovalisporum was isolated from Lake Kinneret in Israel in 1994, the first reported bloom of this cyanobacterium in Israel (Sukenik et al., 1998). The bloom gradually developed during the summer and peaked in October with 4,000 filaments/mL. The cyanobacterium was evenly concentrated in the epilimnetic water column. The cyanobacteria was found in 1995 and 1996, but no massive blooms were reported.

When the body of water is visibly colored by the cyanobacteria, then it is considered a water-bloom and cyanobacteria probably number more than 10,000 cells/mL (Falconer, 1998). The floating scum formed by the floating cells on the water surface usually contains 1,000,000 cells/mL. All blooms do not result in scum being formed on the surface of the water and the position of the cyanobacterium in the water column is dependent on the buoyancy of the cells. Unlike *Microcystis* and *Anabaena*, *C. raciborskii* does not form floating scum, but algal densities may be very high (up to hundreds of thousands per milliliter) (Falconer, 1997). *C. raciborskii* is usually concentrated several meters below the surface in a reservoir and this poses an increased risk in drinking water supplies since water is usually drawn into treatment plants at a depth of several meters. The prevailing winds may concentrate the floating scum into certain areas of surface waters (Carmichael and Gorham, 1977; cited by Thomas et al., 1998).

The production of toxins is strain-specific and not species-specific (Neilan, 1996; cited by Sukenik et al., 1998). Strains of *Microcystis aerugenosa* and *Microcystis flos-aquae* found in Lake Kinneret were not toxic in mouse bioassays although other strains were toxic (Carmeli, unpublished data; cited by Sukenik et al., 1998). Generally, toxins in cyanobacteria are retained in the cell when conditions are favorable. The concentration of toxin within *A. ovalisporum* from Lake Kinneret increased to a plateau concentration during the growth phase and then decreased during the stationary phase (S phase) (Sukenic et al., 1998). The reduction in cylindrospermopsin concentration within *A. ovalisporum* cells during S phase is attributed to cell degradation and the release of the water-soluble toxin into the medium. The concentration of cylindrospermopsin found in the culture medium containing *A. ovalisporum* was positively correlated with the age of the culture. This has also been seen in *C. raciborskii* blooms, where older blooms release more cylindrospermopsin into the water than is retained in the cell (Chiswell et al., 1999) most likely due to cell lysis. It is common to find around 70-98% of the total cylindrospermopsin dissolved in the water column. At the height of a bloom in Hervey Bay, Queensland, the concentration of cylindrospermopsin in the water column was 63 g/L and the cells numbered around 2 million per mL with 29 g cylindrospermopsin/L held within the cells. Only 4 days earlier the concentration of cylindrospermopsin in the water column was 7 g/L and cells numbered about 530,000 and 29 g cylindrospermopsin/L held within the cells. The increase in cylindrospermopsin in the water column was attributed to a lack of rainfall and hence a lack of dilution in the reservoir. During an *A. ovalisporum* bloom in Australia, as much as 85% of the toxin was found in the water column when compared to the interior of the cells, indicating that the toxin is continually released from the cells or that the toxin is relatively stable (Shaw et al., 1999).

C. raciborskii contained between 0.0041 pg (1.5 mg/g dried cells) and 0.026 pg (5.5 mg/g dried cells) cylindrospermopsin per cell in several studies (Hawkins et al., 1997; Ohtani et al., 1992; Saker et al., 1999) indicating an intraspecies difference in the concentration of cylindrospermopsin within *C. raciborskii* cells. In a study using cell extracts of *C. raciborskii* strain AWT 205, four different batches grown at different times contained between 1.3 and 5.4 mg/g extract (Falconer et al., 1999). In a study of an aquaculture pond in Australia containing a *C. raciborskii* bloom, the cylindrospermopsin content of the harvested pond water was 6.6 mg/g freeze-dried cells, the highest concentration of cylindrospermopsin observed in *C. raciborskii* (Saker and Eaglesham, 1999). One milligram of *A. ovalisporum* biomass contained about 2 g of cylindrospermopsin (Sukenic et al., 1998).

Various environmental conditions such as nutrient availability, light conditions, and temperature affect the production of cyanobacterial toxins. The frequency of increased cyanobacterial blooms is related to increased nutrient availability (Falconer, 1998). Domestic and industrial chemicals, human and animal excreta, and agricultural fertilizers can contribute to increased nutrient levels. Recent construction and the exposure of fresh soil, along with the lack of water plants and macrophytic algae to remove excess nutrients (iron and zinc), were thought to have enhanced an *A. ovalisporum* bloom in Australia (Shaw et al., 1999). Optimal concentrations of soluble phosphorus (as phosphate usually) and nitrates result in the increased production of hepatotoxins in cyanobacterial strains; however, strains that do not fix nitrogen do not rely on the presence of nitrogen in the environment for toxin production (Sivonen, 1990; Lehtimaki et al., 1994; both cited by Sukenik et al., 1998). Blooms of *C. raciborskii* are reportedly enhanced by a pH of 8.4 to 9.0 (Fabbro and Duivenvoorden, 1996; cited by Thomas et al., 1998). It should be

noted that although these environmental conditions may affect the formation of blooms, the numbers of cyanobacteria and the amount of toxin produced are not always closely related (Hitzfeld et al., 2000).

Cylindrospermopsin is relatively stable to heat, pH, and light (Moore et al., 1998 abstr.; Chiswell et al., 1999). Cylindrospermopsin is stable at temperatures ranging from 4 to 50 ¡C for up to 5 weeks in the dark. After 4 weeks at 50 ¡C, 83% of an initial (1 mg/L) cylindrospermopsin concentration remained (Chiswell et al., 1999). At temperatures of 50 and 100 ¡C, concentrations of a compound structurally related to cylindrospermopsin increased without a corresponding decrease in cylindrospermopsin concentration. No degradation of cylindrospermopsin occurred when a culture of C. raciborskii was boiled for 15 minutes. Cylindrospermopsin was relatively stable for 8 weeks at pH of 4, 7, and 10 with 75-81% of the initial amount remaining. Microcystins can be degraded by UV light in 10 minutes, provided that cyanobacterial pigments are present; however, cylindrospermopsin had an experimental half-life of 18 hours under shortwave (400 mW/m2) UV light (Chiswell et al., 1999). The pigments are thought to aid decomposition of cylindrospermopsin by free radical mechanisms. Cylindrospermopsin was degraded more rapidly under normal sunlight than under artificial UV light. When cylindrospermospin (1 mg/L aqeous media) was exposed to normal sunlight, 54% remained after 3 hours and only 6% remained after 72 hours. At a higher concentration (4 mg/L), cylindrospermopsin was degraded more rapidly, with 29% of the original concentration remaining after 3 hours and 1% remaining after 72 hours. Two other unidentified bacterial strains have been found in cultures of *C. raciborskii* and may play a role in degradation due to the decreased concentration of cylindrospermopsin in longer established cultures. Toxins released from cyanobacteria into lakes are reportedly decomposed by bacteria (Rapala et al., 1994; cited by Falconer, 1998). No additional information was available to determine the persistence of cylindrospermopsin in the environment. Microcystins can persist in the environment by residing in dried crusts of cyanobacteria for months and this possibility should be investigated for cyanobacteria that produce cylindrospermopsin (Jones et al., 1995; cited by Falconer, 1998).

Copper sulfate is usually applied to lakes and reservoirs containing cyanobacteria to suppress blooms (Falconer, 1998). While it does control the bloom and effectively kills the bacteria, the cyanobacterial toxins are released as the cells are lysed. In two cases of cylindrospermopsin poisoning in Australia, the toxic symptoms of cylindrospermopsin occurred after the application of copper sulfate to dense blooms (Falconer et al., 1983; cited by Falconer, 1998; Hawkins et al., 1985). The use of other algicides can also result in similar increases in toxin release. Compounds to control blooms can only be safely used if the bacterial concentration is still relatively low. It is also recommended that another source of water be made available after treatment of municipal water supplies. The use of copper to treat water is limited in some countries to use only in reservoirs and not in natural rivers and lakes. The most effective method of algae control in reservoirs is though the use of aeration and destratification (Yoo et al., 1995; cited by Falconer, 1998).

Domestic animals and livestock are expected to be exposed to much higher concentrations of cyanobacterial toxins than are humans (Duy et al., 2000). This is because many consume water and scum directly from the sources of the toxic blooms, before they have a chance to be degraded.

6.1 Exposure to Wild and Domestic Animals

Numerous incidents have been reported involving the poisoning of farm and wild animals following drinking water from lakes and ponds containing surface scum from cyanobacterial blooms (Thomas et al., 1998). Most have been documented in Australia. One incident in Northern Queensland involved cattle that drank from a dam showing a heavy growth of blue-green algae. One cow and three calves were found dead at the dam and another eight animals died within the next three weeks. Cultures of the water revealed a predominantly straight-chain strain of *C. raciborskii*, which caused similar symptoms in laboratory mice.

The addition of barley straw to watering areas has been shown to reduce the numbers of *C. raciborskii* and decrease the likelihood of bloom formation (Pillinger et al., 1994; cited by Thomas et al., 1994).

6.2 Water Treatment and Its Utility in Controlling Cyanobacterial Toxins

Drinking water treatment methods commonly used at drinking water plants are inadequate for removal of large amounts of cyanobacteria and may even contribute to the presence of cyanobacterial toxins in drinking water. Current methods of the production of finished drinking water usually involve the pre-chlorination of water as a first step (Falconer, 1998). Chlorination then leads to the lysis of cyanobacterial cells and the release of toxins into the chlorinated water. Subsequent treatment of the chlorinated water by flocculation, rapid sand filtration, and subsequent chlorination is ineffective in removal of the neurotoxins and hepatotoxins from water (Hoffman, 1976; Falconer et al., 1978; Donati et al., 1994; all cited by Falconer, 1998). In studies using current water treatment methods (coagulation/flocculation-sand filtration-chlorination), the efficiency of cyanobacterial toxin removal (11-18%) was too low to remove all cyanobacterial toxins during a bloom (Duy et al., 2000). Some chemicals used for raw water treatment in reservoirs and ponds such as copper sulfate and Simazine may also result in the release of dissolved organic compounds from the cyanobacterial cells (Kenefick et al., 1993; Lam et al., 1994; Peterson et al., 1995; all cited by Sukenik et al., 1998). If the treatment facility is distant from the consumer, lysis of cyanobacterial cells could occur in the distribution system, especially if negative pressure is applied. It has been shown that pressure above 15 atm is sufficient to burst *A. ovalisporum* cells and cause an increase cylindrospermopsin concentrations in water (Sukenik et al., 1998). Hydrostatic pressures in the Israel National Water Carrier can reach 26 atm. The development of more effective methods of water treatment to eliminate the risk of toxin exposure rely on the removal of cyanobacterial cells or by the adsorption and destruction of toxins.

The removal of cyanobacterial cells can be performed by dissolved air flotation of cells in unchlorinated raw water treated with flocculant (Rositano and Nicholson, 1994; cited by Falconer, 1998). Further evaluation of methods and the amount of flocculant needed to effectively remove suspended cells is currently being performed. Certain flocculants have been found to coagulate cells with little (alum) or no (calcium hydroxide) increase in the concentration of microcystins (Kenefick et al., 1993; Lam et al., 1995; both cited by Sukenik et al., 1998). Membrane filtration may remove both cells and toxins from the water, and research is currently

being conducted by membrane manufacturers to determine the types of membranes that are effective.

Alternative water treatment methods have been proposed that will more reliably remove or destroy cyanobacterial toxins (Falconer, 1998). Coagulation may effectively remove cyanobacterial cells, but the highly water-soluble toxins like cylindrospermopsin are not efficiently removed by this method (James and Fawell, 1991; Rositano et al., 1994; both cited by Hitzfeld et al., 2000). A treatment process consisting of prechlorination (1 mg/L)-flocculation with alum-sedimentation-sand filtration-chloramination was shown to remove cylindrospermopsin from drinking water to below the detection limit of HPLC-MS with a contact time of 1 hour (Duy et al., 2000). Activated carbon has been shown to be effective in the removal of cyanobacterial toxins. Toxins were effectively removed by filtration though granular activated carbon and by injection of activated carbon powder. Due to the high cost of activated carbon, modifications to facilities, monitoring effectiveness, and replacement of spent activated carbon it is not a preferred alternative for large municipal treatment facilities, but may be better suited for the home treatment or small municipal treatment works. The formation of a biofilm over the activated carbon during water processing will reduce its efficiency (Hitzfeld et al., 2000). Under experimental conditions, concentrations of microcystin LR/L below 0.15 g/L were not removed by activated carbon in the presence of a biofilm or natural organic matter (Lambert et al., 1996; cited by Hitzfeld et al., 2000). Oxidation and inactivation of toxins may be achieved with excess chlorine, potassium permanganate, and ozone. All of these methods are currently being tested. Chlorination has been shown to decompose cylindrospermopsin provided that chlorine is in excess of amounts that will react with other organic matter and that the pH is 6 or above (Senogles et al., 2000). Relatively low concentrations of chlorine (0.5 mg/L) were found to degrade cylindrospermopsin (>99% degraded) when a low amount of dissolved organic carbon was present. Ozone may be the most promising of these methods because of its ability to render microcystins (UV at high intensities) and ADDA (UV at low intensities) inactive; however, the radiation required to cleave the microcystin peptide ring may be too high to be used on public water supplies (Tsuji et al., 1995; Drikas, 1994; both cited by Falconer, 1998).

7.0 HUMAN EXPOSURE

Human exposure to cylindrospermopsin may occur by ingesting toxin contaminated water during recreational activities in lakes or by ingestion of food or water contaminated with the toxin. Exposure could occur by ingesting agricultural products that have been irrigated with water containing cylindrospermopsin or by the consumption of fish taken from algal-infested waters (DNR, Australia, 2000). The possibility that livestock (e.g. cattle, goats) could retain cylindrospermopsin in their bodies and possibly excrete it in milk should not be overlooked; although this would probably not be a major route of exposure. Dermal contact with toxins may also occur during showering or bathing, or during recreational activities such as wading, swimming, skiing, and canoeing (DNR, Australia, 2000).

The occurrence of cyanobacterial blooms has not been a major concern in the United States, but with emerging information about the occurrence of blooms in water supplies in the United Sates this may be changing. Cyanobacteria are predominantly found in eutrophic water bodies. In the United Sates, many surface reservoirs are at the age (40-50 years) when they start to turn eutrophic as a result of excess nutrient availability. Almost 80% of 50 surface drinking water

reservoirs tested in the United States contained detectable amounts of toxins (unidentified in article); however, the toxins were at low concentrations (not provided) and most treatment processes were capable of removing toxins (Freeman, 2000). The identification of cyanobacteria and cyanobacterial toxins in fresh surface waters of Florida between June 10 and November 4, 1999, indicates that there is now a real potential for human exposure in the United States (Williams et al., 2000 abstr.; Burns, 2000 abstr.). The most frequently observed toxigenic cyanobacteria were *Microcystis*, *Cylindrospermopsis*, and *Anabaena* spp., with significant concentrations (measurements not provided) of these cyanobacteria in 88 of 167 samples, representing 75 individual water bodies in Florida. Cylindrospermopsin was detected in 34 water bodies throughout the state, while another hepatotoxin, microcystin, was found in 77 bodies of water. Eighty percent of these samples were lethal to mice in mouse bioassays. The abstracts did not provide details of the status of these bodies of water as sources for drinking water or recreational use. In a study of five lakes in Florida, the concentration of the dominant algal species *C. raciborskii* was between 88,000 to 176,000 units/mL when counts of all bacterial species ranged from 95,000 to 209,500 (Chapman and Schelske, 1997). *C. raciborskii* was found in past samples from several Minnesota lakes (1965) and one lake in Kansas (1955) and identified as *Anabaenopsis raciborskii* (Chapman and Schelske, 1997)

The risk of exposure to cyanobacterial toxins in drinking water is greater in children since they consume more water per unit body weight than do adults (Burns et al., 2000 abstr.) and also may be more biologically susceptible to cyanobacterial toxicity. This was evident in the Palm Island cylindrospermopsin exposure incident described in detail in **Section 9.1.1 Human Data**. During this incident, 139 children exhibited symptoms of hepatoenteritis with electrolyte imbalance, while only 10 adults were affected (Byth et al., 1980; cited by Burns, 2000). The increased risk to young animals was shown when a greater number of calves succumbed to cylindrospermopsin poisoning when compared to adult cattle that died (Thomas et al., 1998).

Exposure to cylindrospermopsin may occur by the consumption of fish that have been exposed to cyanobacteria which produce the toxin. Eating fish from reservoirs containing blooms could result in exposure to the cyanobacteria and/or cylindrospermopsin. A study reporting the bioaccumulation of cylindrospermopsin in muscle tissue of the redclaw crayfish (*Cherax quadricarinatus*) and visceral tissues of rainbow fish (*Melanotaenia eachamensis*) show that cylindrospermopsin exposure to fish may occur in aquaculture ponds (Saker and Eaglesham, 1999). The concentration of cylindrospermopsin was 0.9 g/g freeze-dried tissue in muscle of the pond-raised crayfish and 1.2 g/g freeze-dried tissue in the viscera of pond-raised rainbow fish. The study also showed that bioaccumulation could be greater in fish due to the longer exposure time in natural bodies of water to cyanobacterial bloom, sometimes as long as 4 months, when compared to shorter acute exposure in the laboratory. Fish are generally more tolerant of algal toxins than mammals and tend to accumulate them over time (Ross, 2000).

Since no oral subchronic or chronic studies of cylindrospermopsin were available at the time, the intraperitoneal (i.p.) no-observable-adverse-effect level (NOAEL) (0.2 mg/kg) in mice after acute exposure was used for the calculation of tolerable daily intake (TDI) and guideline values (GV) for the exposure of humans to cylindrospermopsin in a review by Duy et al. (2000). The authors calculated these values based on formulas provided in the review and were meant to be a reference only (Duy et al., 2000). The guideline values take into account the tumor-promoting effects of some cyanobacterial toxins as well as other toxic endpoints. The TDI for

cylindrospermopsin was 0.02 g/kg/day. It was estimated that GVs for adults, children, and infants were 0.48, 0.16, and 0.11 g/L, respectively, based on drinking water consumption of 2 L for a 60-kg adult, 1 L for a 10-kg child, and 0.75 L for a 5-kg infant. The guideline values for the number of cyanobacterial cells (based on 0.026 pg cylindrospermopsin/cell) were 4231 in infants, 6154 in children, and 18,461 in adults (Falconer et al., 1994; Duy et al., 2000). These values are summarized in **Table 1**.

Since the writing of the review deriving the above TDIs and GVs based on acute studies, one 90-day oral study exposing mice to cylindrospermopsin in drinking water has been performed (Shaw et al., 2000). This new study indicated that different guideline values should be calculated depending on the route and the type of extract (purified cylindrospermopsin, cell-free extract, or cellular suspension) used. It was proposed that an interim guideline used for microcystins in drinking water of 1 g/L be adopted for cylindrospermopsin in Australian drinking water until further studies are conducted (Shaw et al., 2000). All of the guideline values are presented in **Table 1.**

Demands on groundwater supplies in Florida are currently exceeding or threatening to exceed sustainable yields, forcing water managers to rely more often on surface drinking-water supplies. Existing surface-water treatment plants in Florida are currently not required to monitor for cyanobacterial toxins and may not be adequately equipped to treat surface waters for taste, odor, and toxins associated with immense cyanotoxic blooms. The monitoring of reservoirs and drinking-water supplies for cyanobacteria can lead to the early initiation of treatment measures to prevent blooms from occurring. This is currently the most reliable means to reduce the risk of human exposure to cyanobacterial hepatotoxins.

Artificial neural networks, computers that simulate the arrangement of neurons in the brain, are being investigated for the prediction of cyanobacterial blooms weeks or months in advance of occurrence (Freeman, 2000). Researchers have been able to predict blooms of toxic *Anabaena* spp. up to four weeks before occurrence (Maier et al., 1998) in a river in Australia. The government of Australia has been interested in developing models for predicting algae growth in an effort to minimize public exposure and the expense of supplying clean drinking water to areas affected by cyanobacterial blooms. In one bloom (Darling River, Australia, 1991), the bill for emergency water supplies was more than $1 million (Australian currency) (Freeman, 2000).

Table 1. Tolerable Daily Intake (TDI) and Guideline Values (GV) for Human Exposure in Drinking Water

Group	TDI (g/kg/d)	GV (ug/L)	GV (cells/mL)[a]	Based On	Reference
Infants	0.02	0.11	4,231	A NOAEL of 0.02 mg/kg/d for 12 d i.p. in mice (extract type and dose n.p.) (Shaw et al., unpublished data)	Duy et al. (2000)
Children	0.02	0.16	6,154		
Adults	0.02	0.48	18,461		
Infants	0.15	0.8		Cell-free extract of *C. raciborskii* provided *ad libitum* in drinking water to mice for 90 d	Shaw et al. (2000)
Children	0.15	1.2			
Adults	0.15	3.6			
Infants	0.001-0.01	0.005-0.05		Purified cylindrospermopsin i.p daily for 14 d in mice and gavage with cellular suspensions of *C. raciborskii* daily for 14 d	Shaw et al. (2000)
Children	0.001-0.01	0.008-0.08			
Adults	0.001-0.01	0.024-0.24			

Based on weight of 5 kg for infants, 10 kg for children, and 60 kg for adults.

[a] Assuming one cyanobacterial cell contains 0.026 pg of toxin, based on information for *C. raciborski* (Hawkins et al., 1997).

Values were calculated using the Spearman-K ber Method (Wardlaw, 1985).

TDI = NOAEL/Uncertainty factor. Uncertainty factor = 1000 (10 for interspecies variation, 10 for intraspecies variation, and 10 for less-than-lifetime study)

GV = TDI **x** body weight **x** percent of exposure allocated to drinking water (80% [0.8] for cylindrospermopsin)/Daily drinking-water consumption

Daily water consumption is estimated to be 2 L for an adult, 1 L for a child, and 0.75 L for an infant.

8.0 REGULATORY STATUS

No regulations were found that pertain to cylindrospermopsin. Guidelines for tolerable exposure have been described in the previous section. The U.S. Environmental Protection Agency (EPA) has listed cyanobacteria and their toxins as priority contaminants in the Drinking Water Contaminant Candidate List (CCL) (EPA, 2000). Cylindrospermopsin is the product of a biological agent that is not subject to any proposed or promulgated national primary drinking water regulation (NPDWR) but is known or anticipated to occur in public drinking water systems, and may require regulations under the Safe Drinking Water Act (SDWA).

9.0 TOXICOLOGICAL DATA

9.1 General Toxicology

9.1.1 Human Data

In 1979, a major cyanobacterial bloom occurred in a reservoir on Palm Island, off the North Queensland Coast of Australia (Falconer, 1998). This reservoir provided chlorinated, unfiltered drinking water to residents, who complained of a bad taste and smell in the drinking water. The reservoir was treated with the algicide copper sulfate to kill the bloom. Within one week, an outbreak of hepatoenteritis occurred. As many as 139 children (avg. age of 8.4 years) and 10 adults complained of hepatitis-like symptoms including malaise, anorexia, vomiting, tender hepatomegaly, headache, and abdominal pain (Bourke et al., 1983; cited by Duy et al., 2000; Byth, 1980; Hawkins et al., 1985). Kidney malfunction as well as bloody diarrhea and urine were also reported (Byth, 1980; Flaconer, 1998). Kidney damage was severe in those reporting bloody urine (20% of cases) and together with severe damage to the gastrointestinal lining caused fluid and electrolyte loss. This was the most acute clinical aspect of the outbreak. In some cases, the electrolyte loss was so severe that patients suffered from hypovolemic/acidotic shock characterized by extreme lethargy (Falconer, 1998). The severity of the illness was positively correlated with the amount of water ingested from contaminated water supplies (Hayman, 1992; cited by Thomas et al., 1998). A culture of the reservoir water revealed the presence of *C. raciborskii*. When isolate extracts were injected i.p. in mice, they exhibited toxic symptoms similar to those observed in humans. According to these symptoms, which have been observed since colonial times in Australia, it is believed that cylindrospermopsin may be the causative agent of a debilitating and sometimes fatal disease in northern and central outback Australia known as Barcoo spews.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

9.1.2.1 Absorption and Distribution

No mammalian studies on the absorption and distribution of cylindrospermopsin were found. However, acute and subchronic toxicity studies in mice showed that absorption of cylindrospermopsin from the gastrointestinal tract does occur (Shaw et al., 2000; Seawright et al., 1999; Falconer et al., 1999). Trichomes of cylindrospermopsin were found in the gut contents of animals dosed orally with cellular suspensions of *C. raciborskii*. It is not known whether the cyanobacteria can survive or multiply in the gastrointestinal tract.

The absorption and distribution of cylindrospermopsin was assessed in the redclaw crayfish (*Cherax quadricarinatus*) and rainbow fish (*Melanotaenia eachamensis*) during a *C. raciborskii* bloom in an aquaculture pond (Saker and Eaglesham, 1999). Pondwater samples collected during the bloom contained 589 g/L of cylindrospermopsin (93% in the cells and 7% in the water). *C. raciborskii* reached a maximum concentration of 324,640 cells/mL. The highest concentration of cylindrospermopsin was found in the hepatopancreas tissue (4.3 g/g freeze-dried hepatopancreas) of crayfish collected from the pond while the concentration of cylindrospermopsin in muscle tissue was less (0.9 g/g freeze-dried muscle). No cylindrospermopsin was detected in the tissues of the rainbow fish but were evident in the viscera at concentrations similar to cylindrospermopsin in crayfish muscle. Trichomes *of C. raciborskii* were found in the gut contents of the crayfish, but not in the gut contents of the rainbow fish.

A group of crayfish were experimentally exposed to a pure culture isolated from the aquaculture pond described above for 14 days (Saker and Eaglesham, 1999). The concentration of cylindrospermopsin in the hepatopancreas (0.54 g/g freeze-dried hepatopancreas) and muscle (0.12 g/g freeze-dried muscle) tissue of the laboratory-exposed crayfish was less than that observed in the pond-exposed crayfish. However, the same 5:1 ratio of cylindrospermopsin found in the hepatopancreas and muscle tissue of aquaculture pond-exposed crayfish was observed in the laboratory-exposed crayfish. As the concentration of cylindrospermopsin in the water increased so did the concentration in the hepatopancreas and muscle, but the ratio remained 5:1. No histopathologic abnormalities were observed in either the group of crayfish exposed in the pond or the group of crayfish exposed in the laboratory. It was proposed that the difference in the bioaccumulation of cylindrospermopsin between the laboratory- and pond-exposed groups of crayfish may be attributed to the longer length of exposure of the pond-exposed fish.

9.1.2.2 Metabolism

There is evidence for the contribution of metabolites in the toxicity of cylindrospermopsin. Inhibition of cytochrome P450 provided partial protection from cylindrospermopsin in hepatocytes, indicating that metabolites, as well as cylindrospermopsin, may play a role in toxicity (Runnegar et al., 1995; cited by Falconer, 1998). Glutathione depletion in hepatocytes and centrilobular cell injury point towards the role of metabolite(s) in toxicity; however, nonselective damage in tissues that are less likely to metabolize compounds points towards the primary role of cylindrospermopsin in toxicity (Hawkins et al., 1997; Runnegar et al., 1994). In another study, the initial location of liver damage was the periacinar region, where xenobiotic metabolism by the cytochrome P450 system primarily occurs (Shaw et al., 2000). Cylindrospermopsin was toxic only after metabolic activation in cultured rat hepatocytes (Runnegar et al., 1994).

9.1.3 Acute Exposure

9.1.3.1 Toxicity in Wild and Domestic Animals

The toxicity of cylindrospermopsin has been observed in wild and domestic animals more than any other organisms, due to the higher probability of exposure from toxic cyanobacterial blooms in open lakes and ponds.

Symptoms of cylindrospermopsin toxicity in cattle have been reported as weakness, anorexia, palor of the mucous membranes, coldness of the extremities, and diarrhea (Carmichael, 1992; Carmichael and Falconer, 1993; both cited by Thomas et al., 1998). Cattle from Northern Queensland that died after drinking from *C. raciborskii*-contaminated water exhibited extensive fibrosis and bile duct proliferation with isolated groups of hepatocytes remaining (Thomas et al., 1998; Saker et al., 1999). Extensive epicardial hemorrhaging was observed in the heart and subserosal hemorrhaging was seen in the small intestine and omentum. No abnormalities were observed in the lungs, spleen, kidney, and brain. Gross findings on necropsy of one calf showed severe abdominal and thoracic hemorrhagic effusion, hyperemic mesentery, pale and swollen liver, and extremely distended gall bladder containing dark yellow bile. The freeze-dried and sonicated suspended culture of *C. racibroski* isolated from the cattle s pond was injected intraperitoneally in mice (12-165 mg/kg) and found to be toxic at concentrations of 153 mg extract/kg (Saker et al., 1999). The incidence of mortality and morbidity was higher in calves than in adult cattle, indicating that the risk of toxicity of cylindrospermopsin in livestock may be greater in young animals.

9.1.3.2 Experimental Animals

Acute toxicity values for cylindrospermopsin are presented in **Table 2**. The details of studies discussed in this section are presented in **Table 3**.

When given orally or intraperitoneally to mice, cylindrospermopsin can cause gastroenteritis though injury to the gut lining, hepatitis from injury to liver cells, kidney malfunction due to injury to renal cells, and hemorrhage from damage to blood vessels (Falconer, 1994; cited by Duy et al., 2000).

Hepatotoxicity was shown after intraperitoneal administration of purified cylindrospermopsin (0.2 mg /kg) in 24 male ICR mice and was described as a four-step process (Terao et al., 1994; Duy et al., 2000):

- ribosome detachment from the surface of the rough endoplasmic reticulum and accumulation in the cytoplasm of hepatocytes
- a phase that occurs about 24 hours after cylindrospermopsin administration and is characterized by a considerable decrease in the amount of P450 enzymes and membrane proliferation
- accumulation of fat droplets in the central portion of the hepatic lobules, probably induced by free radicals
- terminal phase of severe hepatic necrosis

Low doses of cylindrospermopsin to mice typically result in injury or death of hepatocytes near central veins (centrilobular), pale livers, and fatty degeneration of hepatocytes, all occurring generally more than 24 hours after the initial dose (Ohtani et al., 1992; cited by Runnegar et al., 1994; Hawkins et al., 1985). High doses lead to extensive hepatocyte necrosis (panlobular) and a reddened, swollen liver usually within six to nine hours after the initial dose. Extensive liver damage was observed in every toxicity study reviewed.

Mechanisms for the liver toxicity of cylindrospermopsin were proposed to be by inhibition of protein synthesis and disruption of metabolic activity including hepatocyte death and lipid accumulation in the liver. The inhibition of protein synthesis in the liver by cylindrospermopsin may account for triglyceride accumulation in the liver as a result of decreased lipoprotein synthesis and excretion (Terao et al., 1994). The toxicity of cylindrospermopsin may depend on metabolic transformation in the liver by cytochrome P450 (Runnegar et al., 1995). Glutathione, which may be required to inactivate cylindrospermopsin, or possibly a metabolite, is inhibited in the hepatocytes themselves (Runnegar et al., 1994).

The main target of cylindrospermopsin toxicity is the liver, but other organs such as the thymus, kidneys, adrenal glands, lungs, intestinal tract, and heart may also be affected (Hawkins et al., 1985; Terao et al., 1994;). Adrenal cells exhibit variable scattered epithelial cell degeneration and necrosis (Hawkins et al., 1985). Single cell necroses were occasionally observed 48 hours after administration of 0.2 mg/kg of cylindrospermopsin to male ICR mice (Terao et al., 1994). Lesions in the heart were also observed in MF1 male mice dosed with a culture of *C. raciborskii* (2.5-8.3 mg/kg alkaloid equivalent) and were attributed to the agonal phase of intoxication.

Congestion and edema of the small intestine have been observed in mice, accompanied by slight diarrhea. Mice dosed with cylindrospermopsin also huddle together and experience anorexia. It was thought that liver damage led to a centrally directed depression of gastrointestinal motility and retention of food in the stomach until the gastrointestinal contents were emptied.

In many studies, mice dosed orally with culture suspensions of *C. raciborskii* contained the cyanobacterium in the gastrointestinal tract (Seawright et al., 1999). Gastrointestinal hemorrhaging and anorexia were common in orally dosed mice. Ulcerations of the esophagus were noted when male MF1 mice were gavaged with a freeze-dried culture of *C. raciborskii* (Seawright et al., 1999). Mice killed 48-72 hours after a single administration consumed no food and the stomach still contained food from the initiation of the experiment.

Either pure cylindrospermopsin or an extract of cultured *C. raciborskii* tested in fish (*Rutilus rutilus* L.) at lethal and sublethal doses caused liver damage and inhibited respiration in fish. Slow gasping respiration was also observed in mice dosed i.p. with 800 mg freeze-dried *A. ovalisporum*/kg mouse (Sukenic et al., 1998).

The histopathological observations in the liver and kidney were similar in studies where mice were dosed with cylindrospermopsin purified from cell cultures or the cell cultures themselves were used. In some studies of the toxicity of cell isolates of *C. raciborskii*, the content of cylindrospermopsin in the cell isolates was not known. In these studies, the histopathology of the liver and kidneys were identical to findings in studies where the content of cylindrospermopsin was known. However, a discrepancy between the toxicity of *C. raciborskii* cellular lysates to the kidney and liver of mice and the cylindrospermopsin content of the lysates was observed in two studies (Hawkins et al., 1997; Falconer et al., 1999). A comparison was

made between the experimental LD_{50} results in one study with male Swiss albino mice dosed with cell extracts of C. raciborskii and predicted results (382 mg/kg at 24 h and 36 mg/kg at 5-6 d) based on an earlier study using lysates with the same cylindrospermopsin content (0.026 pg/cell) (Ohtani et al, 1992; cited by Hawkins et al., 1997). It was shown that the 24-h toxicity in mice did not correlate with the amount of cylindrospermopsin in culture extracts. This could be due to the presence of toxins other than cylindrospermopsin that are more rapidly lethal; mouse sensitivity differences; or synergistic effects from other cellular components. The original isolate from a culture of Solomon Dam *C. raciborskii* had an i.p. 24-hour LD_{50} of 64 mg/kg in 3-month-old male white mice (Hawkins et al., 1985). Cultures obtained from the same site seven years after the original poisoning and cultures from other *C. raciborskii* blooms in Australia have shown variable toxicity in mice, both in acute death and in the extent of proximal tubule damage (Hawkins et al., 1997; Falconer, 1998). Kidney damage was observed in the human poisoning, but was not as evident in mice given the purified extract. It is possible that the variation in toxicity in *Cylindrospermopsis* could be due to the presence of other toxins (Falconer, 1998).

The toxic potency of cylindrospermopsin is similar to that of other hepatotoxic and neurotoxic compounds isolated from other cyanobacteria and from paralytic shellfish poisons within a 10-fold range. Unlike microcystins, cylindrospermopsin does not inhibit phosphatases 1, 2A, or 3, but rather affects glutathione synthesis in hepatocytes (Runnegar et al., 1994; Runnegar et al., 1995). Also unlike microcystins, which kill mice relatively quickly within 1-2 hours after a single dose, cylindrospermopsin s toxic effect on hepatocytes is delayed and progressive, causing death usually within 24-120 hours in mice (Runnegar et al., 1994; Hawkins et al., 1985).

Table 2. Acute Toxicity Values for Cylindrospermopsin

Route	Species (sex and strain)	$LD_{50}/LC_{50}/NOAEL$[a]	Reference
In Vivo Studies			
from *C. raciborskii*:			
i.p.	mouse	24-h LD_{50} = 2.1 mg/kg 5-6 d LD_{50} = 0.2 mg/kg	Ohtani et al (1992)
i.p.	mouse	7-d LD_{50} = 0.17 mg/kg (0.1-0.28, 95%CI)	Shaw et al. (unpublished; Wardkaw, 1985; both cited by Duy et al., 2000)
i.p.	mouse (M, Swiss albino)	24-h LD_{50} = 50-110 mg/kg (cell extract) 7-d LD_{50} = 20-65 mg/kg (cell extract)	Shaw et al. (1999)
i.p.	mouse (M, Swiss albino)	24-h LD_{50} = 64 ± 5 mg/kg (freeze-dried culture)	Hawkins et al. (1985)
i.p.	mouse (M, Swiss albino)	24-h LD_{50} = 0.286 mg/kg[b] 7-d LD_{50} = 0.176 mg/kg[b]	Hawkins et al. (1997)
i.p.	mouse (M, Swiss albino)	24-h LD_{50} = 0.1-0.352 mg/kg[b] 7-d LD_{50} = 0.046-0.189 mg/kg[b]	Falconer et al. (1999)
oral	mouse (M, MF1)	2-6-d LD_{50} = 4.4-6.9 mg/kg	Seawright et al. (1999)
oral	mouse	LD_{100} = 8 mg/kg	Shaw et al. (unpublished; cited by Duy et al., 2000))
oral	mouse (M, Quackenbush)	LD_{50} = 6 mg/kg (cell suspension)	Shaw et al. (2000)
from *A. ovalisporum*			
i.p.	mouse	24-h LD_{50} = 116 mg/kg (crude extract) 24-h LD_{50} = 465 mg/kg (freeze-dried culture)	Reed and Muench (1938; cited by Banker et al., 1997)
i.p.	mouse (sex and strain n.p.)	24-h LD_{50} = 0.930 mg/kg[b]	Sukenik et al. (1998)
i.p.	mouse	24-h LD_{100} = 800 mg/kg (dry weight cells)	Banker et al. (1998)

Abbreviations: LC_{50} = concentration lethal to 50% of test animals; LD_{50} = dose lethal to 50% of test animals; M = male; NOAEL = no observable adverse effect level; n.p. = not provided

[a] Doses are pure cylindrospermopsin unless otherwise noted

[b] Calculated as cylindrospermopsin based on the reported content in the freeze-dried culture sample, cell suspension, or crude extract.

Table 3. Acute Toxicity of Cylindrospermopsin in Laboratory Animals*

Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
36 Male ICR mice	*U. natans*/location n.p.	purified cylindrospermopsin injected i.p. (0.2 mg/kg); cyclohexamide (less common name for cycloheximide) injected i.p. (150 mg/kg) in 12 mice	Mice dosed with cylindrospermopsin were sacrificed at 16, 24, 32, 40, 48, 72, 80, and 100 h after toxin administration. Mice dosed with cyclohexamide were sacrificed at 24, 48, 72, and 80 h. At 16 h, hepatic cell microsomes detached from the rough endoplasmic reticulum and accumulated in cytoplasm; hepatocyte nucleoli become dense, rounded, and smaller; no behavioral abnormalities. At 24 h, there was a marked proliferation of membrane systems; often membrane whorls with central accumulation of fat droplets were observed. At 48 h, hepatocytes around the central vein contained numerous autophagic vacuoles in the cytoplasm; fat droplets were scattered in glycogen area of hepatocytes in centrilobular portions; dilation of bile canaliculi and flocculent material filled lumen. At 72 h, livers were swollen and yellowish in color; almost all hepatocytes in hepatic lobules were swollen and pale; necrotic debris was abundant in the centrilobular regions; bleeding lesions were frequently seen in midzonal and centrilobular regions; fat droplets were fused and formed fat lakes; almost all microorganelles were depressed by the fat. At 100 h, all hepatocytes in the hepatic lobules were destroyed. Effects of cyclohexamide were similar up to 48 h, but there was no proliferation of membrane configurations. Kidneys of mice exposed to cylindrospermopsin were unremarkable until 48 h after dosing when proliferative smooth endoplasmic reticulum was observed in the cytoplasm of the proximal convoluted urinary tubules; fat droplets accumulated near brush border; single cell necrosis was seen in proximal and distal convoluted urinary tubules. Necrosis of lymphocytes in the cortical layer of the thymus and single cell necrosis in the heart was observed after 48 h. Cylindrospermopsin inhibited protein synthesis in hepatic microsomes even more than phospholipid synthesis was inhibited. The amount of P450 was significantly reduced in hepatic microsomes. Globin synthesis was completely inhibited by 48 ng cylindrospermopsin/mL The inhibition of protein synthesis was observed in hepatic microsomes. The decrease in the total amount of protein in hepatic microsomes was more significant than the decrease of phospholipid in microsomes. The amount of total P450 was greatly diminished after exposure to cylindrospermopsin.	Terao et al. (1994)

Table 3. Acute Toxicity of Cylindrospermopsin in Laboratory Animals (Continued)*

Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
Mice (number, sex, and age n.p.)	*A. ovalisporum*/Lake Kinneret, Israel (1994)	i.p. injection of a crude extract of *A ovalisporum* (800 mg dw of *A. ovalisporum*/kg bw)	1 mg dw of *A ovalipsorum* contained about 2 g of cylindrospermopsin. A dose equivalent of 800 mg dw of *A. ovalisporum*/kg bw killed all mice within 24 h of inoculation. Toxic symptoms of lethal doses include slow, gasping respiration and occasional limb paddling. An autopsy revealed congestion of the liver, lungs, kidneys, and small intestine. The estimated 24-h LD_{50} of cylindrospermopsin after i.p. administration was116 mg crude extract/kg bw, which is equal to 465 mg freeze-dried culture / kg bw. A toxicity guided fractionation of the active extract led to a polar compound that was determined by mass spectrometry and NMR data to be cylindrospermopsin	Sukenik et al. (1998)
18 MF1 mice (M, age n.p.)	*C. raciborskii*/AWT EnSight, Australia	gavage once with freeze-dried *C. raciborskii* suspended in 1 mL saline (equivalent to 2.5-8.3 mg cylindrospermopsin/mg bw)	Animals were observed for periods ranging from 48 to 8 d. Mice were fasted for 24 h prior to dosing. Mice dose with potentially lethal amounts of cell culture consumed their diet freely during the first 24 h following gavage; however, over the next 24 h, appetite was depressed and body weight loss occurred. Also at this stage mice huddled in the corner of the cage, were depressed, and their coats were rough from pilorection. In succeeding days body weight decreased further as anorexia persisted and water intake ceased as animals became moribund. In all mice a green culture material was found in the stomach and proximal third of the small intestine. In a few mice blood was found in both regions. No diarrhea was present. The liver was swollen and uniformly pale, sometimes with a yellowish, mottled appearance. Kidneys were also pale and swollen. The thymus was invariably atrophic and often the spleen appeared shrunken to about half its normal size. Histologic examination of the liver revealed foaminess of hepatocellular cytoplasm, generalized and severe fatty infiltration associated with periacinar coagulative necrosis, and small areas of hepatocellular necrosis in severely affected livers.	Seawright et al. (1999)

Table 3. Acute Toxicity of Cylindrospermopsin in Laboratory Animals (Continued)*

Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
52 Swiss albino mice (M, age n.p.)	*C. raciborskii*/ Oatley Pond, Sydney, Australia	i.p. injection of sonicated freeze-dried culture sample (10-300 mg dry weight cyanobacteria/kg bw); (cylindrospermopsin content of the freeze-dried cells was 5.5 mg/g dry weight cyanobacteria or 0.026 pg/cell)	Mice were killed as their condition was assessed to be terminal (7.5 to 151 h) or at 168 h. Signs of hemorrhage in the liver, kidneys, and small intestine, along with congestion of the lungs was noted. Centrilobular necrosis of the liver was observed at low doses with generalized liver necrosis at higher doses. Kidney damage was restricted to the tubular epithelium. The 24-h i.p. LD_{50} for the freeze-dried cells was 52 mg/kg (30-77, 95% CI) and a 7-d i.p. LD_{50} of 32 mg/kg (1-48, 95% CI). A comparison was made between the experimental LD_{50} results in this study with predicted results (382 mg/kg at 24 h and 36 mg/kg at 5-6 d) based on an earlier study (Ohtani et al, 1992). It was shown that the 24-h toxicity in mice did not correlate with the amount of cylindrospermopsin. This could be due to the presence of toxins other than cylindrospermopsin, which are more rapidly lethal; mouse sensitivity differences; or synergistic effects from other cellular components. The biochemical mechanism of toxicity was thought to be by inhibition of protein synthesis and disruption of metabolic activity including hepatocyte death and lipid accumulation in the liver. It was not known whether cylindrospermopsin or a metabolite was responsible for the observed effects. Glutathione depletion in hepatocytes and centrilobular cell injury point to a metabolite; however, non-selective damage in various tissue less likely to metabolize compounds points to the role of cylindrospermopsin.	Hawkins et al. (1997)
18 ICR mice (M, 5 wk)	*U. natans*/Lake Mikata, Fukui, Japan (1987)	i.p. injection of 0.2 mg purified cylindrospermopsin/kg bw in saline	Cylindrospermopsin showed toxicity to mice at 7 mg/kg and accounted for all toxicity. The toxin was pure according to NMR results. Morphological changes at 24 h were congestion of the liver, kidneys, and heart. Centrilobular necrosis in hepatic lobules was noted. At 72 hours, one mouse died and the surviving 2 mice were sluggish. The liver and kidneys were swollen and pale yellowish. Histologically, almost all of the hepatic cells in the liver accumulated fat droplets. Occasionally, hepatic cells in the centrilobular portions were dissociated from the hepatic plates.	Harada et al. (1994)

Table 3. Acute Toxicity of Cylindrospermopsin in Laboratory Animals (Continued)*

Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
Swiss albino mice (M, 4- to 14-wk-old)	*C. raciborskii* (AWT 205)/AWT EnSight	cell extracts of C. raciborskii either i.p. (up to 50 mg/kg bw) or gavage (up to 1400 mg/kg bw)	Four different batches of *C. raciborskii* were used. Each batch contained different amounts of cylindrospermopsin ranging from 1.3-5.4 mg/g extract. LD_{50}s were determined in mice dosed i.p. with a single batch. The 24-h LD_{50}s for the batches which contained 1.3, 2.0, 3.2, and 5.4 mg cylindrospermopsin/g extract were 80, 50-100, 90-110, and 50 mg extract/kg bw, respectively. The 7-d LD_{50}s for the batches which contained 1.3, 2.0, 3.2, and 5.4 mg cylindrospermopsin/g extract were 35-65, not determined, 30-50, and 20-35 mg extract/kg bw, respectively. Mice given oral or i.p doses of *C. raciborskii* extracts equal to one half the LD_{50} showed similar pathological findings. The livers exhibited a mottled appearance due to dark red lobule boundaries and blood-filled central vein. Kidneys appeared pale and changes were more apparent as the dose increased. The histological damage in mice was dose dependent regardless of route; however, the i.p. toxicity was 25-fold higher than the oral toxicity. Liver damage was centrilobular. Hepatocytes had increased cytoplasmic vacuolation, intercellular spaces, and darker nuclear and cytoplasmic staining. The extent and severity of the liver damage became more widespread as dose and time after dose increased. Kidney damage was marked by a reduction of erythocytes in the glomerulus, more space around the glomerulus, a larger diameter of the tubule lamina, epithelial cell necrosis in the proximal tubules, and proteinaceous material in the distal tubules. The renal effects of cylindrospermopsin occurred within 24 h of administration and tissue repair ensued within 5 d. The extent of kidney damage was more consistent across batches than was liver damage. While cylindrospermopsin administered by the i.p. route resulted in deaths, no deaths were observed over the seven day observation period in mice dosed orally. Because of the variability in renal toxicity of extracts containing comparable amounts of cylindrospermopsin it was implied that more than one toxin may be present in *C. raciborskii*. It was proposed that glomerulur blood cell content and tubule luminal diameter may be useful in determining the extent of cylindrospermopsin exposure.	Falconer et al. (1999)

* Only studies that provided the amount of cylindrospermopsin are summarized in this table.
Abbreviations: AWT = Australian Water Technology; bw = body weight; d = day(s); dw = dry weight; h = hour(s); i.p. = intraperitoneal route; M = male; n.p. = not provided

9.1.4 Short-Term and Subchronic Exposure

No pathological changes were observed in the lungs, liver, kidney or heart of mice administered cylindrospermopsin (0.02 mg/kg bw/day) i.p. for 12 days (Shaw et al., unpublished study; cited by Duy et al., 2000). Deaths were observed at doses greater than 0.05 mg/kg. In a published study by the same authors, purified cylindrospermopsin administered i.p. to white Quackenbush mice, the NOAEL was 0.001 mg/kg bw/day. The 0.02 mg/kg/bw/day dose was considered the NOAEL of cylindrospermopsin by the i.p. route, with a 95% confidence interval of 0.1-0.28 mg/kg/bw/day. This i.p. NOAEL is comparable to that of microcystins (Duy et al., 2000).

The subchronic oral toxicity of cylindrospermopsin was evaluated in mice after exposure for 90 days *ad libitum* in drinking water (Shaw et al., 2000). The 90-day oral NOAEL was found to be 0.15 mg/kg/day in male white Quackenbush mice. Details of this study are provided in **Table 4**.

9.1.5 Chronic Exposure

No studies have been conducted on chronic exposure to cylindrospermopsin in any species.

9.1.6 Synergistic and Antagonistic Activity

No studies were found on the synergistic and antagonistic effects of cylindrospermopsin.

9.2 Reproductive and Teratological Effects

No mammalian reproductive or teratogenicity studies were found for cylindrospermopsin. A study of alligators in Lake Griffin, Florida, showed that *C. raciborskii* had no effect on the viability of alligator eggs even when the presence of *C. raciborskii* in the lake was in concentrations (not provided in the abstract) great enough to kill laboratory mice (Ross, 2000).

9.3 Carcinogenicity

No studies were found on the carcinogenicity of cylindrospermopsin. Cylindrospermopsin was proposed for evaluation in future IARC Monographs but was deleted from consideration due to inadequate animal data (IARC, 2000).

9.4 Neurotoxicity

The neurotoxicity of cylindrospermopsin was studied in two snail species (*Helix pomatai* and *Lymanea stagnalis*) (Vehovszky et al., 1997 abstr.). The electrical responses of identified neurons were characterized using intracellular microelectrophysiological methods. An extract of a *C. raciborskii* culture had a direct depolarizing or hyperpolarizing membrane effect on a *C. raciborskii* culture had a direct depolarizing or hyperpolarizing membrane effect on neurons. Also, responses of the neurons to acetylcholine were inhibited. The results suggested that the cylindrospermopsin extract had specific membrane effects on snail neurons and neurotransmitter receptors. Interestingly, the hepatotoxin microcystin did not have any effect on snail neurons.

Table 4. Subchronic Toxicity of Cylindrospermopsin in Laboratory Animals

Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
White Quackenbush mice (M)	*C. raciborskii* (AWT 2 05/1)/Australia	The following dosing was employed: either i.p. with purified cylindrospermopsin, in drinking water with cell-free extract, or gavage with cellular suspension	A single oral dose (gavage) of freeze-dried cellular suspension of C. raciborskii resulted in an LD_{50} of approximately 6 mg/kg bw. Swollen and pale livers were consistently observed in mice receiving the single gavage dose, which corresponded to lipid vacuolization in the hepatocellular cytoplasm. Extensive areas of hepatocyte necrosis were seen in severely affected livers. Other pathological observations were: thymic atrophy and necrosis, necrosis in the tubules of the kidney cortex, and thombohemorrhagic lesions in one or both eye orbits. Intraperitoneal dosing for 14 days resulted in a NOAEL of 0.0001 mg/kg/d. Repetitive dosing by gavage over 14 d produced NOAEL of 0.01 mg/kg bw/d. Mice receiving cylindrospermopsin in drinking water exhibited NOAELs of 0.15 mg/kg bw/d as shown by no observable histopathological or morphological changes after receiving drinking water for 90 d. Lymphophagocytosis in the spleen was observed in mice dosed either i.p. or by gavage. Periacinar coagulative necrosis was also commonly observed. The periacinar region of the liver is also the region of metabolism of xenobiotic metabolites and the appearance of lesions in this region supports the theory that metabolic activation of cylindrospermopsin may be responsible for toxicity. The lower toxicity of cylindrospermopsin with 90-d oral administration when compared to 14-d gavage administration could be due to increased absorption due to gastrointestinal tract damage. Another possible explanation was resistance to cylindrospermopsin due to inhibition of metabolic enzymes or activation or induction of enzymes capable of cylindrospermopsin degradation. The presence of lymphophagocytosis is indicative of an immunotoxic response and was seen in only with 14-d administration and not from 90-d administration	Shaw et al. (2000)

Abbreviations: bw = body weight; d = day(s); M = male

Alligators in Lake Griffin (Florida) which experienced algal blooms dominated by *C. raciborskii* exhibited abnormal behavior including innanation, paralysis, and reluctance to submerge (Ross et al., 2000). All of the alligators demonstrated one or more signs of severe neurological impairment such as depressed clinical responses, reduced nerve conduction velocities, axonal degeneration, and necrosis of specific foci in the midbrain. These sick alligators did not exhibit any visible sign of gross organ or tissue abnormality. These neurological symptoms could not be directly attributed to *C. raciborskii*. It is known that some strains of *C. raciborskii* can also produce the potent neurotoxin saxitoxin.

9.5 Anticarcinogenicity

No studies were found on the anticarcinogenicity of cylindrospermopsin.

9.6 Genotoxicity

Cylindrospermopsin or its metabolite(s) have been shown *in vivo* to bind to liver DNA in mice, forming adducts (Shaw et al., 2000). The binding of cylindrospermopsin or a metabolite to DNA, or possibly RNA, in liver cells has been proposed as a possible mechanism for the inhibition of protein synthesis that occurs with cylindrospermopsin toxicity.

The details of this study are presented in **Table 5**.

Table 5. Genotoxicity of Cylindrospermopsin

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form and Purity	Dose	Summary	Reference
Male white Quackenbush mice (age n.p.)	Detection of DNA adducts/binding using ^{32}P-postlabelling assay with separation of radiolabelled nucleotides with TLC	cylindrospermopsin purified by HPLC (purity n.p.) from cell extracts of *C. raciborskii*	Mice were given single i.p doses of cylindrospermopsin in cell-free extracts of *C. raciborskii* (dose n.p.)	Observed adduct spots in liver DNA from mice killed 24 to 96 h after dosing. A single adduct spot was observed in each case	Shaw et al. (2000)

9.7 Cogenotoxicity

No studies were found on the cogenotoxicity of cylindrospermopsin.

9.8 Antigenotoxicity

No studies were found on the antigenotoxicity of cylindrospermopsin.

9.9 Immunotoxicity

A recent study has shown that lymphophagocytosis in the spleen occurred in white Quackenbush male mice repetitively dosed daily either i.p. with purified cylindrospermopsin extract for 14 days or gavaged with a cellular suspension of *C. raciborskii* for 14 days (Shaw et al., 2000).

In another study of male ICR mice dosed once with 0.2 mg/kg cylindrospermopsin extract showed massive necrosis of lymphocytes in the cortical layer of the thymus after 72 hours; however, lymphocytes in the epithelial reticular cells of the cortical layer and large lymphocytes in the medulla survived (Terao et al., 1994). The involution of thymus lymphocytes occurred immediately after the proliferation of hepatocyte membrane components. This evidence may support the speculation that the proliferative membrane system plays a role in steroid metabolism and exert an influence on the involution of the thymus.

These same effects on the thymus and spleen were also seen in male MF1 mice dosed with 2.5-8.3 mg/kg of cell suspensions of C. raciborskii, but these were considered to be normal response of the immune system to the stress of severe intoxication and is not specific to cylindrospermopsin toxicity (Seawright et al., 1999).

9.10 Other Data

9.10.1 Effects on Enzyme or Protein Synthesis

The effects of cylindrospermopsin on protein synthesis are summarized in **Table 6**. Glutathione (GSH) is an important nonprotein thiol in the cell, protecting against oxidative damage and acting in the detoxification of many xenobiotics by serving as a substrate for GSH transferases and GSH peroxidases. Severe depletion of GSH can result in cellular injury or death.

Cylindrospermopsin (≥1.6 M) significantly decreased the concentration of GSH in rat hepatocytes *in vitro* (Runnegar et al., 1994 and 1995). The decrease in GSH was dependent on the length of incubation and the concentration of cylindrospermopsin. In all cases, the decrease in GSH preceded signs of toxicity in the cells as determined by the presence of lactate dehydrogenase in the medium.

The total protein content (11.2 ± 1.2 mg/g liver) of hepatic microsomes from ICR mice dosed i.p. with 0.2 mg/kg of cylindrospermopsin extract was much less than the total protein content in livers of control mice (16.6 ± 1.3 mol/g liver). The difference between the phospholipid concentration in liver after cylindrospermopsin administration (3.0 ± 1.1 mol/g liver) when compared to control mice mg/g liver and (3.8 ± 0.4 mol/g liver) was not as great as the difference in protein content (Terao et al., 1994). There was also a significant decrease in hepatic P450 of mice dosed with cylindrospermopsin (4.6 ± 2.8 nmol/g liver) when compared to controls (14.4 ± 2.8 nmol/g liver).

Cylindrospermopsin (48 ng/mL) completely inhibited globin synthesis in a rabbit reticulocyte cell-free *in vitro* system (Terao et al., 1994).

9.10.2 *In Vitro* Cytotoxicity of Cylindrospermopsin

The cytotoxicity of cylindrospermopsin is presented with effects of cylindrospermopsin on protein synthesis in **Table 6**. Cylindrospermopsin (6.26-3200 ng/mL) was toxic to rat

hepatocytes and ketone body cells when incubated for 24, 48, and 72 hours. The LD_{50} of cylindrospermopsin in hepatocytes was 50 ng/mL after 72 h and 200 ng/mL after 48 h. The LD_{50} of cylindrospermopsin in ketone body cells was 2000 ng/mL after 72 h and 200 ng/mL after 48 hours.

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Currently only two deoxy isomers of cylindrospermopsin have been isolated and characterized, but like microcystins, anatoxins, and paralytic shellfish poisons (PSP), it is possible that more congeners of cylindrospermopsin exist (Hawkins et al., 1997). This may explain the varying toxic potencies from different blooms.

Two deoxycylindrospermopsin congeners isolated from *C. raciborskii* were administered intraperitoneally (i.p.) to three male white Quackenbush mice and found to be nontoxic at a concentration of 0.8 mg/kg after 5 days (Norris et al., 1999). The amount of deoxycylindrospermopsin found in cells was 0.02% of the mass of lyophilized *C. raciborskii* and the purity was estimated to be about 80%. Based on the comparison of the toxicity of deoxycylindrospermopsin and cylindrospermopsin (~0.2 mg/kg) and their relative abundances in cells, it is unlikely that deoxycylindrospermopsin contributes significantly to the toxicity of cylindrospermopsin (Ohtani et al., 1992; Norris et al., 1999).

A relatively nontoxic deoxygenated analog of cylindrospermopsin, in which the hydroxyl group on the uracil bridge (C-7) has been removed, was isolated in all samples from *C. raciborskii* and *A. ovalisporum* (Moore et al., 2000). When the toxicity of cylindrospermopsin was compared to that of the deoxycylindrospermopsin analog, it was suggested that the presence of the hydroxyl on the uracil bridge or perhaps the keto-enol status of the uracil moiety is critical for the hepatotoxic action of cylindrospermopsin (Norris et al., 1999).

The accumulation of fat after administration of cylindrospermopsin mimics that of another xenobiotic, carbon tetrachloride (CCl_4), and the formation of fat droplets in hepatocytes in the central portion of hepatic lobules could be attributed to free radicals generated in the injury (Terao et al., 1994). An exotoxin produced by *Nocardia otitidiscaviarum*, HS-6 [1-((3--(aminocarbonyl)pyridinio)methoxymethyl)-2-((hydroxyimino)methyl)pyridinium dichloride], attacks the rough surface endoplasmic reticulum like cylindrospermopsin and induces single cell necroses in the myocardium, and proximal and distal convoluted urinary epithelium (Terao et al., 1992; cited by Terao et al., 1994). Furylfuramide, a compound used as an anti-microbial food additive in Japan until 1974, when given to rats (0.1% of the diet) resulted in a marked increase in liver weight, and a concomitant reduction of microsomal mixed-function oxidase activity attributed to decreases in heme and cytochrome P450 (Terao et al., 1994).

Table 6. Cytotoxicity and Effects of Cylindrospermopsin on Protein Synthesis

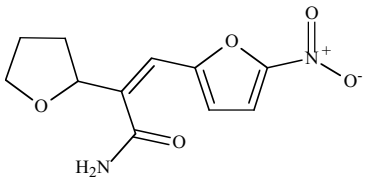
Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
Hepatocytes isolated from male Sprague-Dawley rats	*C. raciborskii*/Palm Island, Australia	Purified cylindrospermopsin extract	The release of lactate dehydrogenase (LDH) into the medium after cell lysis was used as the indicator of *in vitro* toxicity. Glutathione (GSH) and LDH concentrations in the medium were determined 20-22 h after initial plating. Cells were incubated in the presence of air (95%) and CO_2 (5%). Incubation of hepatocytes with 10-100 M cylindrospermopsin for 18-20 h resulted in complete cell death. The toxicity window of cylindrospermopsin is very narrow as determined by the release of LDH (39±4%, 20% in controls) when cells were incubated with 3.3 M cylindrospermopsin for 18 h and 6±7% LDH released when cells were incubated with 5 M cylindrospermopsin for 18 h. No cell lysis was detected as LDH when cells were incubated with 5 M cylindrospermopsin for 12 h; however, a significant increase of LDH (40±5%) was seen when cells were incubated for 16 h. The pronounced inhibition of GSH by cylindrospermopsin was dose dependent. Cylindrospermopsin (1.3 M) caused a 50% drop in cell GSH after incubation for 18 h, but was even more dramatic after incubation for 18 h with 5 M cylindrospermopsin (87.5% GSH inhibition). Lowering cell GSH predisposed the cell to cylindrospermopsin toxicity. The GSH reduction might be attributed to conjugation with metabolites or toxic peroxides, or by decreased GSH synthesis. Cylindrospermopsin was directly cytotoxic resulting in the lack of hepatic cord structures and a rounded appearance.	Runnegar et al. (1994)
Cultured hepatocytes from male Sprague-Dawley rats	*C. raciborskii*/location of isolation n.p.	Purified cylindrospermopsin extract (2.5 and 5.0 M)	The release of LDH into the medium after cell lysis was used as the indicator of *in vitro* toxicity. GSH and LDH concentrations in the medium were determined 12 h after initial plating. GSH efflux, fate, and activity, uptake of methionine, as well as cystein concentrations in the hepatocytes were also measured. Cylindrospermopsin was found to result in a concentration-dependent decrease in intracellular GSH, but without any significant increase in toxicity (LDH in medium).	Runnegar et al. (1995)

Table 7. Cytotoxicity and Effects of Cylindrospermopsin on Protein Production (Continued)

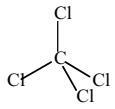
Species/Strain	Source of Cylindrospermopsin/ Location	Dose	Summary	Reference
Wistar rat liver hepatocytes (strain n.p.)	*C. raciborskii* (AWT 2 05/1)/Australia	6.25-3200 ng cylindrospermopsin/mL	Ten thousand viable hepatocyte cells and 5,000 ketone body cells were added to 50 L of medium. Final concentrations of cylindrospermopsin were between 6.25 and 3200 ng/mL. Plates were incubated for 24, 48, and 72 h. Viability of cells was determined by using formazan absorbance. The LD_{50} of cylindrospermopsin in hepatocytes was 50 ng/mL after 72 h and 200 ng/mL after 48 h. The LD_{50} of cylindrospermopsin in ketone body cells was 2000 ng/mL after 72 h and 200 ng/mL after 48 h.	Shaw et al. (2000)
6 ICR mice (M, 4-wk-old)	*U. natans*/location n.p.	0.2 mg cylindrospermopsin/kg bw	Four mice were administered normal saline and used as controls. The total protein content of hepatic microsomes from ICR mice dosed i.p. with 0.2 mg/kg of cylindrospermopsin extract (11.2 ± 1.2 mg/g liver) was much less than the total protein content in livers of control mice (16.6 ± 1.3 mol/g liver). The difference between the phospholipid concentration in liver after cylindrospermopsin administration (3.0 ± 1.1 mol/g liver) when compared to control mice mg/g liver and (3.8 ± 0.4 mol/g liver) was not as great as the difference in protein content. There was also a significant decrease in hepatic P450 of mice dosed with cylindrospermopsin (4.6 ± 2.8 nmol/g liver) when compared to controls (14.4 ± 2.8 nmol/g liver)	Terao et al. (1994)
Rabbit reticulocyte cell-free system	*U. natans*/location n.p.	48 ng/mL, other doses were n.p.	The inhibition of globin synthesis by cylindrospermopsin was studied. Globin synthesis was completely inhibited by cylindrospermopsin at a concentration of 48 ng/mL. This evidence reaffirmed the inhibition of protein synthesis observed in hepatic microsomes of mice.	Terao et al. (1994)

Abbreviations: AWT = Australian Water Technology; bw = body weight; GSH = glutathione; h = hour(s); LDH = lactate dehydrogenase; n.p. = not provided

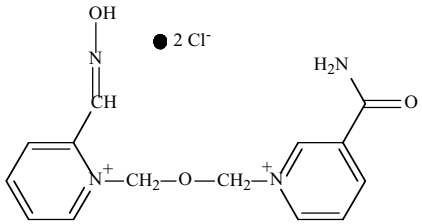
Figure 1. Some Compounds That Have Similar Biological Activity as Cylindrospermopsin



Furylfuramide



Carbon Tetrachloride



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11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

STN International Files

AGRICOLA	EMBASE	TOXLINE
BIOSIS	MEDLINE	
CA	NIOSHTIC	
CABA	NTIS	
CANCERLIT	Registry	

TOXLINE includes the following subfiles (which do not always have all the records in the

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC¤	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

In-House Databases
CPI Electronic Publishing Federal Databases on CD
Current Contents on Diskette¤

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APPENDIX A: UNITS AND ABBREVIATIONS

°C = degrees Celsius
µg/L = microgram(s) per liter
µg/m^3 = microgram(s) per cubic meter
µg/mL = microgram(s) per milliliter
µM = micromolar
ACGIH = American Conference of Governmental Industrial Hygienists
atm = atmosphere
AWT = Australian Water Technology
bw = body weight
d = day(s)
DOT = U.S. Department of Transportation
ELISA = enzyme-linked immunosorbent assay
EPA = U.S. Environmental Protection Agency
F = female(s)
g = gram(s)
g/mL = gram(s) per milliliter
GC = gas chomatography
GV = guideline value (for exposure)

h = hour(s)
HPLC = high-performance liquid chromatography
i.p. = intraperitoneal(ly)
i.v. = intravenous(ly)
kg = kilogram(s)
L = liter(s)
LC_{50} = lethal concentration for 50% of test animals
LD_{50} = lethal dose for 50% of test animals
lb = pound(s)
M = male(s)
mg/kg = milligram(s) per kilogram
mg/m^3 = milligram(s) per cubic meter
mg/mL = milligram(s) per milliliter
mL/kg = milliliter(s) per kilogram
mm = millimeter(s)
mM = millimolar
mo = month(s)
mol = mole(s)
mol. wt. = molecular weight
MS = mass spectrometry
NA = not applicable
NIEHS = National Institute of Environmental Health Sciences
nm = nanometer(s)
NOAEL = no observable adverse effect level
n.p. = not provided
PCR = polymerase chain reaction
s = second(s)
TDI = tolerable daily intake
TLC = thin-layer chromatography
UV = ultraviolet (light)
wk = week(s)