appendix G

Data Supporting Determination of Adverse Affect Thresholds for Potentially Harmful Algal Bloom Species

MICROCYSTIS AERUGINOSA EFFECTS TRESHOLD

A substantial body of literature deals with the negative effects of toxic cyanobacteria on the feeding, growth, behavior and survival of micro- and mesozooplankton. Numerous studies have documented the avoidance of ingestion of toxic and nontoxic strains of *Microcystis aeruginosa* by specific taxa of zooplankton (Clarke 1978; Lampert 1981; Gilbert and Bogdan 1984; Fulton and Paerl 1987, 1988; DeMott and Moxter 1991) while others indicate physiological and behavioral problems associated with the ingestion of *Microcystis aeruginosa* (Lampert 1981, 1982; Nizan et al. 1986; Fulton and Paerl 1987; DeMott et al. 1991; Henning et al. 1991).

Fulton and Paerl's study (1987) indicated that a unicellular strain of *Microcystis aeruginosa* (concentrations of 100,000 cells milliliter^{-1}) was toxic to and failed to support populations of *Keratella mixta*, *Diaphanosoma bracyurum*, *Daphnia ambigua* and *Bosmina longirostris* (a rotifer and three cladocerans, respectively). Other studies have shown additional evidence of inhibitory effects of *Microcystis aeruginosa*. For instance, Penaloza et al. (1990), showed that water-soluble fractions of *Microcystis aeruginosa* were toxic to several rotifers, a copepod and a cladoceran. De Mott et al. (1991) showed that a calanoid copepod was more sensitive to purified microcystin than the cladoceran that he used in his experiments. Nutritionally, many zooplankton have been shown to grow poorly on *Microcystis aeruginosa* because it lacks certain fatty acids (Ahlgren et al. 1990). The results of these studies indicate a deleterious effect exerted by blooms of *Microcystis aeruginosa* on zooplankton communities. Two studies were chosen in the context of deriving thresholds for impairment because they used densities of cells that could be used to evaluate data from the Chesapeake Bay Monitoring Program and ultimately translated into chlorophyll *a* concentrations. Without doing direct experiments on inhibitory effects of the Chesapeake Bay strains of *Microcystis aeruginosa* on zooplankton populations, certain assumptions of comparable toxicity were made for the purposes of setting thresholds.

Numerous laboratory studies also have documented the acute effects of toxins from the cyanobacterium *Microcystis aeruginosa* on fish (Erickson et al. 1986; Rabergh et al. 1991; Keshavanath et al. 1994; Beveridge et al. 1993; Tencalla et al. 1994; Bury et al. 1995). Several instances of fish kills resulting from cyanobacterial blooms also have been documented (Erickson et al. 1986; Penaloza et al. 1990; Rabergh et al. 1991). These studies indicate a variety of negative effects, including inhibition of filtering rate, liver damage, disturbed ionic regulation, behavioral changes and mortality. However, these studies addressed the potential damage from the standpoint of toxin concentrations, not actual cell densities of the phytoplankton species itself. Therefore, it was not possible to deduce a specific quantitative chlorophyll *a* threshold whereby fish can be assessed as being negatively affected by blooms of *Microcystis aeruginosa*.

Two laboratory studies were chosen to determine the threshold at which a negative impact on the zooplankton community occurs–an impact in which the zooplankton community structure is altered by the poor food quality, large particle size of the colonies, increased density of particles in the water column or directly by the toxin. Lampert (1981) conducted a laboratory feeding study in which densities as low as 1,400 cells milliliter^{-1} of *Microcystis aeruginosa* resulted in the feeding inhibition of zooplankton. Similarly, Fulton and Paerl (1987) conducted grazing experiments in which the inhibitory threshold of *Microcystis aeruginosa* ranged from 10,000-100,000 cells milliliter^{-1}, but was most clearly demonstrated at concentrations of 100,000 cells milliliter^{-1}. Since there is a difference of two orders of magnitudes between the two studies, an intermediate concentration of 10,000 cells milliliter^{-1} was chosen for exhibiting an inhibitory effect on zooplankton feeding.

It should be noted that a third study has been identified which documented negative impacts on zooplankton at *Microcystis aeruginosa* cell densities of 50,000 cells milliliter^{-1} which is an intermediate value compared to the two previously cited studies (Smith and Gilbert 1995).

PROROCENTRUM MINIMUM EFFECTS THRESHOLD

Certain strains of *Prorocentrum minimum* are toxic. In Japan in 1942, *Prorocentrum minimum* was attributed as the cause of a shellfish poisoning in Japan in which 114 people died (Nagazima 1965, 1968). *Prorocentrum minimum* isolated from a 1998 bloom in the Choptank River and subsequently grown in the laboratory was found toxic to scallops (Wickfors, personal communication). Blooms of *Prorocentrum minimum* in the source intake water to Virginia and Maryland oyster hatcheries were suspected to have caused oyster larvae mortality at the two hatcheries in 1998 (Luckenbach and Merritt, personal communication). There has been no documented case of shellfish toxicity or mortality as a result of the 1998 *Prorocentrum minimum* bloom in the Chesapeake Bay, but clearly the potential exists for toxic repercussions to shellfish and other organisms as a result of this bloom.

Embryonic development of the Eastern oyster (*Crassostrea virginica)* was not affected by living cells or extracts of *Prorocentrum minimum*, however, larvae showed poor growth and poor development of the digestive system when fed *Prorocentrum minimum* (approximately 4,000 cells milliliter^{-1}) (Wickfors and Smolowitz 1995). Juvenile oysters adapted to digesting *Prorocentrum minimum*, but only after a two-week period. The study concludes that feeding *Prorocentrum minimum* to oyster larvae resulted in clear detrimental effects, but it was not apparent whether the effects were from toxicity or starvation. In addition, it was concluded that some component of the *Prorocentrum minimum* cell interfered with cellular digestive processes in oyster larvae and spat.

The Wickfors and Smolowitz (1995) study also showed detrimental effects of various diets containing different proportions of *Prorocentrum minimum* to oyster larvae and newly set spat. The larvae showed consistently poorer survival and growth in the different experimental diets and only those fed the diet with no *Prorocentrum minimum* or one-third the maximum concentration developed into pedi-veligers and set. Both life stages showed difficulties in the digestive system after *Prorocentrum minimum* became a major component of their diet. The study concludes that *Prorocentrum minimum* blooms impaired the survival, growth and development of oyster larvae. That the study did not reveal whether the cause of these detrimental effects was toxicity or starvation is important to the derivation of numeric chlorophyll *a* criteria or target concentrations. The highest density used in the study was 3,900 cells milliliter^{-1} and detrimental effects were seen at densities of ~ 2,600 cells milliliter^{-1} in a mixed diet. The study is, however, useful in establishing that 1) *Prorocentrum minimum* is detrimental to oyster life stages and 2) specific densities of cells cause impairment.

Another laboratory study indicated more intense impairment of Eastern oyster life stages when they were subjected to bloom concentrations of *Prorocentrum minimum* (Luckenbach et al. 1993). Growth rates were minimal at cell densities of 3,000 cells milliliter^{-1}, as an inverse relationship was documented between grazing rate and cell density. Ultimately, mortality resulted for 43 percent of the juvenile oysters that were subjected to this same density of *Prorocentrum minimum* cells.

The 1993 Luckenbach study was designed to test the effects of *Prorocentrum minimum* on the growth and survival of the Eastern oyster. The momentum for this study came from observations over many years made at the Virginia Institute of Marine Science oyster hatchery over many years on the impact of dinoflagellate blooms on the oyster populations in the hatchery. These observations are unpublished but still noteworthy. They include the observation that adult oysters do not spawn in the presence of bloom densities of *Prorocentrum minimum* and that early larval development is impaired and high mortalities occur in the presence of high densities of this dinoflagellate. The study used densities between 8,900-25,000 cells milliliter^{-1} for the 100 percent bloom density and 2,964-8,250 cells milliliter^{-1} for a 33 percent bloom density. Mortalities of 100 percent for juvenile oysters took place

in the 100 percent bloom diet, while 43 percent mortality was observed in the 33 percent bloom diet.

The density of 3,000 cells milliliter^{-1} that was chosen as a threshold for the chlorophyll *a* criteria analysis is based on the results of these two studies, whereby detrimental effects were documented at cell densities of 2,600 cells milliliter^{-1} in one study and 2,964-8,250 cells milliliter^{-1} in the other study. Neither study was aimed specifically at determining the threshold of impairment for *Prorocentrum minimum*, but impairments took place in both studies at a bloom density of around 3,000 cells milliliter^{-1}. The fact that two different strains of *Prorocentrum minimum* were used in the two studies and negative effects occurred at a very similar density, gives credence to using 3,000 cells milliliter^{-1} as a threshold for impairment.

COCHLODINIUM HETEROLOBATUM EFFECTS THRESHOLD

This species forms intense blooms in warm months at the mouth of the York River and in the lower Chesapeake Bay (Mackiernan 1968; Zubkoff and Warriner 1975; Zubkoff et al. 1979; Marshall 1995). Laboratory studies indicated a threshold concentration of ~ 500 cells milliliter^{-1} whereby calcium uptake was depressed and mortality of larvae was significantly elevated (Ho and Zubkoff 1979). Above densities of ~ 1000 cells milliliter^{-1}, calcium uptake was negligible and mortality extremely high. Mortality was attributed to 'spatial competition' rather than a 'toxic secretion' (although this chain-forming dinoflagellate produces copious amounts of mucilage; Lacouture, personal communication). The densities of this organism during bloom conditions far exceeds these values and the extent of these densities can cover tens of square miles (Mackiernan 1968; Zubkoff et al. 1979; Marshall 1995).

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