TOXIC ALGAE IN WISCONSIN LAKES

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INTRODUCTION

As early as 1878, recorded deaths of domestic animals were ascribed to "water bloom" poisoning. Francis (1878) gave the first accurate account of death in domestic animals resulting from consumption of algae laden water from Lake Alexandrina near Adelaide, Australia. He described the abundant growth of algae as ".....a thick scum like green oil paint some two to six inches thick and as thick and pasty as porridge .....". Wind conditions had caused shoreline accumulations of algae. Sheep died in one to six hours, horses in eight to twenty-four hours, dogs in four to five hours, and pigs in eight to twenty-four hours. Toxicity symptoms were stupor, unconsciousness, prostration, convulsions, and evident spasms under certain conditions. The dominant alga was identified by Francis as Nodularia spumigena Mert.

In general, toxic algae blooms have resulted when wind conditions have concentrated blue green algae (Cyanophyta) along windward areas of surface waters. However, not all blue-green algae produce toxins. Ingram and Prescott (1954) concluded that six species of Cyanophyta are known to contain toxin. These are Microcystis aeruginosa and Microcystis flos-aquae, Nodularia spumigena, Coelosphaerium Kuetzingianum Näeg and Coelosphaerium Naegelianum Unger, and Gloeotrichia echinulata (J.E. Smith). Pigments of blue-green algae unlike other groups are not in chloromatophores, but are in solution and confined to the peripheral portion of the cell. Pigments include chlorophyll-a, carotenes, distinctive xanthophylls, a blue pigment called c-phycocyanin, and a red pigment, c-phycoerythrin. The blue-greens have a primitive nucleus called the central body, which

lacks a nucleolus and a nuclear membrane. This makes the Cyanophyta similar to bacteria.

Prescott (1968) refers to the Cyanophyta as "the most ancient of all chlorophyll-bearing organisms on earth". This phylum dates back to the Archaeozoic era of more than 2,000,000,000 years ago. Fuller and Tippo (1954) report there are 1,500 genera and 17,400 species. Much like the bacteria, the blue-green algae have the ability to withstand temperature extremes. Their gelatinous sheath helps them withstand long periods of desiccation and has earned them the name "slime algae". Internally, their protein molecule bonding and compactness aid the cells in withstanding these extremes.

Blue-green algae are solitary or colonial. The cells can be round, oval, globular or lamellated in shape. The majority are in a trichome but unbranched, false branched, and true branched filaments also occur.

ALGAE AND MAN

Algae's relationship with man is both beneficial and detrimental to his welfare and are the basic link for conversion of the inorganic nutrients into organic matter. Algae may be useful in oxidation ponds used for secondary sewage treatment. In these ponds complex organic compounds rapidly oxidize into substances such as carbon dioxide and ammonia. Photosynthesis supplies the oxygen to this system up to three or four times the saturation level. This supersaturation takes place only during the daylight hours. As the organic wastes are changed

or stabilized some nutrients are used by the algae and aerobic bacteria for growth and reproduction. This system is ideal for small communities in mild climates where algae can grow all year. Advantages are minimal odor, little maintenance, and low operational cost compared to other secondary sewage treatment processes. In addition, a portion of available nutrients are bound up by the growing algae before discharge, thus lessening further down stream eutrophication.

Algae can benefit man through its usage in assaying biological substances and by its poisonous nature in cancer research and as a future source of food. Marine algae serves as a food and supplements the diet of maritime people. Except for Nostoc (Wood, 1967) which is eaten in the Fiji Islands by the natives, fresh water algae have not yet been developed for use as human food. It is however used to feed domestic animals.

The detrimental role played by algae is extensive and diversified. From the medical standpoint, human ailments such as skin allergies and sensitization, digestive complaints, and pulmonary diseases are caused by algae. Algae can also poison domestic animals, ruin recreational sites, kill fish either with toxin or by suffocation (Red Tide, e.g.), and kill humans that eat toxic shellfish or ciguateric fish. In addition, algae can spoil domestic and industrial water supplies and clog filters in water treatment plants.

In the natural environment, algae also plays a beneficial and detrimental role. Carbon dioxide removal and oxygen production during daylight is a benefit of photosynthesis. This plant process thus keeps

the gaseous biological balance between plants and animals. Carbon dioxide is continually produced by respiring algae, but during active photosynthesis more oxygen is produced than carbon dioxide. At night or on cloudy days when photosynthesis stops, oxygen production stops also.

Algal growths are important because they constitute a source of food for aquatic fish-food organisms. However, when algae over produce to a condition of bloom, anoxic conditions can develop in an entire body of water. Death and decomposition of a dense algal concentration depletes the dissolved oxygen and kill fish by suffocation. This triggers a series of events which ends in the death of most aerobes in the water. Death of higher animals in the food chain, i.e. aquatic vertebrates and invertebrates, leads to increased bacterial decomposition which accordingly lowers the oxygen content still further. Algae, therefore, can even destroy themselves. Dense algal blooms develop during the summer months when the water is warm and its oxygen content is low compared to saturation levels of cold water. Prescott (1968) states that during the hours of darkness, photosynthesis halts and the continuous respiration of the algal mass leads to oxygen tensions of 0.3 ppm or less.

Algae can be detrimental to the aesthetic value of a body of water also. Algae may congregate at or near the water surface and impart a green, yellow, red or blue green color to the water. Extensive growths of microscopic algae which blanket the surface of a lake, pond, or stream are often called "Wasserbluthe" in Germany, "Vasserbloom" in Holland and "waterbloom", "scum" or "paint pot" in North America. The

latter terms apply to the blue-green algae which make recreational waters less desirable for swimming, boating, water skiing, and general usage. Dense growths of algae may limit the photosynthetic activity to only a few centimeters beneath the water surface. Shading the bottom muds may prevent germination and growth of practically all rooted submergent and emergent aquatic plants. As a consequence an important food source for water fowl is eliminated.

PUBLIC HEALTH

Blue-green algae can affect public health in various ways including medical, public water supply treatment for toxin, taste and odor, and recreation. Cohen and Rief (1953) reported skin and respiratory allergies may be produced in humans who consume algae laden water while swimming. They referred to a 1950 case of "erythematons papulovesicular contact dermatitis" in a four-year-old child who bathed in Lake Carey, Pennsylvania. The lake contained c-phycocyanin, the blue pigment of Anabaena. Cohen and Reif also reported the development of skin irritations with similar symptoms to "swimmers' itch" in swimmers during 1953 in Pennsylvania. An unidentified blue-green algae was theorized the causative agent of swelling and itching skin along with redness of the eye conjunctivae. Heise (1949) reported two cases of "systemic allergic eruption" caused by Oscillatoracae, experienced by people swimming in Wisconsin lakes in 1934-35 and 1945-46: 1.) a 57 year-old man swimming in North Lake and Muskego Lake, Waukesha County, developed itching eyes, conjunctivitis, blocked nasal passages, and bronchial asthma; 2.) a 39 year-old woman swimming in Lake Keesus, Waukesha County, developed swollen eyelids,

blocked nasal passages, itching hives, and welts. Heise (1951) also reported on many other positive skin reactions to Microcystis by his office patients.

Human gastric disturbances can also be attributed to drinking blue-green algae infested waters. Several genera which have been linked to this problem are: Anabaena, Anacystis, Aphanizomenon, Coelosphaerium, Gloeotrichia, Microcystis, Nodularia, and Nostoc. Schwimmer and Schwimmer (1955) characterized the symptoms of gastro-intestinal disorders arising from drinking water as diarrhea, abdominal pain, nausea, and vomiting. Ingram and Prescott (1954) reported outbreaks of human gastroenteritis which have not been positively traced to algae, although it has been implied in the literature, may be due to contaminated drinking water. No bacteriological evidence, however, supports this hypothesis. Tisdale (1931) stated the following in reference to a human gastroenteritis outbreak in Charleston, West Virginia, where 8,000 to 10,000 people were affected.

> "Bacteriological tests indicate that measured by the present accepted U.S. Treasury standards for bacteria counts, the water was safe. Microscopical tests during August and September revealed growths of blue-green algae of several different types capable of causing the objectional odor. Cycles of algae growth were followed by development of crustacea, daphnia, and other allied water flea organisms being present in such great numbers that a glassful of the raw river water appeared to be actively vibrating from the infinite number of organisms present. Whether the intestinal disorders in the city during the latter part of October and in early November can be attributed to the water is still a mystery, but it is a fact that widespread disorders were noted wherever the water supply mains went."

Human symptoms included diarrhea and symptoms generally associated with stomach flu. Tisdale (1931) further stated:

> "Whether products of anaerobic decomposition of nitrogenous

> or carbonaceous matter could be sufficiently concentrated in the water has not been shown. Neither the U.S. Public Health Service nor the State Health Department of West Virginia has stated definitely that the bacteriologically safe water supply was responsible for the epidemic, but it is certain that most unusual conditions have existed with reference to intensity of raw water pollution."

Wheeler et al. (1942) stated that humans would seek other drinking water sources before they would consume enough algae-laden water to be harmful, because of tastes and odors associated with algae in water. Gorham (1960) agreed with Wheeler et al. that humans would unlikely consume enough water for toxic effects to develop. He concluded that associated bacteria - rather than algae - may be the direct cause of gastro-intestinal disorders in humans. Hammer (1968) emphasized the small possibility of drinking algae toxins in a public water supply which has a surface water source i.e. Great Lakes. Water inlets are well off shore and below the water's surface where algal cells accumulate along with possible toxins.

Various authors disagreed on the effects water treatment procedures have in eliminating algal toxins in a public water supply. Gorham (1964) reported water treatment procedures inactivate any toxins which may be present. However, Ingram and Prescott (1954) along with Palmer (1960) indicated algal toxins may survive laboratory treatments utilizing precipitation processes which include alum coagulation, filtration, and chlorination, equivalent to public water treatment. They also reported algal toxins may survive carbon filtration treatment after the toxins are treated with large amounts of Norite A, a common chemical used in water treatment.

Blue-green algae also secrete oily substances which produce undesirable tastes and odors in public drinking water supplies. Prescott (1968) named Microcystis and Aphanizomenon along with certain diatoms as the culprits. The highly proteinaceous nature of these algae cause very foul odors to develop upon decomposition of the cells. Bodies of water described as "smelling fishy" is caused by oil-producing algae in the water rather than fish. Schwimmer and Schwimmer (1955) give four general causes of odors and tastes in water supplies. These were oils of living algae, products of decomposition, products of active photosynthesis, and death of fish and other aquatic organisms caused by toxins or suffocation. Products of decomposition included such compounds as hydrogen sulfide, methane, ethereal sulfates, nitrogenous substances, along with other volatile gases.

Toxic algae is a potential problem to water skiers, swimmers, and others who may come in contact with surface waters containing toxic algal scum. Hammer (1968) reported swimmers who ingested algae became ill. No toxic fatalities were reported. He stated that surface water accumulations of algae should definitely be avoided by humans if toxic conditions are suspected. The algal cells can release their endotoxins at any time after cell lysis which can be caused by high light intensity or bacterial action. This happens near shore where algae accumulates. Hammer suggested the use of algicides on small bodies of water only, because marginal treatments on larger lakes are of a temporary nature due to wind action. Precautions should be taken for at least twenty-four hours after chemical treatment of toxic algae, because the toxin is released to the surrounding water upon cell lysis while the cells

sink into the profundal zone.

DOMESTIC AND LABORATORY ANIMAL TOXICITY

Records show cattle, sheep, hogs, horses, and fowl have been victims of toxins produced by blue-green Anabaena, Aphanizomenon, Coelosphaerium, Gloeotrichia, Microcystis, Nodularia, and Nostoc. Since 1878, there have been many references to domestic animal death attributed to drinking algae-contaminated water in the United States, Canada, South Africa, Australia, and Germany.

Fitch et al. (1934) reported the first toxic algae experiment was conducted on a dog in 1918. The dog was fed an algal bloom containing Anabaena flos-aquae and Coelosphaerium Kuetzingianum from Oakes Lake, Windom, Minnesota, resulting in death to the dog. These same investigators examined the deaths of domestic animals including turkeys, ducks, geese, cattle, pigs, horses, and chickens caused by toxic algae blooms of Microcystis flos-aquae, Anabaena flos-aquae, and Aphanizomenon flos-aquae between September and October, 1933. They observed in detail the effect of similar blooms by injecting algal cells into laboratory animals i.e. mouse, guinea pig, rabbit, chicken and pigeon. Chronic spasms, restlessness, coughing and salivation, tear production, and hind limb weakness symptoms were noted before death. Foamy-white tears were produced in guinea pigs.

Wheeler et al. (1942) used mice and guinea pigs in their laboratory experiments with the toxic alga, Microcystis aeruginosa, but were unable to observe the snydrome of foamy tears. Using a fresh culture of M.

aeruginosa they tried oral feeding experiments with no success. Intraperitoneal injections (0.25 ml of filtrate) caused mouse death within 16 to 36 hours. Intraperitoneal injections of 2.0 ml dosages were needed to cause death in a guinea pig. A 5.0 ml injection into a rabbit produced no effects. Since Fitch's and Wheeler's work on laboratory animal toxicity, Olson (1960) like Fitch et al. has observed "the syndrome of foamy, white, fat-staining tears" in mice which were injected with a lethal dose of toxic algae, Microcystis aeruginosa. Olson also agreed with Wheeler et al. on the time of death for various laboratory animals injected with toxic Microcystis.

Another reference on domestic animal deaths in the United States was made by Brandenburg and Shigley (1947). They reported on livestock poisoning due to drinking blue-green algae-contaminated water from Upper Des Lacs Lake, North Dakota. Numerous other accounts of toxic blooms and animal poisoning are in the literature.

Many Canadian studies i.e. Gorham (1960) and Hammer (1968), have reported Aphanizomenon, Anabaena, and Microcystis toxins as causative agents for death of livestock in Canada. Death usually came shortly after consumption of the water-algae combination. Aphanizomenon was found most often among the three genera but was not the major cause for toxicity.

FISH TOXICITY

The literature has cited many instances of fish deaths associated with toxic blooms of algae. Prescott (1939) commented on fish deaths

and toxic blue-green algae by saying:

> ".....it is apparently possible for algae to bring about the death of fish through the liberation of substances toxic to them during the decay process. When highly proteinacious blue-green algae undergo decay, sufficient quantities of hydroxylamine and other derivatives are produced to poison any fish caught in the shallow water of a bay by masses of decaying algae."

Prescott (1948) reported fish deaths caused by the toxic action of decomposing Aphanizomenon flos-aquae (l.) Ralfs. Experimental data revealed the fish were killed by an endotoxin and not low dissolved oxygen, as oxygen tests showed 4 to 6 ppm concentrations present. In one experiment crappies (Pomoxis nigo-maculatus L.S,) and perch (Perca flavescens Mitchell) were placed in an aquarium containing toxic Aphanizomenon and 8.0 ppm oxygen concentration. Resulting death ruled out suffocation. All the fish except one crappie (Pomoxis nigo-maculatus L.S.) were killed in another experiment using 20 gallons of decomposing Aphanizomenon flos-aquae in aquaria. All fish involved (crappie, Pomoxis nigro-maculatus, imported gizzard shad, Dorosoma cepedianum L.S., golden shiner, Notemigonus crysoleucas Raf., orange-spotted sunfish, Lepomis humilis Gir., fathead minnow, Pimephales p. promelas Raf., blue gill, Lepomis macrochirus Raf., and buffalo Megastomatobus cyprinella Vol.) were killed within six hours. Another experiment using five gallons of decaying Aphanizomenon flos-aquae in an aquarium containing a satisfactory dissolved oxygen concentration produced dead fish within five hours. Sheephead, Aplodinotus grunniens (Raf.); minnow,Hyborhynchus notatus Raf.; perch, Perca flavescens (Mitchell); bullhead, Amerinus m. melas Raf.; pumpkinseed, Lepomis gibbosus L.; and carp, Cyprinus carpio L. were used in this experiment.

Prescott (1948) used small ponds instead of aquaria in a fourth experiment and obtained similar results. Dissolved oxygen levels at no time were low enough to cause fish suffocation. The ponds located on the shores of Storm Lake, Iowa, were filled with water from the lake which contained a heavy concentration of Aphanizomenon flos-aquae. The decaying algae in the shallow water killed all fish, i.e. crappie (Pomoxis nigro-maculatus), carp (Cyprinus Carpio) and bullhead (Amerinus m. melas) within two hours after their introduction. From these experiments plus chemical data obtained, Prescott found "hydroxylamine", a poisonous protein decomposition product, and hydrogen sulfide (8.5 ppm) present in sufficient amounts to kill fish. Actual pond concentration of hydrogen sulfide was theorized to be much higher because gas was lost during the distillation procedure.

Gorham (1960) reported fishkills may be caused by oxygen depletion in the water as well as the release of toxic amines during algal cell decay. He referred to the presence of the fast death factor when carp were injected intraperitoneally with non decomposing Microcystis aeruginosa cells. He reported fish may be susceptible to other factors beside anoxia and amines.

Hammer (1968) reported a large number of perch were killed by an Anabaena flos-aquae (L.) Ralfs bloom on July 3-9, 1961 on Lake Burton Saskatchewan. Oxygen concentrations were adequate for fish life during the mortality period. He reported the fish kill was due to Gorham's fast death factor. Midge fly larvae, Chironomus spp. were observed to be "very green in color" in the lake during this period. Hammer

hypothesized the perch ate the midge larvae which contained the toxin given off to the water by the blue-green algae. He reported Anabaena flos-aquae caused most toxic algae cases in Saskatchewan. Microcystis aeruginosa was the causative agent in only a few cases. Hammer placed emphasis on weather conditions i.e. wind direction and velocity during the time of toxic bloom conditions. Calm weather with no wind causes algae to surface and accumulate in large floating masses while light wind action accumulates algae along shoreline and bay areas. Cell decomposition releases the algal toxin resulting in fish mortality.

FOWL TOXICITY

Through the years, the literature has referred to duck and goose deaths resulting from toxic blue-green blooms. Hammer (1968) documented the effects on fowl for the Saskatchewan lakes. He cited two significant causative species, Anabaena flos-aquae and Microcystis aeruginosa. In 1951, turkeys, geese and chickens died from drinking algae-laden water from Duck and Baptiste Lakes at Edmonton, Alberta. In 1949, 1950, and 1951 wild ducks were killed by algal blooms on Whitewater Lake, Manitoba, consisting of Microcystis aeruginosa and Aphanizomenon flos-aquae. Hammer noted Clostridium botulinum was also present in the injected samples. In 1959, one goose died at a Saskatchewan resort as a result of drinking water containing Aphanizomenon flos-aquae and Anabaena flos-aquae. In 1961, a wild duck died from drinking water containing blue-green algae and botulinum bacteria. Hammer concluded blue-green algae along with botulinum bacteria can cause "duck sickness" symptoms. Hammer reported algal injections of mice along with botulism studies can be used to find

the "true cause of duck sickness". Earlier Bossenmaier et al. (1954) purposed the same hypothesis.

Gorham (1960) found in his Canadian studies of 1959 that ducks were not killed by toxic strains of Microcystis aeruginosa. Meanwhile in the United States Olson (1964) and Bossenmaier et al. (1954) were working on the effect of toxic algae on ducks. Ducks treated with a toxic Anabaena died within one to two hours. Observed symptoms were similar to symptoms of "duck sickness". "Lumberneck" symptom caused by botulinus poisoning was also present before death. Bossenmaier et al. used botulinus antiserum on the affected ducks which failed to counteract the poison effect.

Kalbe and Tiess (1964) reported on the death of 400 ducks at a duck farm in Rudgen, Germany in July 1963. A bloom of Nodularia spumigena Mertens (var. vacuolata, F.E. Fritsch) covered the drinking water supply causing toxic conditions and death. The chemical, biological, and toxicological aspects of the bloom were examined. They concluded algae decomposition products containing toxin were the causative agent of death.

Olson (1964) emphasized the effect of algal toxins on wild birds and waterfowl when he stated that if "a toxic strain becomes predominant in a water bloom, hundreds of birds may die in a few hours. Then any living creature that drinks the water is a potential victim, and shorelines may be stewn with bodies of mammals, land birds, and waterfowl". Olson ran controlled tests to find the time of death and generally found the dosage size was proportional to death time. Four teaspoonfuls of unconcentrated Anabaena lemmermanni suspension killed a great blue heron

in 14 minutes. Olson further stated, "Extensive algae blooms are potentially dangerous to waterfowl, especially where the principal component is Anabaena flos-aquae or Anabaena lemmermanni,..... To forestall waterfowl losses it would be desirable to keep surface waters free of heavy algae growths."

Konst et al. (1965) also experimented with bioassay animals under laboratory conditions. Toxicity tests on Microcystis aeruginosa were run on mice, guinea pigs, rabbits, chickens, ducks, calves, and a lamb. Greater oral dosages were needed to kill the larger animals compared to the smaller lab size animals by a factor of 3 to 5 times on an equivalent weight basis. Ducks and chickens were found to require four times higher oral dosages for lethal effects than any other animal studied. Up to 16 g/kg of body weight was needed to obtain results and both showed no toxic effects till six hours after feeding. Toxic symptoms observed were quiet behavior and complete paralysis before death. Konst et al. differed from Bossenmaier's et al. (1954) and Hammer's (1968) conclusions given above by claiming "duck sickness" was "probably not" the result of Microcystis aeruginosa toxin.

It seems likely work in the future will show advances in settling the difference in opinion on duck sickness causation, whether it be algal toxin, botulinum bacteria or a combination of both. The resistance to toxin by fowl, compared to other animals, requires further study in the area of physiological make up.

MARINE TOXICITY

Ichtyotoxism

Although animal toxins are widely distributed throughout the marine environment, they are most abundant in tropical and subtropical waters. Halstead (1968) lists such marine life as sponges, hydroids, jellyfish, corals, mollusks, worms, arthropods, starfish, turtles, mammals, and fish as being toxic. Mosher et al. (1964) refers to these animal toxins as being 3,000 times more potent than the best synthetic war gases man has at present and 10,000 times more potent than sodium cyanide.

At present many questions are left unanswered about marine toxins. Many studies have been made on "ichtyotoxism" which is caused by ciguatoxic and clapeotoxic fishes (Halstead et al., 1954, Halstead, 1959, 1964, Russell et al., 1968, Banner et al., 1958, 1960, and 1963, and Barnes, 1969). The unpredictable nature of the occurrence of poisonous fish and their enormous phylogenetic spread is not fully understood. The external appearance of the fish presents no evidence to its toxic nature. Halstead (1968) and Randall (1958) noted ciguatera fish usually developed around sunken ocean ships and islands in the ocean. Thus ciguateric fish are localized in small sectors rather than large areas in the ocean. Randall hypothesized these small areas developed toxic blue-green algae which were eaten by the herbivorous fish. These fish became toxic along with larger predaceous fish which fed on the toxic herbivors. Sunken ships play an initial role in the entire chain of events by creating a

new substrate for toxic algae to grow on. Halstead et al. (1963) and Halstead (1968) indicated from food chain studies that toxic blue-green algae (Lyngbya and other related genera) were responsible for producing ciguatera toxin or biotoxic precursor substances. This phenomenon developed in a series of steps in which "each trophic level ingests the poisons that are found in the flesh or viscera of the level of organism beneath it". Man becomes poisoned by eating large toxic fish such as reef fishes which are high on the food chain. The large toxic fish contain accumulative poisons that have resulted from the total feedings of all the lower trophic food levels reaching down through the food chain to the initial toxic algae. Halstead (1968) referred to unanswered questions relating to the elemental requirements for algae to become toxic and whether these substances could be artificially supplied. At present these answers are unknown. Randall (1968) reported toxic algae cells must be fine in size because certain toxic surgeonfishes, Acanthurus triostegus, can not feed on course cell types. Randall (1958), Banner et al. (1960), and Helfrich et al. (1963) have reported surgeonfish are selective in their food habits. It was concluded Acanthurus triostegus were less likely to become toxic because they usually avoid blue-green algae in lieu of certain preferred red and green algal species. Clenochaetus sp. were found to be nondiscriminating in their food habits and consistently more toxic. Naso sp. tended to feed on course algae only and were found to be least toxic of all surgeonfishes studied.

In general, the chemical nature of various ichtyotoxins are unknown and research is needed to determine their exact identities. Halstead

and Bunker (1954), Halstead and Mitchell (1963), and Russell (1965) reviewed various toxins from a variety of marine fishes including the sting ray, horned sharks, catfishes, scorpionfishes and toadfishes. Venom extracts from each fish type were prepared and injected intravenously, intraperitoneally and subcutaneously into test mice, guinea pigs, rabbits, rats, and fowl for observation of poisoning symptoms. All three types of injections resulted in muscular spasms, respiratory distress, and eventual death. Venom from the scorpionfish contained considerable quantities of protein or protein-like material which was nondialyzable and heat stable (Halstead and Mitchell). Potency of the poison after one year storage at -20° C was excellent. Hypertension, increased respiratory rate, and heart injury were symptoms noted in injected rabbits. Venom from the sting ray especially caused auricle and ventricle malfunctions. Toadfish venom caused convulsions and paralysis.

Fogg (1966) worked with an algal toxin produced by Prymnesium parvum (Chrysophyta). He found the toxin had characteristics somewhat like those found by Halstead and Mitchell in their ichtyotoxin work. The toxin was nondialyzable, poorly water soluble and thermo-unstable. Research on the toxin proved its proteinaceous nature corresponded with the scorpionfish toxin. Fogg found co-factors such as spermine and other polyamines greatly increased the toxin's activity. Magnesium and calcium ions produced increased activity while sodium ions inhibited activity. The toxin was inactivated when it was maintained at an alkaline pH (7.0-9.0).

Banner (1967) concluded that several ciguateric fish toxins were similar in nature, chemical structure and pharmocological activity to blue-green toxin. He also correlated the presence of toxic fish in the vicinity of the Hawaiian and Gilbert Islands to growth distributions of toxic Schizothrix calcicola Agardh, a blue-green species. He reported the distribution of toxic fish corresponded to the appearance of the algal species around the islands. Banner chemically isolated one important ciguateric fish toxin which he called "anticholinesterase". This compound has yet to be identified structurally and studied in pure form for its pharmacological properties. Banner showed the toxin was neuro in character and measured it quantitatively as a function of the inhibition of cholinesterase activity. Mouse injection bioassays were used for observing toxic symptoms. Rise and fall in blood pressure, increased rate and depth of respiration, bronchial constriction and painful breathing were usual symptoms before death. He concluded death was caused by neuro-respiratory failure.

Banner (1967) also experimented with toxin extraction and identification from the alga Schizothrix. Upon extraction two groups of toxins were obtained, a water soluble group and a polar benzene soluble group. After attempted purification procedures, Banner found the water soluble group yielded an impure mixture which was toxic to mice but showed no inhibition of cholinesterase activity as "anticholinesterase" did. The polar benzene soluble layer yielded two toxins or possibly one toxin plus its degradation product. A very low cholinesterase inhibition activity was observed for these two toxins.

Shellfish Toxism

Toxic shellfish poisoning is another area in the marine environment which closely associates man's health to toxic organisms i.e. dinoflagellates. For more than a century "paralytic shellfish poisoning" has been recognized in Europe and well known along the Atlantic and Pacific coasts of North America (Prakash, 1963, McFarren, 1960 and Quayle, 1969). Shellfish ingest microscopic toxic marine dinoflagellates which do not harm the shellfish per se but make the mollusks toxic for man's consumption. Shellfish toxicity much like ciguatera develops upon consumption of toxic food by the toxic organism. Halstead (1959) listed general physical factors which affect toxic dinoflagellate populations. Seasonal periods (March to November), availability of chemical nutrients in the water, and warm water temperature may cause large populations to develop. Prescott (1968), Halstead (1959, 1965), Wood (1969) and Quayle (1969) signified various geographical locations where the "red tide phenomenon" has occurred. Gulf of Mexico, California and New Jersey coasts, New Brunswick, Nova Scotia and British Columbia coasts, along with European and South African coasts were cited. Several species of toxic dinoflagellates including Gymnodinium brevis are known to cause fish kills along continental shelves. Prescott (1968) refers to this phenomenon as "red tide". Other toxic species i.e. Gonyaulax catenella, G. moniliata, G. polyedra and G. tamarensis are also responsible for causing shellfish paralysis.

Halstead (1959, 1968) and Russell (1965) classified the medical aspects of "shellfish poisoning" into three categories. The gastrointestinal

category developed symptoms in about 10-12 hours in a victimized man. Common symptoms included nausea, vomiting, diarrhea and abdominal pain. Halstead believed bacterial contamination caused this type. The allergic category resulted from man's sensitivity to shellfish and is dependent upon the individual person's make up rather than an actual toxic condition. Typical symptoms included skin redness, swelling, hive-like rash, itching, headache, nasal congestion, abdominal pain, throat dryness, tongue swelling, heart palpetation, and respiratory difficulty. The paralytic category resulted from toxic dinoflagellates which are consumed by shellfish and the concern of this paper. Symptoms in man included tingling or burning sensation of the gums and lips, tongue, and face which spreads to the entire body. Tingling areas became numb and body movement very difficult for the victim. Muscle paralysis increased in severity till death. Additional symptoms to those mentioned above included weakness, dizziness, joint aches, salivation, intense thirst and swallowing difficulty.

As with ciguatera poisoning, no known antidote is now available for shellfish poisoning and the exact identity of the toxin is not known. Halstead (1959) referred to the toxin as water soluble with the dark colored meat containing more toxin than the white. Halstead concluded the dark meat of the shellfish i.e. digestive organs, gills and siphons, came in direct contact with the toxic dinoflagellates and thus able to absorb more toxin than the white muscular tissue could. Sommer et al. (1937) published the first extensive investigation of paralytic shellfish poisoning. A direct relationship between the number of Gonyaulax

catenella Whedon et Kofoid and the degree of toxicity in the mussel Mytilus californianus Conrad was demonstrated. Methods for extracting and assaying the toxin was given as well as a suggested clinical and experimental approach to the problem. This piece of work has been recognized through the years as a guide for many other researchers, Medcof et al. (1947), Schantz et al. (1958), McFarren et al. (1960), Halstead (1965), Prakash (1963) and Quayle (1969). Sommer and Meyer (1937) developed a qualitative test using intraperitoneal mouse injections to demonstrate the toxin potency. They found "an average lethal dose" which they called "mouse unit" killed a 20 gram mouse in 10 to 20 minutes. They concluded after a nine year study "an average lethal dose" varied from 0.017 mg to 60 mg of toxin which was extracted from 3,000 Gonyaulax. Meyer (1953) later modified this method expressing "10 mouse units" per 50 gram of mussel as the amount in 1 ml of supernatant extract which when injected killed a 20 gram mouse in 15 minutes. Meyer concluded the purest preparation obtained from toxic mussels had a toxicity of "4 Sommer mouse units per microgram".

Schantz et al. (1958) working with California mussels and Alaska butter clams obtained high yields of pure toxin. They concluded approximately 0.2 micrograms of purified toxin was equal to "1 mouse unit". A "mouse unit" was defined as the amount of toxin that would kill a 20 gram mouse in 15 minutes with signs of paralysis or respiratory failure. Stability studies revealed the toxin was stable for two years. Toxin characteristics included the following: basic in nature, formed salts with mineral acids, stable in acid solutions but not in alkaline

solutions when exposed to air, very soluble in water and insoluble in fatty solvents and had a molecular formula of $C_{10}H_{17}N_7O_4 \cdot 2HCl$ and a molecular weight of 372. Oxidation of the toxin took place in mild alkaline solutions and toxicity reduction was in direct proportion to the oxygen uptake. Examples of oxidation products formed were guanidoproprionic acid, urea, ammonia, carbon dioxide and guanidine. Schantz et al. compared the mussel poison to those from dinoflagellates and found the toxins similar in properties.

Russell (1965, 1968) indicated the toxin isolated from Gonyaulax catenella was a metabolic product and not the result of a symbiotic relationship between bacteria and the dinoflagellate species. He found isolated dinoflagellates free of bacteria were still capable of producing the poison. McFarren and Bartsch (1960) worked on oral LD_{50} per kg of body weight and concluded the dinoflagellate toxin was one of the most lethal biological toxins known to man. Their figures indicated the human was twice as susceptible to the toxin as a dog or four times more susceptible than a mouse. Prakash (1963, 1967) worked with Gonyaulax tamarensis and reported on a similar toxin which he isolated. The toxin had ichtyotoxic activities and was classified as an endotoxin. Release of the toxin by the dinoflagellate cells resulted from cell lysis of a dense population. Prakash (1967) found bacteria play no direct role in toxin production, but may indirectly affect "toxigenesis" by promoting dinoflagellate growth.

INVESTIGATION OF TOXIC ALGAE IN WISCONSIN

INTRODUCTION

It was not until the summer of 1967 that a toxic algae problem in Wisconsin became apparent. Surface samples from Lake Delton, Sauk County were sent into the State Laboratory of Hygiene for toxin verification following a complaint from a resident living on the lake. A pet poodle died after drinking lake water laden with blue-green algae (Anabaena and Microcystis). An autopsy on the dog revealed that the lower bowel was full of blue-green algae. Algal injections into mice verified the toxicity of the algae in the lake.

The purpose of the present study has been to provide information needed to design a more efficient system of monitoring and predicting danger periods in recreational lakes during the summer months. The relative importance of the environmental factors that regulate the production of toxic algae in particular areas have been and will continue to be explored. This study determines the genera involved in toxicity and the geographic distribution of toxicity. In addition a simple laboratory method for the detection of toxic algae was developed.

METHODS

The methods in this study consisted of three stages: 1.) field collection of algae samples; 2.) extraction and preparation of algal extracts for injection; 3.) mice bioassay for toxin verification.

Field Sampling

Thirty-nine samples of planktonic algae were collected during the summers of 1967 thru 1969 from 20 Wisconsin lakes (Table I and Figure I). These lakes are eutrophic and have a history of blue-green blooms. They were chosen as likely sources for toxic algae.

Samples were collected in bays or along windward shorelines where wind accumulated the algae. When wind conditions were not present during an algal bloom, samples were collected from a boat, long piers, or an accessible outlet. All samples were collected in 250 cc sterilized glass bottles.

Field notes recorded physical conditions present at the time of sampling (Appendix I) -- wind direction, wave action, air and water temperature, weather observations and algal conditions.

Samples were packed in ice within two hours after collection and taken to the Wisconsin Laboratory of Hygiene where they were refrigerated until extracts were prepared for bioassays. Transporting samples from outlying areas delayed bioassays for 72-96 hours. When possible, extracts were prepared on the collection date and injected into mice immediately. Hopefully rapid processing prevented excessive bacterial growths in the samples.

Extraction

At the laboratory samples were microscopically examined and the algae identified. Smith (1950) was used for all identifications. Species were not identified for some genera because good keys were not available.

TABLE I

Toxic Samples

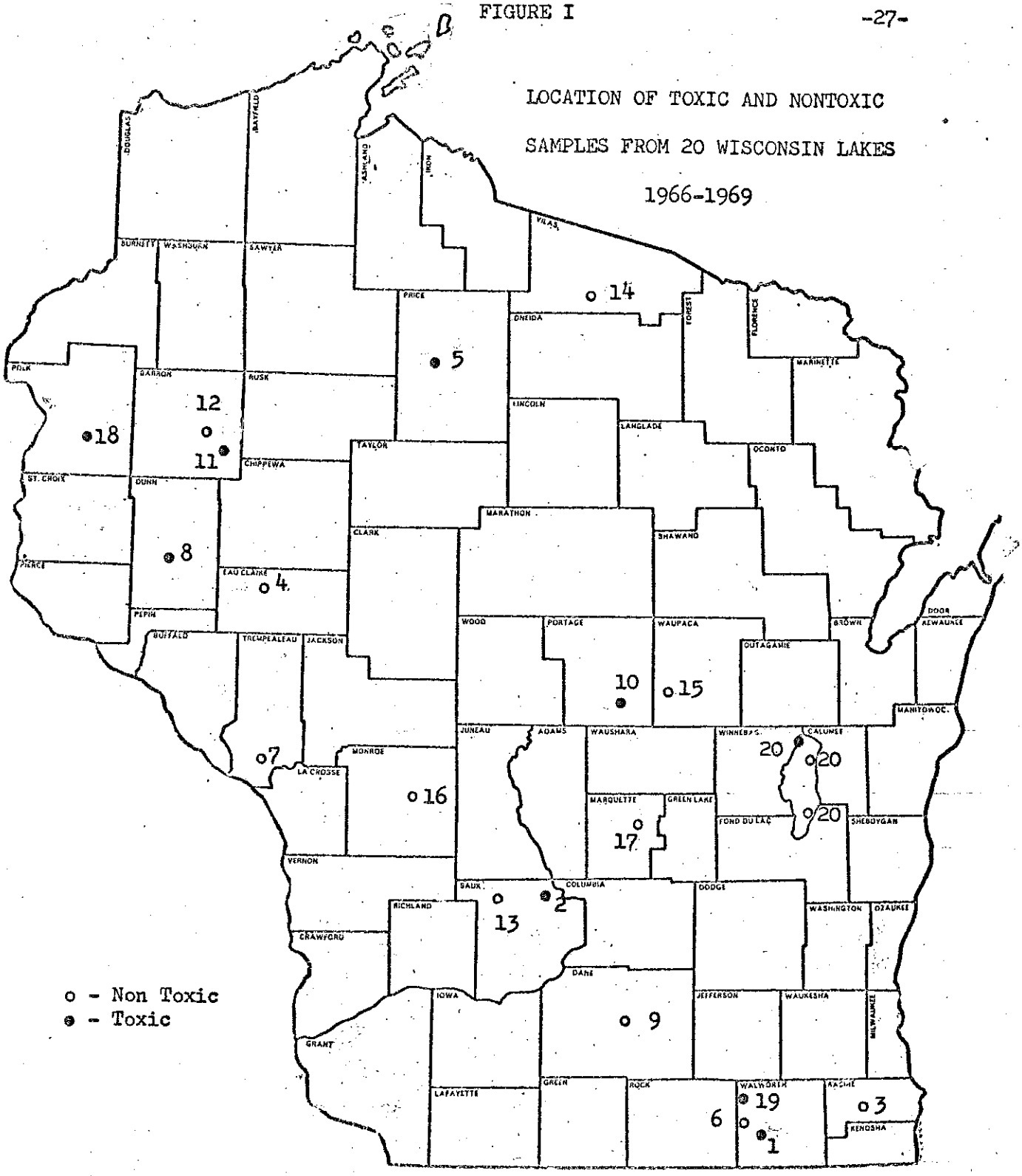
Lake	County	Death Factor Time	Year Bioassayed
Delavan	Walworth	Fast	1968
Delton	Sauk	Very Fast	1967
Long 8/10	Price	Fast	1969
Long 9/10	Price	Fast	1969
Menomin	Dunn	Slow	1969
Pickerel	Portage	Slow	1969
Pokegama 8/11 - 1	Barron	Slow	1969
Wapogasset	Polk	Slow	1967
Whitewater	Walworth	Slow	1968
Winnebago 8/29	Winnebago	Fast	1969
Winnebago 9/5	Winnebago	Fast	1969

NonToxic Samples

Eagle	Racine	1968
Eagle 8/5	Racine	1969
Eagle 8/11	Racine	1969
Half Moon	Eau Claire	1968
Lorraine	Walworth	1969
Marinuka	Trempealeau	1968
Menomin	Dunn	1967
Monona	Dane	1969
Pokegama 8/10	Barron	1967
Pokegama 8/16	Barron	1967
Pokegama 6/19	Barron	1968
Pokegama 7/25	Barron	1968
Pokegama 8/11 - 2	Barron	1969
Pokegama 8/26	Barron	1969
Prairie	Barron	1967
Prairie	Barron	1969
Redstone 7/29	Sauk	1969
Redstone 8/2 -A	Sauk	1969
Redstone 8/2 -B	Sauk	1969
St. Germain	Vilas	1968
Silver	Waupaca	1967
Tomah	Monroe	1969
Tuttle	Marquette	1969
Wapogasset	Polk	1969
Whitewater	Walworth	1967
Winnebago	Calumet	1967
Winnebago	Fond du Lac	1968
Winnebago 8/3	Fond du Lac	1969

FIGURE I

LOCATION OF TOXIC AND NONTOXIC SAMPLES FROM 20 WISCONSIN LAKES 1966-1969



o - Non Toxic
● - Toxic

1 Delavan
2 Delton
3 Eagle
4 Half Moon
5 Long
6 Lorraine
7 Marinuka
8 Menomin
9 Monona
10 Pickerel
11 Pokegama
12 Prairie
13 Redstone
14 St. Germain
15 Silver
16 Tomah
17 Tuttle
18 Wapogasset
19 Whitewater
20 Winnebago

A sample was thoroughly stirred before aliquots were drawn and placed on a slide for viewing. Percent composition of each genera in the sample was estimated grossly by eye with a microscope. From this information, toxic samples could be compared with one another in an attempt to determine toxic genera.

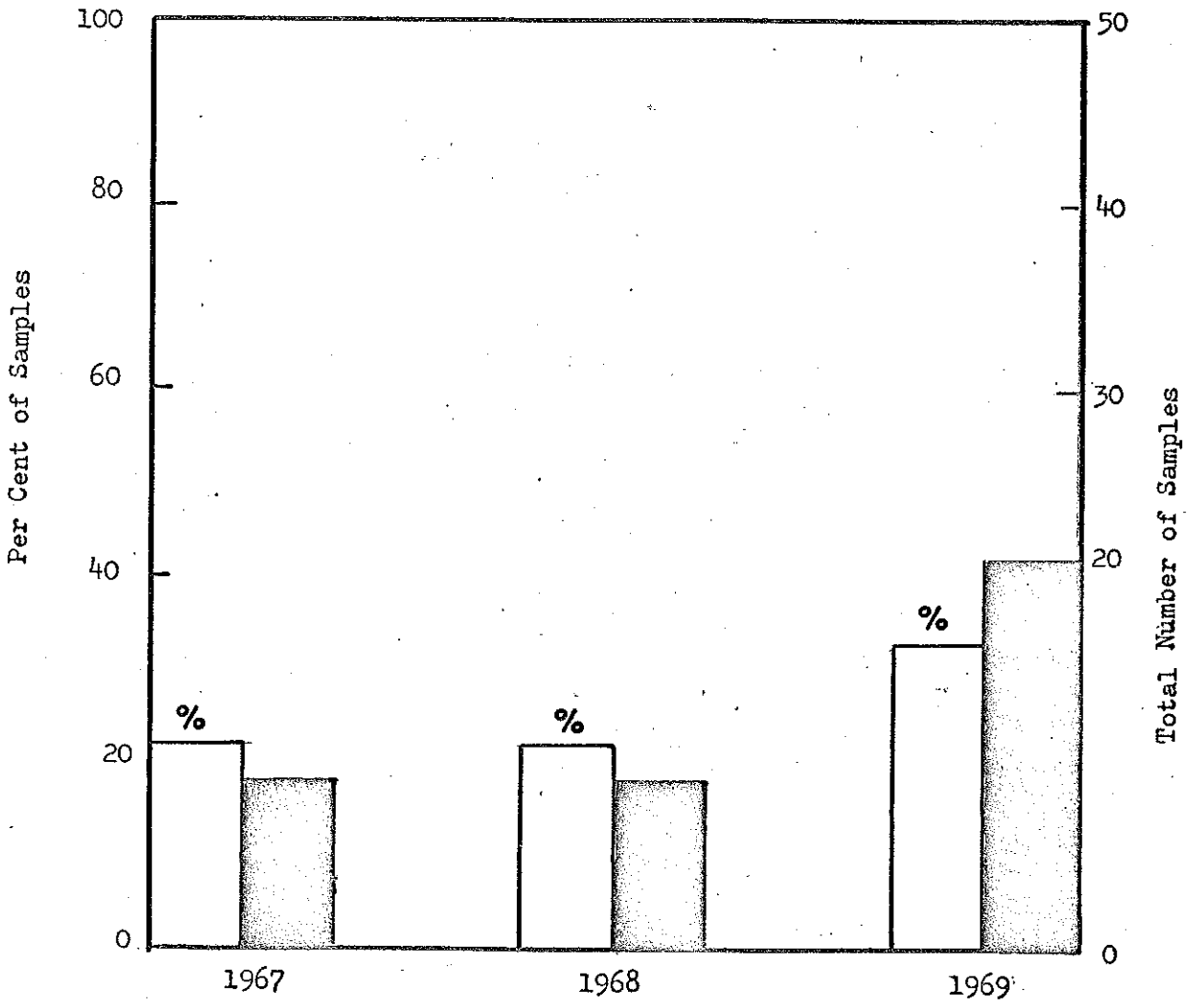
Next a sample was blended in a Waring table top blender, Model B, for 10 minutes to break up the cells and release endotoxin (s). Five minutes of blending was insufficient to break up the cells based on microscopic examination.

Each blended sample was centrifuged for 10 minutes at 2,000 R.P.M. in an International Centrifuge, Universal Model UV. This technique seperated the supernatant containing endotoxin (s) from the solid portion of each sample. This blending and centrifugation method replaced a filtration technique used in 1967 and part of 1968.

The latter method filtered samples twice by gravity flow. The first funnel contained Reeve Angel Number 202 filter paper which removed debris and organisms. The second funnel contained Whatman Number 40 filter paper. The replacement of this method could have accounted for the increased percentage of toxic samples i.e. 33% (1969) and 22% (1967, 1968) (Figure II). In 1967 the filtration method yielded 2 toxic samples out of 9 tested while in 1969 the blending-centrifugation method yielded 7 toxic samples out of 21 samples tested (Table I). Since cells were not broken up in the filtration method the endotoxin (s) may not have been released in sufficient concentration to demonstrate toxicity. The above discussion however pertains only to the very fast and fast death toxins as the slow death toxin is of bacterial origin and not an endotoxin.

FIGURE II

OCCURRENCE OF TOXIC SAMPLES



Another difficulty with the filtration method was the amount of time needed per sample i.e. 2-5 hours. The gravity filters were slow and often plugged with algae concentrate. The blending-centrifugation method only took 30 to 45 minutes.

Toxin Bioassay

The extracts were injected intraperitoneally into white mice. This bioassay was effective in demonstrating toxicity.

Feeding experiments were tried initially but were unsatisfactory. Algae producing very fast death were combined with mouse chow, "Lab Blox", by grinding meal pellets and pouring in algae with a minimum of water from the sample. The algae-chow combination was fed to test mice which often refused to eat it. After two trials using four mice, this bioassay method was replaced by the injection method. Results obtained from the initial method were all negative. However force drinking and feeding methods were not tried. The literature also indicates a preference for the intraperitoneal injection method over oral feeding. Konst et al. (1965) compared the two methods and found the oral LD_{100} of "Microcystin" (fast death toxin) was "approximately 40 times higher than the corresponding intraperitoneal LD_{100}" using mice, guinea pigs, and rabbits as test animals. Wheeler et al. (1942) also tried oral feeding experiments using a toxic Microcystis aeruginosa culture on laboratory animals and obtained negative results. Intraperitoneal injections (0.25 ml) using the same algal culture produced death in 16-32 hours.

White laboratory mice from an inbred Swiss-Webster strain were used.

Mice were 4 to 6 weeks old and weighed 20 - 35 g (mean 27 g). They were housed in sheet metal cages covered with hardware cloth. Cages contained an inverted watering bottle, mouse chow, and two mice. Initially two mice each were injected with an algal extract to test for the sample's toxicity. If negative results were obtained in both mice, no further testing was done. Duplicate injections were made on two more mice to confirm toxicity of the sample if either one or both mice died initially. A total of four mice were bioassayed for all positive samples. Periodic controls were run to check on injection technique and viability of the mouse stock. Normal medical saline solution (0.85%) was used as the control. Both sexes were used but males were more available and were used predominately.

The belly of each mouse was wiped with a cotton-alcohol swab and an intraperitoneal injection was made with a disposable 2.5 cc syringe and 27 gauge - ½ inch needle. All refrigerated extracts were returned to room temperature prior to injection. Each mouse was injected with 0.5 ml extract and placed in a cage for observation. Observations were closely spaced for the first 1.5 hours and periodic thereafter. Observations were limited to 48 hours in all bioassays as this was considered the critical time for toxic symptoms to develop in test mice (Hammer, 1968, and Gorham, 1960, 1962 and 1964). Bacterial infections can become critical after 48 hours and bias results.

DISCUSSION AND RESULTS

Toxicity Classification

Toxins were classified into three levels based on time until death (Table II). Death during the first hour after injection was called very fast death toxin. Death between one hour and four hours was called fast death toxin and death between four hours and forty-eight hours was called slow death toxin. Various authors are cited who have used a similar classification system (Table II). Hours to death in all except the present study left openings in the time sequence.

A misclassification could occur in this study and in previous work from variations in dilution of the toxin. A toxin leading to very fast death (0 to 1 hour) when concentrated could cause slow death (4 to 48 hours) when diluted. A toxin producing death in 6 minutes (Delton Lake in 1967) was diluted to a 50,32,18, and 1 per cent solution in sterile distilled water (Figure III). Time to death for the above dilutions were 14, 27, 1260, and 2400 minutes respectively. The 50 and 32 per cent solutions could be called the very fast death toxin, whereas the 18 and 1 per cent solutions could be called the slow death toxin. Thus time to death could be a result of different dilutions rather than different toxins. It was observed that the 50 per cent solution killed mice in 14 minutes or approximately double the time of the original undiluted sample. The linear relationship between sample concentration and death time stopped at 32 per cent solution. This solution killed mice in 27 minutes rather than an 18 minute time period for which a solution 1/3 the

TABLE II

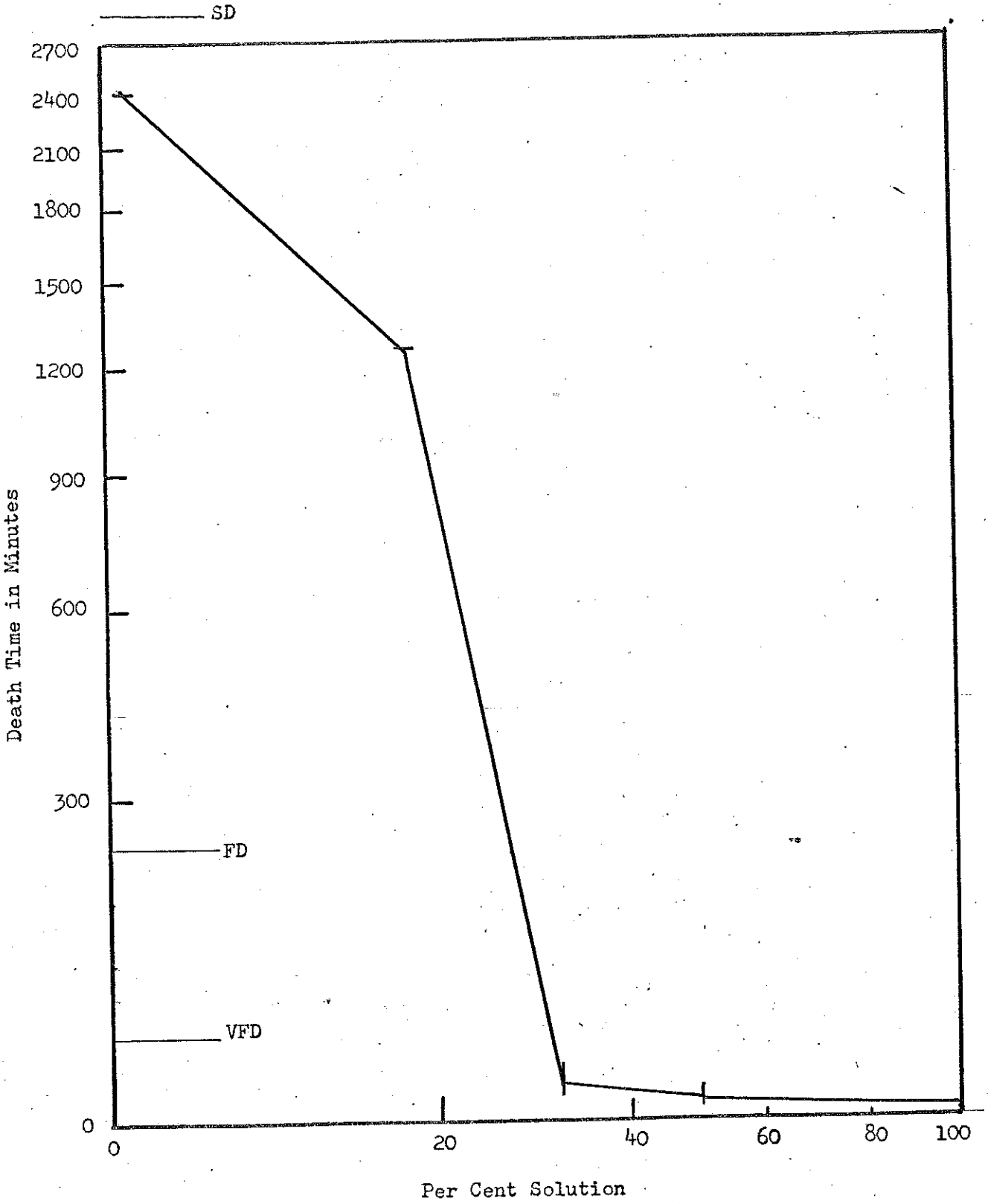
Classification System by Time until Death

Used in Present Study and by other Authors

Toxin Classification	Hours to Death	Author
VFD	0.10 - 1	Present study
	0.07 - 0.83	Hammer (1968)
	0.02 - 0.17	Gorham (1962, 1964)
	0.5 - 1	Olson (1951, 1952)
	0.5 - 1	Hughes et al. (1958)
FD	1 - 4	Present study
	1 - 2.5	Hammer (1968)
	1 - 2	Konst et al. (1965)
	1 - 2	Gorham (1960, 1964)
	1 - 2	Olson (1951, 1952)
	1 - 2	Hughes et al. (1958)
	1 - 2	Ingram et al. (1954)
	1 - 2	Fitch et al. (1934)
SD	4 - 48	Present study
	5 - 48	Hammer (1968)
	4 - 48	Gorham (1960, 1964)
	16 - 36	Olson (1951, 1952)
	4 - 48	Hughes et al. (1958)
	16 - 36	Wheeler et al. (1942)
	16 - 36	Fitch et al. (1934)

FIGURE III

DILUTION OF VERY FAST DEATH TOXIN*



* Delton Lake - 80% Anabaena

original strength should have caused. Therefore the death time increased 4.5 x rather than 3 x. The 18 per cent and 1 per cent solutions went still farther from a linear relationship with respective death times of 21 hours (1260 minutes) and 40 hours (2400 minutes); i.e. 210 x rather than 6 x and 400 x rather than 100 x.

Toxin Occurrence in Wisconsin

The very fast death toxin (VFD) occurred in 5% of the total samples (Figure IV). A sample containing 100% Anabaena from Delton Lake killed laboratory mice within 6 minutes. Another sample containing 80% Anabaena from the same lake produced the same results (Figure V). The blue-green toxin from this lake was also responsible for death in a pet dog as verified by an autopsy.

Gorham (1960) identified this toxin as a "very fast death factor" while working with Anabaena flos-aquae which killed mice in 0.02-0.17 hours (Table II). Gorham et al. (1964) first cultured this species from bloom samples obtained by Hammer from Canadian lakes. This alga has been associated with fast and slow deaths but the fast deaths occurred much sooner than those caused by Microcystis aeruginosa. Hammer (1968) confirmed Gorham's work by carrying out toxicity tests using Anabaena flos-aquae, Microcystis aeruginosa, and Aphanizomenon flos-aquae. He found Anabaena flos-aquae obtained from three Canadian lakes contained very fast death toxin which produced "rapid deaths within 4 - 50 minutes" in test mice.

The fast death toxin (FD) occurred in 13% of the total samples

FIGURE IV

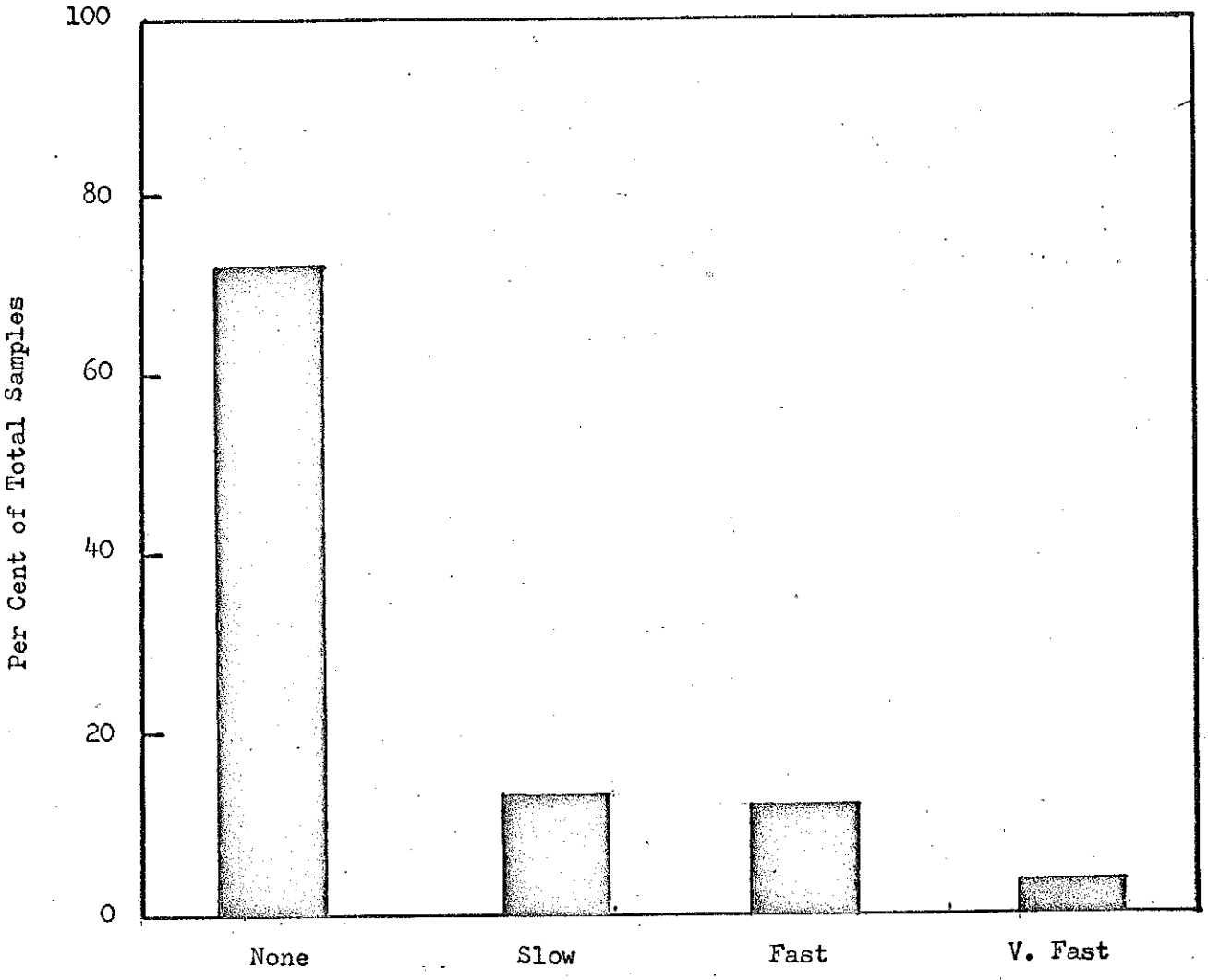
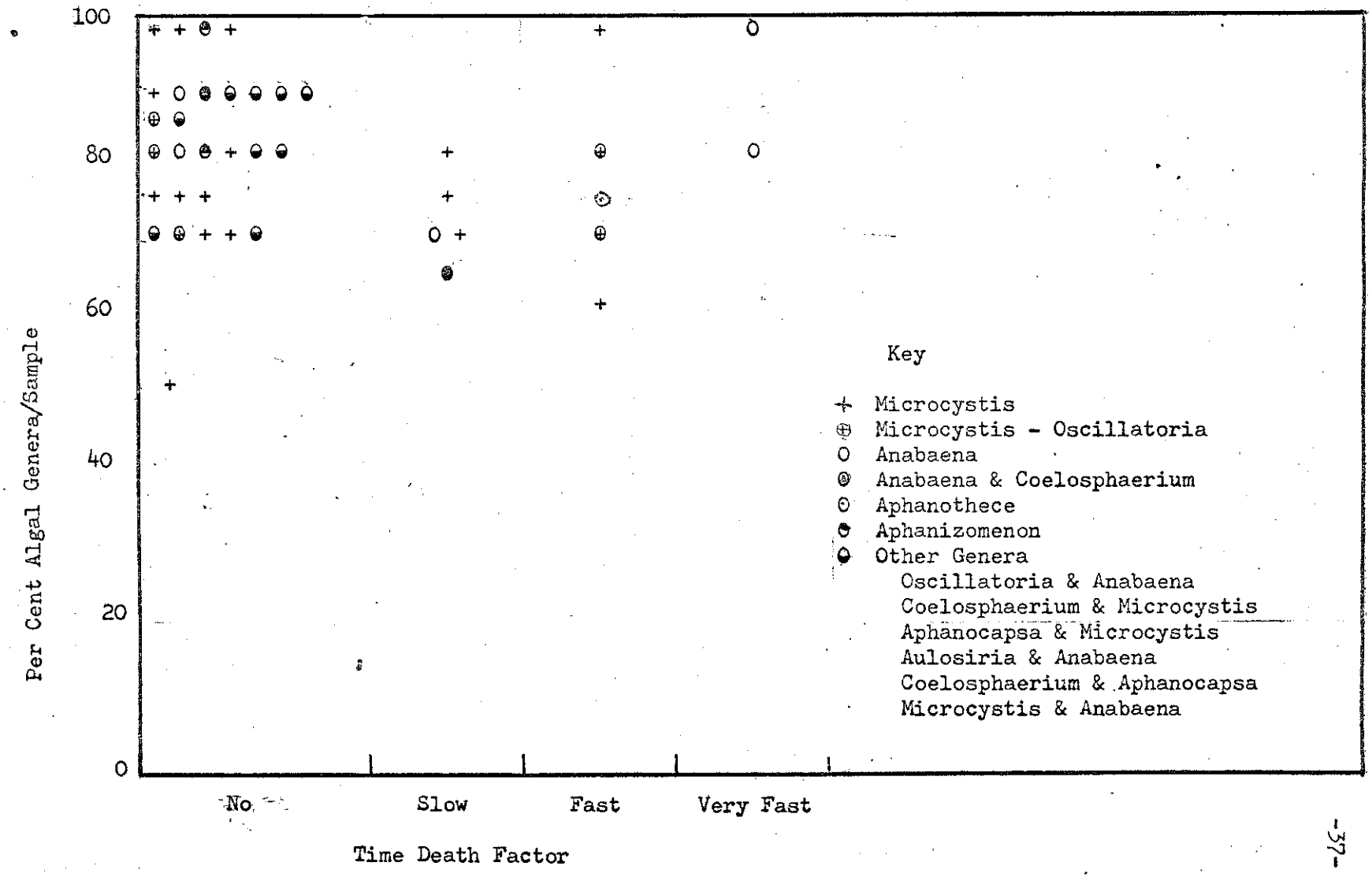

100
80
60
40
20
0
Per Cent of Total Samples
None
Slow
Fast
V. Fast
TOXICITY TIME FOR DEATH

FIGURE V


100
80
60
40
20
0
Per Cent Algal Genera/Sample
No
Slow
Fast
Very Fast
Time Death Factor
Key
Microcystis
Microcystis - Oscillatoria
Anabaena
Anabaena & Coelosphaerium
Aphanothece
Aphanizomenon
Other Genera
Oscillatoria & Anabaena
Coelosphaerium & Microcystis
Aphanocapsa & Microcystis
Aulosiria & Anabaena
Coelosphaerium & Aphanocapsa
Microcystis & Anabaena

(Figure IV) and was found in lakes Delavan, Long and Winnebago (Table I). The Delavan Lake sample contained 80% Microcystis - Oscillatoria combination as the dominant algae producing fast death (Figure V). Wind conditions on Long Lake caused algal concentrations along shorelines twice during 1969 which produced the fast death toxin (Appendix I and Table I). These samples contained approximately 75% Aphanothece and 70% Microcystis - Oscillatoria combination (Figure V). Brighton Drive Lagoon, a back water of Lake Winnebago, produced fast death toxin (Table I) from a 60% Microcystis sample on one sampling date and 100% Microcystis sample on another date (Figure V).

The literature reports that Gorham (1960) defined "the fast death factor" as the toxin which killed injected mice within 1 to 2 hours (Table II), and was present in both algal cells and filtrates. Further information was obtained on growth of toxic algae by Bishop et al (1959) and Gorham from pure toxic laboratory cultures of M. aeruginosa called NRC-1. The best yields of algae occurred at 28° to 32.5° C with maximum cell production taking about 4 days at 25° C before a sharp decline in the population curve was detected. Water temperature ranged from 22° to 26° for lakes Delavan and Winnebago (Appendix I). This temperature range was slightly lower than the optimum from Bishop's and Gorham's studies. Water temperature was not recorded for Long Lake.

The slow death toxin (SD) occurred in 13% of the total samples (Figure IV) and was found in lakes Wapogasset, Whitewater, Pickerel,

Pokegama and Menomin (Table I). Dominant genera in samples causing slow death were Microcystis, Anabaena, and Anabaena-Coelosphaerium combination (Figure V). Microcystis predominated samples collected from Wapogasset (80%), Pokegama (75%), and Menomin (70%). Anabaena-Coelospharium predominated the Whitewater Lake sample (65%), while Anabaena alone made up the Pickerel Lake sample (70%).

The literature reports that slow death toxicity is more common than very fast death or fast death toxicity. Hammer (1968) made an ecological study of blue-green algae in various Saskatchewan lakes and stated "Slow deaths were of more frequent occurrence". Both Gorham et al. (1964) and Hammer found Anabaena and Microcystis associated with slow death toxicity. Coelosphaerium Kuetzingianum Näeg, and C. Naegelianum Unger were reported toxic by Ingram and Prescott (1954). Gorham (1962) identified these species as causing slow death toxicity.

Aphanizomenon in the present study was nontoxic (Figure V) although it was the predominant genera in two samples (100% and 80%) collected from Eagle Lake in 1968 (Appendix II). Wheeler et al. (1942) working with a pure culture of Aphanizomenon flos-aquae also found no toxic effect on laboratory mice. Other authors obtained negative results also from laboratory bioassays (Ingram et al., 1954; McLeod et al., 1952; Nelson, 1903-04; Phinney et al., 1961; Prescott, 1948, 1960; and MacLachlan et al., 1963). Canadian studies by Gorham et al. (1959) and Hammer (1968), however, cite this genera as occassionally producing slow deaths in bioassayed laboratory animals. After carrying out many toxicity tests Hammer stated " .....this species forms more blooms than any other blue-

green alga in Saskatchewan.....". Thus Aphanizomenon is a major bloom producer but is usually nontoxic.

The number of samples and the percentage of toxic ones are summarized in Figure II. During both 1967 and 1968 22 per cent of the samples collected in the Wisconsin lakes were toxic. During 1969, 33 per cent of the samples were toxic. The higher number of toxic samples in 1969 can be attributed to increased sampling compared to the 1967 and 1968 years, but the higher percentage of toxic samples in 1969 probably resulted from the change of extraction methods. (See extraction above.)

Symptoms of Toxicity

Symptoms of very fast death (VFD) are dramatic and characteristic compared to many symptoms observed for fast and slow death (Table III). Symptoms were back arching; violent convulsions, tremors and contractions; total lack of co-ordination and leg paralysis; rapid clawing; salivation and coughing; and collapse with paralysis were observed in a VFD sample from Delton Lake (Appendix II). Except for the symptoms of back arching, rapid clawing, and salivating - coughing, Hammer (1968) and Gorham (1960, 1964) observed similar symptoms. Hammer also observed quiet behavior, general weakness, or no obvious symptoms at all for very fast death.

Symptoms of fast death (FD) were total lack of co-ordination and leg paralysis; collapse with paralysis; poor leg co-ordination; muscle twitching; loss of color in the eyes, ears, and tail; respiration

TABLE III

Comparison of Toxicity Symptoms in Laboratory Mice

Symptom	Toxicity VFD	FD	SD	No	Author
Arching of back	X				Present study
Violent convulsions, tremors and contractions	X				Present study
	X	X	X	X	Hammer (1968)
		X			Konst et al. (1965)
	X				Gorham (1960, 1964)
		X			Hughes et al. (1958)
		X			Ingram et al. (1954)
		X			Olson (1951)
			X		Wheeler et al. (1942)
			X		Fitch (1934)
Total lack of co-ordination and leg paralysis	X	X			Present study
	X	X		X	Hammer (1968)
		X			Konst et al. (1965)
	X			X	Gorham (1960, 1964)
		X			Ingram et al. (1954)
		X			Olson (1951)
			X		Wheeler et al. (1940)
			X	X	Fitch (1934)
Rapid clawing (bicycling)	X				Present study
		X			Konst et al. (1965)
Salivating and coughing	X				Present study
			X	X	Wheeler et al. (1942)
			X	X	Fitch (1934)
Collapse with paralysis	X	X			Present study
	X				Hammer (1968)
Poor leg co-ordination		X	X		Present study
				X	Hammer (1968)
		X			Konst et al. (1965)

Symptom	Toxicity VFD	FD	SD	No	Author
Muscle twitching		X	X		Present study
		X			Konst et al. (1965)
Huddling together			X		Present study
		X			Konst et al. (1965)
Loss of color: eyes, ears, tail.		X	X		Present study
		X			Konst et al. (1965)
		X			Hughes et al. (1958)
		X			Ingram et al. (1954)
		X			Olson (1951)
Respiration difficulty		X	X		Present study
		X			Konst et al. (1965)
			X		Gorham (1962)
		X			Ingram et al. (1954)
		X			Olson (1951)
Excitability		X			Present study
		X			Konst et al. (1965)
			X	X	Wheeler et al. (1942)
			X	X	Fitch (1934)
Quiet behavior and general weakness		X	X		Present study
	X	X	X	X	Hammer (1968)
		X			Konst et al. (1965)
			X		Gorham (1962)
		X			Hughes et al. (1958)
Ruffled coat		X	X		Present study
		X			Konst et al. (1965)
			X		Gorham (1962)
		X			Hughes et al. (1958)
Foamy white tears			X	X	Present study
			X		Olson (1951, 1960)
			X		Ingram et al. (1954)
			X		Fitch (1934)
No obvious symptoms				X	Present study
	X	X	X	X	Hammer (1968)

difficulty; excitability; quiet behavior and general weakness; and ruffled coat (Table III). These symptoms were observed in mice injected with algal samples from Delavan, Long and Winnebago Lakes. Three of the five fast death samples tested, produced leg paralysis particularly in the hind quarters of the injected mice i.e. Long 8/10, Winnebago 8/29, and Winnebago 9/5. The other two samples produced poor co-ordination without leg paralysis i.e. Delavan and Long 9/10 (Appendix II). No explanation can be given for the symptom variability among the five samples even though death times were in the range of 1 to 2.25 hours.

Such symptoms as violent convulsions, tremors, and contractions; bicycling; huddling together, and foamy white tears were observed by other authors but not in this study (Table III). Three symptoms considered important by all authors were violent convulsions, tremors, and contractions (not observed in this study); total lack of co-ordination and leg paralysis; and loss of color in the eyes, ears and tail.

Konst et al. (1965) found comparable symptoms to those mentioned above. They intraperitoneally injected lethal doses of freeze-dried cells from toxic Microcystis into mice. Early symptoms were huddling together, slightly ruffled coats, and rapid respiration. Later symptoms were muscular twitchings of the head and neck, convulsive jumping movements, and periods of alternating hyperexcitability and quietness. Symptoms just prior to death were gradual weakness and complete quietness, slow and labored respiration, lack of co-ordination, and paralysis of the hind limbs, occassional "bicycling" leg movement, and

general loss of color.

Symptoms of slow death (SD) were poor leg co-ordination; muscle twitching; loss of color from the eyes, ears, and tail; respiration difficulty; quiet behavior, general weakness and ruffled coat (Table III). These symptoms were observed in mice injected with algal samples from Wapogasset, Whitewater, Menomin, Pickerel and Pokegama Lakes (Appendix II). Usual SD symptoms are variable and not as pronounced as they are in the FDF and VFDF toxins. The 1967 Wapogasset Lake sample produced labored breathing together with poor co-ordination in the front legs of the injected mice. Both mice looked sick within 24 hours and remained quiet until their death. The other four SDF samples from Lakes Whitewater, Menomin, Pickerel, and Pokegama produced labored breathing, quiet behavior and general sick appearance, loss of color in the eyes, ears and tail, and ruffled coats. Poor leg co-ordination was not produced as it was in the Wapogasset sample.

Pronounced symptoms of slow death consisting of convulsions, tremors, contractions, leg paralysis, rapid clawing, and salivating with coughing, however, were not observed in this study but by other authors (Table III). Hammer (1968) also observed no symptoms in mice injected with the slow death toxin. The quiet behavior and general weakness symptom was observed most often by various authors.

Some symptoms consisting of violent convulsions, tremors, contractions; leg paralysis; salivating with coughing; poor leg co-ordination; excitability and quiet behavior with weakness were observed by various authors in bioassayed mice with no deaths recorded (Table III). Hammer

(1968) reported that mice in this classification recovered. In my work mice that lived 48 hours had not developed any obvious toxic symptoms.

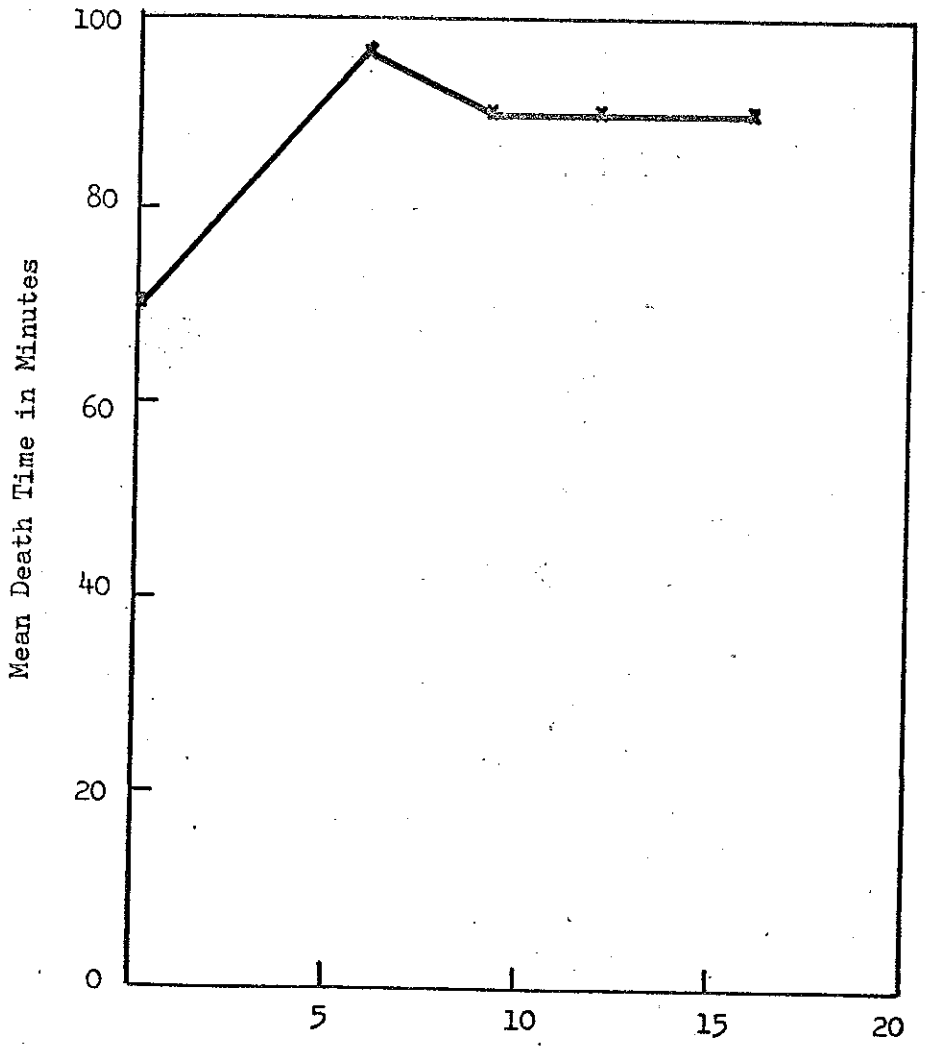
Stability of Toxin Causing Fast Death

Stability studies of the fast death toxin were made using a 1968 Delavan Lake sample (Appendix II). The sample contained a 80% Microcystis - Oscillatoria combination (Figure V) which initially killed mice in one hour. The toxin was refrigerated for 6 months at 2° C, brought up to room temperature and intraperitoneally injected. This procedure was followed for 9-month, 12-month, and 16-month periods. No appreciable loss in toxin potency could be observed during the storage time. The toxin proved very stable after refrigeration for 16 months. During the first 6 months of storage, mean time till death increased from an initial 70 minutes to 97 minutes. After 6 months mean time till death was 90 minutes (Figure VI). Symptoms observed during each study period were the same as those of the initial injections. The mice appeared sick and became quiet within the first hour. Poor co-ordination and hind leg paralysis were common among all mice within 40 to 60 minutes. Hopefully, this toxin breaks down faster under natural lake conditions and warm temperatures.

The literature reports a toxin producing fast death and associated with Anacystis cyanea (Kuetz) was demonstrated to be stable for twenty days at 5° C (McBarron and May, 1966). Toxin potency was also unaffected by boiling for five minutes.

FIGURE VI

FAST DEATH TOXIN STABILITY UNDER REFRIGERATION *



* Delavan Lake - 80% Microcystis - Oscillatoria

Toxicity Identification by Various Authors

The literature reports on various authors and their work concerning the biochemical identification of the very fast, fast and slow death toxins.

Olson (1951, 1952) and Rose (1953) first recognized the very fast death toxin (VFD) as being seperate from the fast death toxin in their work with waterfowl and Anabaena. The former toxin killed waterfowl whereas the fast death toxin did not. Olson seperated the VFD toxin into several fractions, one of which had a strong light absorption between 210 and 290 millimicrons.

Gorham (1962, 1964) while working with toxic Anabaena flos-aquae and Microcystis aeruginosa found the very fast death toxin came only from the former species. He suggested both the very fast and the fast death toxins were endotoxins. The VFD toxin permeated cell membranes faster by its lower molecular weight than the fast death toxin. Both toxins had similar solubilities in solvents such as water and ethanol and exhibited similar nonsolubilities in acetone, ether, and chloroform.

Extraction and purification of the very fast death toxin from a selected clone of Anabaena flos-aquae proved Olson's earlier work i.e. the VFD toxin was made up of several fractions (Starvic et al., 1966). A hot absolute alcohol, water and chloroform extraction followed by diethyl ether precipitation produced a major and two minor fractions. The hydrochloride of the major fraction was stable and hygroscopic while

the toxin was unstable to heat and light and inactivated by 2N NaOH. Starvic et al. concluded the major toxin was ".....a tertiary amine of low molecular weight with a strong absorbance at 229 mμ". Its chemical structure appeared to be made up of a six-membered ring with a beta-unsaturated ketone. In comparison to botulinal toxin's lethal dosage, the major fraction of the very fast death toxin was high as it had a LD_{50} (IP) of 0.25 mg/kg bodyweight in mice (Starvic et al., 1965 and Bishop et al., 1959).

Early work on the fast death toxin (FD) suggested the toxin was an alkaloid (Louw, 1950). Confirmation of this, however, was not proven by later workers (Hughes et al., 1958 and Gorham, 1960). Bishop et al. (1959) listed and identified 7 amino-acids making up the "cyclic polypeptide". Upon hydrolysis the amino acids yielded the following molar proportions: aspartic, 1; glutamic, 2; D-serine, 1; valine, 1; ornithine, 1; alanine, 2; and leucine, 2, for a total of 10. The presence of aspartic, glutamic, and D-serine acids accounted for the weak acid character of the molecule. Bishop et al. found the molecule dialyzed slowly proving a low molecular weight in the order of 1300 to 2600. The molecule was proven cyclic from negative results obtained from the dinitrofluorobenzene test for terminal amino groups. The LD_{50} (IP) for mice was 0.47 mg/kg bodyweight. This figure was considered high compared to botulinal toxin i.e. LD_{50} (IP) for mice was 5×10^{-6} mg/kg bodyweight (Bishop et al., 1959 and Gorham, 1960).

The fast death toxin has been proven to be an endotoxin since Gorham's work (McBarron et al., 1966). No antibiotic activity has been displayed

by the toxin on such bacteria as Bacillus subtilis, Staphylococus aureus, Escherichia coli, and Pseudomonas hydrophila (Gorham, 1960 and Shelubsky, 1951). The literature also reports the toxin is produced only by certain genetic strains (Gorham, 1960 and Stephens, 1945). Simpson et al. (1958) failed to induce production of the FD toxin in a nontoxic strain of Microcystis aeruginosa. Present studies are concerned with comparing the fast death toxin with known marine toxins. Indications are the same methabolic pathways may be used (Jackim and Gentile, 1968).

Unlike the other two toxins mentioned above, the slow death toxin (SD) has been associated with and attributed to bacterial growths with algae (Gorham, 1960, 1962). Thompson et al. (1957) and Thompson (1958) reported finding Gram-negative bacterium most common with toxic Microcystis and a Gram-positive Bacillus with Anacystis. The chemical structures of the toxin (s) are unknown at present. However, the Gram-negative bacterium were found to produce a heat stable and acid stable acetone precipitate identified as a neurotoxin (Gorham, 1960, 1964). Various authors indicate there is more than one slow death toxin produced by the different bacteria involved (Thompson et al., 1957; Gorham, 1960, 1962 and Simpson et al., 1958).

CONCLUSIONS

Experimentation is needed to better understand the toxicity of blue-green algae. The following list of conclusions can be made from this paper:

1. Some algal strains are toxic while others are not and in most cases bioassays are necessary to determine toxicity or lack of it. Therefore, every bloom condition or shoreline algal concentration must be treated as toxic until proven otherwise.
2. Toxicity can be confirmed rapidly by procedures outlined previously in this study (Methods).
3. Such areas as public water supplies, swimming beaches and various recreational waters should be monitored in the future; especially during times of high algal concentration. The above study definitely shows toxic algae are present in Wisconsin lakes, but falls short of an extensive monitoring program which could be set up to forewarn the public of toxic conditions. To date, no known human deaths are directly attributed to algal toxins; however, cases of human illness have been traced to algae in public water supplies. Ingestion of algae and their toxins tends to be accidental for man but not always so for domestic and wild animals. It is, therefore, concluded that swimming in or near algae blooms should be discouraged and preventive measures taken to keep domestic animals away from these waters. No method is known by which natural fish populations, waterfowl, and other wildlife can be prevented from drinking toxic waters.

4. Presumably, man could die consuming fresh water fish or other freshwater aquatic life which may have ingested toxins from lower levels of the food chain. Fresh water toxic algae could, therefore, be indirectly important to man from a health standpoint in much the same way marine toxic algae are via the shellfish carrier.

Research Proposals

The following list of research proposals is suggested for future research on toxic algae and its environmental effect. Many unanswered questions in this area need to be solved.

1. Genetic interpretation of where and how the three death toxins develop in various algal species is needed. Why one algal strain is toxic and another is not is still not understood.
2. An extensive study is needed on all Wisconsin eutrophic recreational waters to determine toxic algae prevalences and establish a basis for warning swimmers and skiers who might be using toxic waters. In relation to this study, accumulation of data might be used to evaluate the statistical reliability of the mouse bioassay as opposed to some more quantitative chemical assay (as yet undeveloped).
3. Medical research is needed to discover antidotes which can be given to toxic algal victims such as pets or livestock.
4. More extensive bacteriological research is needed to isolate and identify all the bacteria which are associated with causing the slow death toxin (s). It is still not known why ducks and other fowl are more resistant to these toxins.

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APPENDIX I

Lake and County	Collection Date	Lake Surface Conditions	Weather	Wind (mph.)	Temperature ° C. Air	Water
Delavan Walworth	7-11-68	Planktonic bloom at inlet-outlet end of lake; grassy odor; "paint pot" conditions along shoreline at resort.	Warm and sunny.	NE @ 2-3	--	22
Delton Sauk	6-3-67	Shoreline accumulation in bay areas along southeast shoreline. Planktonic scum over most of surface.	Warm, sunny and partly cloudy.	light and variable	--	17
Eagle Racine	7-19-68	Wind and wave accumulations in east bay.	Humid, sunny and clear.	SW @ 10-20	29	28
Eagle Racine	8-5-69	Wind blown accumulations along northeast bay shoreline.	Hazy with 80% humidity. Sunny and partly cloudy.	SW @ 5	28	28
Eagle Racine	8-11-69	Wind accumulations along east bay. Algae very thick and jelly like, "paint pot" conditions.	Partly cloudy, and bright but hazy.	SW @ 5-7	--	26
Half Moon Eau Claire	5-15-68	Planktonic bloom covering entire surface; foul odor, decaying algae.	Bright, clear and humid.	light and variable	--	11
Long #19691 Price	8-10-69	Wind accumulation in southeast bay. Wave action breaking up bloom condition. "Paint pot" conditions.	Bright and partly cloudy.	W @ 10	29	27
Long #19695 Price	9-10-69	Wind accumulations along eastern shoreline.	Sunny and bright.	W @ 10-15	24	25
Lorraine Walworth	7-25-69	Wind accumulations on northeast corner of lake; wave action causing mixing.	Bright and sunny.	SSW @ 7-10	21	21

Lake and County	Collection Date	Lake Surface Conditions	Weather	Wind (mph.)	Temperature ° C. Air	Water
Marinuka Trempealeau	6-17-68	Wind accumulations in bay area on east shore. Wave action causing surface bloom to break up.	Sunny, warm and clear.	WSW @ 5-7	--	22
Menomin Dunn	8-17-67	Wind accumulations along north shoreline at inlet, decaying algae and white foam on surface.	Sunny, clear and humid.	SSW @ 6-8	22	20
Menomin Dunn	8-15-69	Wind accumulations along west shoreline. Water looks green with algae suspension.	Sunny, clear and humid.	NE @ 10	28	22
Monona Dane	6-24-69	Wind accumulations along north shoreline. Wave action stirring up surface algae on shore. Grassy odor, "paint pot" conditions.	Sunny, partly cloudy with 90% humidity.	SSW @ 10	20	20
Pickerel Portage	7-29-69	Wind and wave algal accumulations on northeast shore. Algae decomposing, "paint pot" conditions.	Sunny, warm and clear.	S @ 8-10	--	--
Pokegama Barron	8-10-67	Wind accumulations off Lake Chetek into Pokegama; wave action, dense accumulation.	Sunny, hot and humid.	S @ 10	--	--
Pokegama Barron	8-16-67	Wind accumulation along eastern shoreline and bay areas.	Warm and humid.	SW @ 5-10	--	--
Pokegama Barron	6-19-68	Wind accumulations along west shoreline and in Sandy Bay area. Green water.	Sunny and clear.	ENE @ 7-10	25	24

Lake and County	Collection Date	Lake Surface Condition	Weather	Wind (mph.)	Temperature ° C. Air	Water
Pokegama Barron	7-25-68	Calm surface conditions. Bloom condition over entire surface.	Humid and sunny.	none	28	24
Pokegama (1) Barron	8-11-69	Wind accumulations along northeast shoreline. Wave action.	Bright and sunny.	SW @ 5	--	--
Pokegama (2) Barron	8-11-69	Wind accumulation on east shoreline.	Bright and sunny.	SW @ 5	--	--
Pokegama Barron	8-26-69	Wind accumulations very dense in bay areas. Planktonic algal growths over entire surface.	Sunny.	S @ 5-10	29	22
Prairie Barron	8-10-67	Entire surface coverage.	Sunny, warm & clear.	W @ 3	--	--
Prairie Barron	8-11-69	Entire surface coverage.	Sunny, warm and clear	W @ 2-3	--	--
Redstone Sauk	7-29-69	Entire surface covered with a planktonic bloom.	Sunny, warm and clear.	NNW @ 3-5	--	--
Redstone (1) Sauk	8-2-69	Wind driven algae accumulations along east shore. Large quantity.	Sunny, warm and clear.	W @ 8	--	--
Redstone (2) Sauk	8-2-69	Wind accumulations along east shoreline.	Bright, sunny and warm.	NNW @ 5-8	--	--
Silver Waupaca	8-7-67	Wind accumulation on east side of lake. Planktonic bloom over entire surface. Choppy water stirring up surface.	Warm, sunny and bright.	WSW @ 5 -7	--	20

Lake and County	Collection Date	Lake Surface Condition	Weather	Wind (mph.)	Temperature ° C. Air	Water
St. Germain Vilas	8-6-68	Wave action driving algae to north-northeast shore; dense accumulation along shore in east bay. Entire surface appears green from mixing action.	Hot, humid and partly cloudy.	SSW @ 5-10	--	20
Tomah Monroe	6-19-69	Wind accumulations along entire south shoreline to outlet, dense accumulation in residential bay. Surface is mixing.	Cloudy, looks like rain.	NNE @ 5-7	21	19
Tuttle Marquette	8-17-69	Bloom condition early in A.M. before wind accumulations developed.	Sunny, warm and partly cloudy.	W @ 5-10	--	--
Wapogasset Polk	8-11-67	Surface accumulation in North bay; 5 dead perch and 1 dead crappie noted in sampling area.	Bright, sunny and clear.	S @ less than 5	--	--
Wapogasset Polk	8-28-69	Water ripple accumulations along south and west shorelines. Surface is calm.	Warm, sunny and clear.	NE and E @ 2-4	--	--
Whitewater Walworth	8-1-67	Wind accumulations along Eastern shoreline. Ripple action breaking up bloom. Entire surface looks green.	Hot, humid and partly cloudy.	SSW @ 5-7	--	20
Whitewater Walworth	6-19-68	Bloom covering entire surface, grassy odor. Calm surface conditions.	Partly cloudy, warm and sunny.	light and variable.	--	19
Winnebago Calumet	8-15-67	Wind accumulations on east shoreline; whitecaps much wave action, decaying algae at State park; " paint pot".	Bright, sunny and humid.	SW @ 10	--	21

Lake and County	Collection Date	Lake Surface Condition	Weather	Wind (mph.)	Temperature ° C. Air	Water
Winnebago (1) Fond du Lac	8-7-68	Wind accumulations along east shoreline. Decaying algae. Water looks green with algae suspension.	Warm and sunny.	W @ 5-7	--	20
Winnebago (2) Fond du Lac	8-7-68	do	do	do	do	do
Winnebago Fond du Lac	8-3-69	Wind accumulations on south -southeast shoreline. Very dense along residential shoreline. Grassy odor. White capping out on lake.	Bright, sunny and humid.	NW @ 10	31	28
Winnebago Winnebago	8-29-69	Dense algal mass covering entire surface. Dead white bass and leeches. Foul smell. Decaying algae, wind accumulations.	Hot, humid clear and sunny.	SSW @ 5	--	26
Winnebago Winnebago	9-5-69	Dense accumulations over entire surface with some improvement from 8-29-69.	Sunny, warm and bright.	W @ 10-15	--	--

APPENDIX II

Lake and County	Collection Date	Algal Type & Ident.	Approx. %	Time of Death	Remarks
Delavan Walworth	7-11-68	BG Microcystis BG Oscillatoria BG Aphanocapsa BG Other	50 30 20 trace	50 minutes	Sick in 30 min. Labored breathing, muscle twitching, poor co-ordination.
Delavan Walworth	7-11-68	do	do	1½ hrs.	Reinjection. Semi-conscious at 1 hr.
Delavan Walworth	7-11-68	do	do	1¾ hrs.	Algae refrigerated 6 mo. at 2° C. Sick in 1 hour, little movement.
Delavan Walworth	7-11-68	do	do	1½ hrs.	Reinjection of 6 mo. old sample. Poor co-ordination, can't stand at 1 hr.
Delavan Walworth	7-11-68	do	do	1½ hrs.	Algae refrigerated 9 mo. at 2° C. Paralyzed at 40 - 60 minutes.
Delavan Walworth	7-11-68	do	do	1½ - 2 hrs.	Reinjection of 9 month old sample.
Delavan Walworth	7-11-68	do	do	1½ hrs.	Algae refrigerated 1 yr. at 2° C. Became paralyzed in 1 hour.
Delavan Walworth	7-11-68	do	do	1½ hrs.	Algae refrigerated 1 yr. 4 mo. at 2° C. Little movement at 1 hr.
Delton #48812 Sauk	6-3-67	BG Anabaena	100	6 min.	Arched back, convulsions, tremors and total lack of co-ordination, salivating, rapid clawing, collapse.

Lake and County	Collection Date	Algae Type & Ident.		Approx. %	Time of Death	Remarks
Delton #48813 Sauk	6-3-67	BG	*Anabaena &	80		Arched back, convulsions, tremors and total lack of co-ordination, salivating, rapid clawing, collapse.
		BG	Microcystis	15		
			* 100% Solution		6 min.	
			50% Solution		14 min.	
			32% Solution		27 min.	
			18% Solution		21 hrs.	
			1% Solution		40 hrs.	
Eagle Racine	7-19-68	BG	Aphanizomenon	100	alive at 48 hrs.	Fish kill. Reinjections also negative.
		G	Other	trace		
Eagle Racine	8-11-69	BG	Microcystis	45	18½ hrs.	One mouse lived 48 hrs.
		BG	Anabaena	35		
		BG	Aphanizomenon	20		
Eagle Racine	8-11-69		do	do	alive at 48 hrs.	Reinjection.
Eagle Racine	8-11-69		do	do	alive at 48 hrs.	Reinjection.
Half Moon Eau Clair	5-15-68	BG	Anabaena	95	alive at 48 hrs.	Reinjection also negative.
		D	Actinocyclus	5		
Long #19691 Price	8-10-69	BG	Microcystis	40	1½ hrs.	Had paralysis of legs, occassional twitching, labored breathing and glassy eyes at 1 hour.
		BG	Oscillatoria	30		
		BG	Coelosphaerium	20		
		BG	Aphanocapsa	10		
Long #19691 Price	8-10-69		do	do	1¼ hrs.	Reinjection.

Lake and County	Collection Date	Algae Type & Ident.	Approx. %	Time of Death	Remarks
Long #19695 Price	9-10-69	BG Aphanothece BG Microcystis BG Oscillatoria	75 25 trace	2¼ hrs.	Sick, labored breathing, poor co-ordination, quiet at 1 hour.
Long #19695 Price	9-10-69	do	do	2 hrs.	Reinjection. Sick at 1½ hrs.
Lorraine Walworth	7-25-69	BG Aulosiria BG Anabaena G Scenedesmus D & BG Oscillatoria	60 30 10 trace	alive at 48 hrs.	
Lorraine Walworth	7-25-69	do	do	alive at 48 hrs.	Reinjected twice.
Marinuka Trempealeau	6-17-68	BG Microcystis BG Anacystis G Ulothrix G Microspora D Fragilaria D&G Other	30 20 20 10 10 10	alive at 48 hrs.	
Menomin Dunn	8-17-67	BG Microcystis	100	alive at 48 hrs.	
Menomin Dunn	8-15-69	BG Microcystis BG Aphanocapsa Diatoms	70 20 10	16 hrs.	Poor co-ordination, labored breathing and muscle twitching.
Menomin Dunn	8-15-69	do	do	19 hrs.	Reinjection.

Lake and County	Collection Date	Algae Type & Ident.	Approx. %	Time of Death	Remarks
Menomin Dunn	8-15-69	do	do	19 hrs.	Reinjection.
Monona Dane	6-24-69	BG Anabaena BG Microcystis	80 20	alive at 48 hrs.	
Monona Dane	6-24-69	do	do	alive at 48 hrs.	Reinjected twice.
Pickerel Portage	7-29-69	BG Anabaena BG Microcystis G & D Other	70 20 10	15 hrs.	
Pickerel Portage	7-29-69	do	do	21 hrs.	Reinjection.
Pickerel Portage	7-29-69	do	do	20 hrs.	Reinjection.
Pokegama Barron	8-10-67	BG Anabaena BG Microcystis	90 10	alive at 48 hrs.	
Pokegama Barron	8-16-67	BG Anacystis BG Oscillatoria BG Coelosphaerium BG Other	75 10 10 5	alive at 48 hrs.	
Pokegama Barron	6-19-68	BG Oscillatoria BG Anabaena BG Microcystis	60 30 10	alive at 48 hrs.	
Pokegama Barron	7-25-68	BG Aphanocapsa BG Microcystis BG Anabaena	50 40 10	alive at 48 hrs.	

Lake and County	Collection Date	Algae Type & Ident.	Approx. %	Time of Death	Remarks
Pokegama (1) Barron	8-11-69	BG Microcystis	75	16 hrs.	Sample was blended and centrifuged.
		BG Aphanocapsa	25		
Pokegama (1) Barron	8-11-69	do	do	alive at 48 hrs.	Blended and centrifuged.
Pokegama (1) Barron	8-11-69	do	do	18 hrs.	Reinjection.
Pokegama (1) Barron	8-11-69	do	do	25 hrs.	Reinjection.
Pokegama (2) Barron	8-11-69	BG Microcystis	50	alive at 48 hrs.	
		BG Coelosphaerium	30		
		BG Aphanocapsa	20		
Pokegama (2) Barron	8-11-69	do	do	alive at 48 hrs.	Reinjection.
Pokegama #16174 Barron	8-26-69	BG Microcystis	50	alive at 48 hrs.	
		BG Anacystis	50		
Pokegama #16174 Barron	8-26-69	do	do	alive at 48 hrs.	Reinjection.
Prairie Barron	8-10-67	BG Microcystis	90	alive at 48 hrs.	Chetek Chain. Reinjection also negative.
		BG Aphanizomenon	5		
		BG Other	5		
Prairie (1) Barron	8-11-69	BG Microcystis	75	alive at 48 hrs.	
		BG Aphanocapsa	25		

Lake and County	Collection Date	Algae Type & Ident.	Approx. %	Time of Death	Remarks
Prairie (2) Barron	8-11-69	BG Microcystis	40	19 hrs.	One mouse lived 48 hrs.
		BG Aphanocapsa	30		
		G Other	20		
		BG Coelosphaerium	10		
Prairie (2) Barron	8-11-69	do	do	alive at 48 hrs.	Reinjection.
Redstone Sauk	7-29-69	BG Coelosphaerium	70	alive at 48 hrs.	
		BG Aphanocapsa	20		
		BG Microcystis	10		
Redstone Sauk	7-29-69	do	do	alive at 48 hrs.	Reinjection.
Redstone (1A) Sauk	8-2-69	BG Coelosphaerium	40	alive at 48 hrs.	
		BG Microcystis	30		
		BG Aphanocapsa	20		
		BG Aphanizomenon	10		
Redstone (1A) Sauk	8-2-69	do	do	alive at 48 hrs.	Reinjection.
Redstone (1B) Sauk	8-2-69	BG Microcystis	45	alive at 48 hrs.	
		BG Coelosphaerium	40		
		BG Aphanocapsa	10		
		BG Aphanizomenon	5		
Redstone (1B) Sauk	8-2-69	do	do	alive at 48 hrs.	Reinjection.
Silver Waupaca	8-7-67	BG Microcystis	60	alive at 48 hrs.	
		BG Oscillatoria	20		
		BG Gomphosphaeria	15		
		BG Other	5		

Lake and County	Collection Date	Algae Type & Ident.		Approx. %	Time of Death	Remarks
Silver Waupaca	8-7-67	BG	Microcystis	60	alive at 48 hrs.	
		BG	Oscillatoria	20		
		BG	Gomphospaeria	15		
		BG	Other	5		
St. Germain Vilas	8-6-68	BG	Anabaena	40	alive at 48 hrs.	
		BG	Coelosphaerium	50		
		BG	Aphanocapsa	10		
Tomah Monroe	6-19-69	BG	Microcystis	70	35 hrs.	One mouse was alive at 48 hrs.
		BG	Aphanocapsa	30		
		BG	Anabaena	trace		
Tomah Monroe	6-19-69		do	do	alive at 48 hrs.	Reinjection was done twice with same results.
Tuttle Marquette	8-17-69	BG	Microcystis	75	alive at 48 hrs.	
		BG	Coelosphaerium	25		
		BG	Rivularia	trace		
Wapogasset Polk	8-11-69	BG	Microcystis	80	27 hrs.	Sick in 24 hrs. Breathing hard with poor co-ordination in front legs.
		BG	Coelosphaerium	20		
Wapogasset Polk	8-28-69	BG	Microcystis	80	alive at 48 hrs.	[illegible].
		BG	Aphanocapsa	15		
		BG	Coelosphaerium	5		
Wapogasset Polk	8-28-69		do	do	alive at 48 hrs.	Reinjection.
Whitewater Walworth	8-1-67	BG	Coelosphaerium	40	alive at 48 hrs.	
		BG	Microcystis	30		
		BG	Anabaena	10		
		BG	Anacystis	10		
		BG	Aphanizomenon	5		
		BG	Other	5		

Lake and County	Collection Date	Algae Type & Ident.	Approx. %	Time of Death	Remarks
Whitewater Walworth	6-19-68	BG Anabaena	40	19 hrs.	
		BG Coelosphaerium	25		
		BG Gomphosphaeria	15		
		BG Aphanothece	10		
		BG Anacystis	10		
Whitewater Walworth	6-19-68	do	do	19 hrs.	Reinjection. Mouse very quiet after 3 hours.
Winnebago Calumet	8-15-67	BG Microcystis	100	alive at 48 hrs.	
Winnebago (1) Fond du Lac	8-7-68	BG Microcystis	70	alive at 48 hrs.	
		BG Anabaena	25		
		BG Oscillatoria	5		
Winnebago (2) Fond du Lac	8-7-68	BG Microcystis	60	alive at 48 hrs.	
		BG Anabaena	20		
		BG Oscillatoria	5		
		-- Organic Debris	15		
Winnebago Fond du Lac	8-3-69	BG Microcystis	75	alive at 48 hrs.	
		BG Aphanizomenon	10		
		BG Anabaena	10		
		BG Aphanocapsa	5		
		BG Coelosphaerium	trace		
Winnebago Fond du Lac	8-3-69	do	do	2½ hours	Reinjection. One mouse was alive at 48 hours.
Winnebago Fond du Lac	8-3-69	do	do	alive at 48 hrs.	Reinjection for second time.

Lake and County	Collection Date	Algae Type & Ident.	Approx. %	Time of Death	Remarks
Winnebago Winnebago	8-29-69	BG Microcystis BG Aphanothece D -----	60 30 10	2 hrs.	Sick at 1½ hrs., leg paralysis, labored breathing, ruffled coats.
Winnebago Winnebago	8-29-69	do	do	1½ hrs.	Sick at 1 hour. Reinjection.
Winnebago Winnebago	9-5-69	BG Microcystis	100	2 hrs.	Sick at 1½ hours, labored breathing, and leg paralysis.
Winnebago Winnebago	9-5-69	do	do	1-1/3 hrs.	Sick at 25 minutes.