'FUNCTIONAL AND PATHOLOGICAL RESPONSES OF SELECTED AQUATIC ORGANISMS TO CHRYSOTILE ASBESTOS'

by

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(ABSTRACT)

Functional and pathological responses of larval, juvenile, and adult Asiatic clams (Corbicula sp.), juvenile and adult fathead minnows (Pimephales promelas), and egg, larval, and juvenile Japanese Medaka (Oryzias latipes) to chrysotile asbestos were investigated in 96-hour to 91-day tests. Chrysotile significantly reduced siphoning activity and shell growth of adult clams and siphoning, shell growth, and weight gain of juveniles at 10^5 fibers/liter during 30-day tests. Larval Corbicula suffered significantly greater mortality and lower release by brooding adults at 10^2-10^8 fibers/liter. Adult and juvenile Corbicula exposed to 10^8 fibers/liter for 30 days exhibited deteriorated gill tissue and significantly greater tissue water content. Corbicula accumulated up to 1000 fibers/mg in visceral tissue at 10^8 fibers/liter. Clams collected from the California Aqueduct System exposed to 10^9 fibers/liter accumulated up to 10^5 fibers/mg in viscera. Corbicula can be used as a monitor for chrysotile contamination due to its ability to concentrate fibers.

Adult and juvenile fathead minnows did not suffer acute toxicity at 10^{12} fibers/liter and differential mortality relative to controls up to 10^{8} fibers/liter for 30 days. At the conclusion of the 30-day tests the length, weight, and swimming performance of adult minnows exposed to asbestos were not significantly affected relative to controls. Juvenile minnows exposed to 10^{6}-10^{8} fibers/liter had significantly lower weight. Fish exposed to 10^{8} fibers/liter for 30 days accumulated up to 390 fibers/mg in kidney tissue.

Egg and larval Medaka were exposed to 0-10^{10} fibers/liter of chrysotile until hatching and for thirteen weeks, respectively. Eggs responded erratically to asbestos exposure and no conclusive trends could be drawn. Larval Medaka exposed to 10^{6}-10^{10} fibers/liter had reduced growth by 14 days. Fish exposed to 10^{10} fibers/liter suffered 100% mortality by 60 days. Fish exposed to asbestos developed epidermal tumors, thickened epidermal tissue, increased mucous cell density in the intestinal tract, constricted kidney tubules, and abnormal levels of lipid and endoplasmic reticulum in the liver. Maximum asbestos uptake occurred in fish exposed to 10^{8} fibers/liter for 91 days (400 fibers/mg).

The extent of damage to fish and clams at levels greater than 10^{4} fibers/liter in the laboratory suggests that aquatically transmitted asbestos is a potential hazard to these species in the field.

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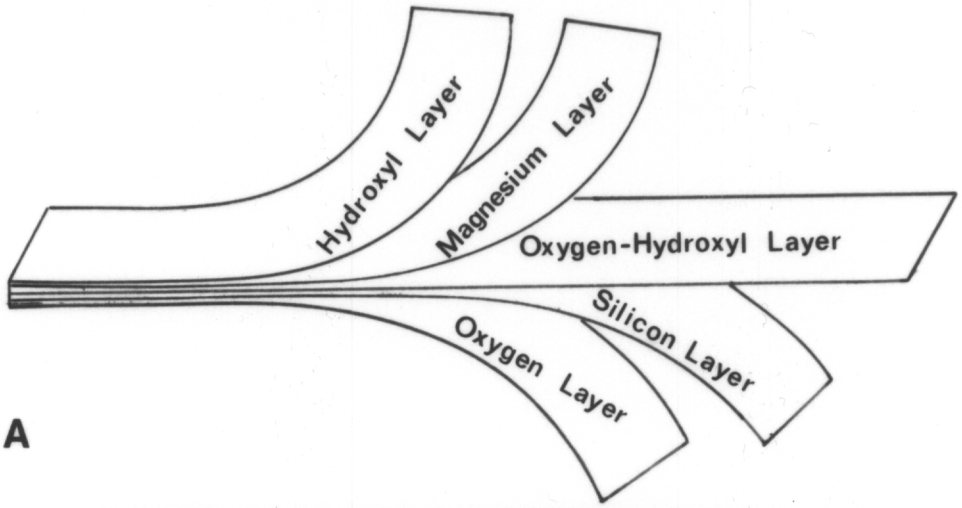
CHAPTER ONE: INTRODUCTION

DEFINITION OF ASBESTOS

Asbestos is a generic term for a group of intergrading hydrated silicate minerals. Asbestos fibers are defined as having a length to width ratio of 3:1 or greater (Anderson and Long 1980). The major groups of asbestiforms are serpentines and amphiboles. Many serpentines exist, but only one is fibrous, called chrysotile (Speil and Leineweber 1969). Fibrous amphiboles are of several types: actinolite-tremolite, amosite, anthophyllite, and crocidolite. Chrysotile fibers differ from amphiboles by forming hollow tubules of layered magnesium-hydroxide tetrahedra mismatched to silica oxide tetrahedra (Fig 1). Chrysotile has the general formula $Mg_2Si_3(OH)_4$ (Speil and Leineweber 1969). Amphiboles are highly variable and consist of sheeted silica-oxygen tetrahedra bounded by heavy metal ions (Al, Mn, Fe, Ti).

The importance of asbestos as an industrial insulation material, fire retardant, and strengthening agent have led to a dependence of modern societies on this mineral (Levine 1978). Asbestos has over 3000 commercial applications ranging from brake linings to filters to asbestos-impregnated cements (Ellis and Leif 1985). It is also a contaminant of

Figure 1. The internal structure of chrysotile asbestos (A) and a transmission electron micrograph of a chrysotile asbestos fiber (B).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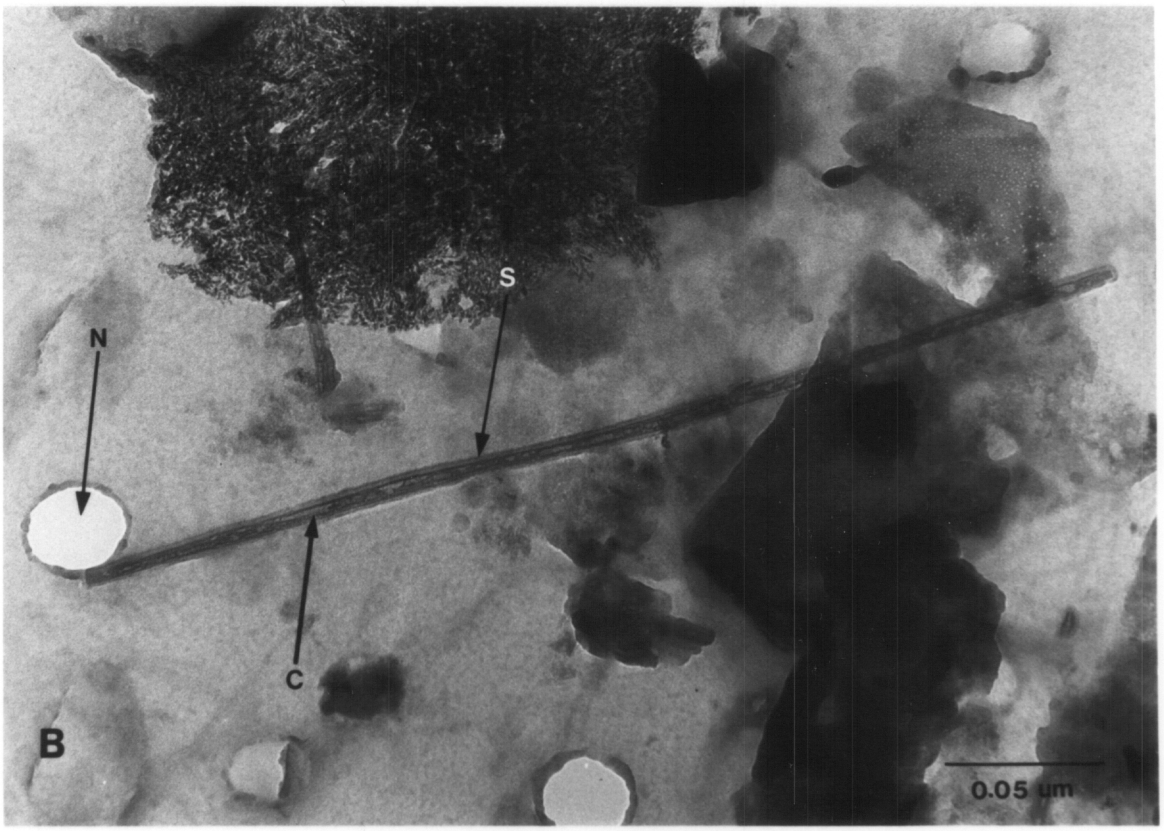
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talc (ranging from 5-80% of raw talc by weight). Talc is employed as a dispersing agent in aerially applied pesticides, processed rice and gum, and cosmetics (Rohl and Langer 1974; Selikoff and Lee 1978).

MEASURING THE PRESENCE OF ASBESTOS

The diminutive nature of asbestos fibers has created problems relative to accurate measurement and mineral species identification by a variety of analytical techniques. Polarized light microscopy (PLM) using dispersion staining techniques is able to detect fibers down to 0.25 µm in width. These methods are utilized in sampling industrial worksites for aerial and construction contamination (McCrone 1985); however, the majority of fibers which are environmentally transmitted are much smaller than 0.25 µm in width and may represent up to 90% of the available fibers for analysis in a sample (Flickinger and Standridge 1976; Buchan et al. 1984). The development of electron optical methods, such as scanning (SEM) and transmission electron microscopy (TEM) and x-ray techniques, has lowered the limit at which smaller fibers can be detected to 0.0005 µm for high resolution/performance instruments. The development of the Jaffe-Wick Nuclepore method for TEM has made this the instrument of choice for environmental samples (Miller 1978; Anderson and Long 1980; Grasserbauer et al. 1984). Chrysotile

asbestos is uniquely distinguishable from all other asbestiforms by the parallel hyaline pattern with a slightly greater electron dense canal (Miller 1978, Fig. 1). The use of TEM poses limitations in its own right by increasing fiber detection limits due to sample processing time and size (Anderson and Long 1980). Generally, 10^4 fibers/liter is considered favorable, but is mitigated by other particles present and fiber-fiber overlap (Iles and Johnston 1983).

DISTRIBUTION OF ASBESTOS IN AQUATIC SYSTEMS

Asbestos has been found to occur in aquatic systems worldwide (Table 1). Millette et al. (1983) found that asbestos occurred at concentrations of 10^6 fibers/liter or greater in 18% of 406 water supplies in the U.S. and territories. McGuire et al. (1982, 1983) reported the presence of billions of fibers/liter in the California Aqueduct system. Reservoirs at the south end of the State Water Project allow settling out of fibers, but, water treatment plants in the region take in up to 1.3×10^9 and release 2×10^8 fibers/liter.

Commercial mining of fibrous chrysotile asbestos occurs principally in Quebec, Canada and the western United States. Smaller mines are in operation in the USSR and Rhodesia (Speil and Leineweber 1969; Selikoff and Lee 1978). Amphibole asbestos is mined principally in Australia and South Africa

Table 1. Asbestos concentrations in various aquatic systems in the world.

Location	Asbestos Concentration (fibers/liter)	Literature Source
S. Lake Michigan-raw water	$5\text{-}45 \times 10^6$	Hesse and Hallenbeck 1978
Chicago, Illinois-rainwater	$2\text{-}200 \times 10^6$	Hesse and Hallenbeck 1978
Ottawa, Canada-snowmelt	33.5×10^6	Cunningham and Pontefract 1971
33 Cities in West Germany-drinking water	$0.2\text{-}2.0 \times 10^6$	Spurny and Schormann 1983
Thetford Mines, Quebec raw water	172.7×10^6	Cunningham and Pontefract 1971
Hudson Bay, Drainage Basin-asbestos mine	$10^6\text{-}10^8$	Batterman and Cook 1981
Sultan River, Washington-raw water	$10^4\text{-}10^8$	Severson et al. 1982
Philadelphia, Pennsylvania-drinking water	1.7×10^7	Levine 1978
Duluth, Minnesota-drinking water	$10^6\text{-}10^8$	Cook et al. 1976
Silver Bay, Lake Superior-raw water	$10^9\text{-}10^{12}$	Cook et al. 1976
California Aqueduct System-raw water	$10^6\text{-}10^{10}$	Hayward 1984

(Speil and Leineweber 1969; Selikoff and Lee 1978). Additionally, asbestos is found in other mined ores such as iron taconite (Cook et al. 1974), nickel and chromite (Langer et al. 1979), and a host of talc-carbonate associations (Veblen and Buseck 1979; Buseck 1983). Environmental distribution has been attributed to several sources in addition to mining: (a) industrial emissions and waste, and car brake emissions (Cook et al. 1974; Williams and Muhlbaier 1980; McCrone 1985); (b) the use of crushed rock gravel on road surfaces which contain chrysotile (Puffer et al. 1980; Ase et al. 1982); (c) deposition of aerially suspended fibers by rain and dry fallout (Cunningham and Pontefract 1971; Hesse and Hallenbeck 1978; Mizota 1982); and (d) erosion of naturally occurring parent serpentines which contain chrysotile (McGuire et al. 1982; Hayward 1984).

EFFECTS OF ASBESTOS ON AQUATIC ORGANISMS

Several researchers have recently begun to address the effects of asbestos, particularly chrysotile which accounts for 95% of asbestos used industrially (Levine 1978), on aquatic life. The investigations span from primary producers to top level consumer fish species in the field and laboratory. Table 2 provides a summary of the present breadth of knowledge on effects to aquatic biota. In laboratory studies, Pfister (1980) and Lauth and Schurr (1983, 1984) have shown

Table 2. Summary of laboratory investigations of asbestos toxicity to aquatic organisms

Organisms	Asbestiform	Fiber Concentration (fibers/liter)	Duration of Exposure	Effects	Citation
Mytilus edulis	Chrysotile	10-100 mg/l	120 hrs	entrance into intestinal wall	Halsband 1974
Artemia salina	chrysotile crocidolite	10^7-10^8	24 hrs	mortality	Stewart and Schurr 1980
Elodea canadensis	chrysotile	150 x 10^6	10 wks	bioaccumulation	Dempster 1980
Ictaluris lacustris	chrysotile amphibole	less than 10^6 50 x 10^6	? ?	kidney and muscle accumulation	Batterman and Cook 1981
Salvelinus fontinalis	as above				
Poecilia formosa	chrysotile	0.1-10 mg/l	6 mths	kidney, gill, and heart lesions, vacuolation	Woodhead et al. 1983
Oncorhynchus kisutch *Lepomis cyanellus*	chrysotile	1.5-3.0 x 10^6	40-70 days	epidermal and lateral line lesions, loss of activity, bioaccumulation	Belanger et al. 1985
Diaptomus sicilus	amosite	0.0001-100 x 10^6	1 hr	no alteration of filtering ability	Keen et al. unpublished
Cryptomonas erosa	chrysotile	1.5-30.0 x 10^6	24-72 hrs	clumping, accumulation	Lauth and Schurr 1983, 1984

macrophytes and algae to be excellent accumulators of chrysotile without suffering toxic consequences. Cryptomonas erosa, a planktonic alga, was found to deposit fibers internally near starch granules, and externally fibers adhered to the cell wall causing cells to become clumped together (Lauth and Schurr 1983, 1984). Stewart and Schurr (1980) exposed Artemia salina, a saltwater microcrustacean, to chrysotile and crocidolite and found 20% excess mortality at 10^7 fibers/liter. However, Keen et al. (unpublished data, Biology Department, Michigan Tech, Houghton, Michigan, personal communication) found Diaptomus sicilus, an herbivorous copepod, to be unaffected relative to feeding ability up to 10^8 fibers/liter and suggested that Stewart and Schurr's (1980) Artemia data was closely related to the highly mucous-lined feeding apparatus of Artemia which caused mechanical clogging during feeding (W.J. Keen, personal communication). Halsband (1974) exposed mussels, Mytilus sp. to high concentrations of chrysotile (10-100 mg/liter) and found penetration and permanent embeddment of fibers in the gut wall after 5 days exposure. Recovery periods up to 10 days did not result in removal of accumulated asbestos. Several species of fish have been investigated and show a diverse array of responses to chrysotile. Batterman and Cook (1981) exposed brown bullheads (Ictaluris lacustris and brook trout (Salvelinus fontinalis) to contaminated Lake Superior water (undocumented concentrations) and found excess burdens in

kidney and muscle. Further, Belanger et al. (1985) documented larval coho salmon (Oncorhynchus kisutch) and juvenile green sunfish (Lepomis cyanellus) accumulate chrysotile asbestos after exposure to 10^6 fibers/liter for 40-80 days resulting in epidermal and lateral line lesions, hypertrophy and hyperplasia in the branchial region, vacuolationof epidermal cells, and a loss of motor activity. Woodhead et al. (1983) in a similar study found that the Amazon molly (Poecelia formosa), developed heart, kidney, and epidermal lesions and vacuolation after six months exposure to 0.1-10 mg/l chrysotile asbestos.

Field studies of asbestos toxicity and effects have been rare (Table 3). Macrobranchium rosenbergii, a freshwater prawn, was found to have lower production in asphalt reinforced asbestos ponds compared to dirt basins in Costa Rica (Bartlett and Enkerlin 1983). Bioaccumulation was evident in fish from western Lake Superior exposed to amphiboles (lake trout, Salvelinus namaycush) and fish from Deception Bay an arm of Hudson Bay) exposed to chrysotile (lake trout and arctic char, Salvelinus alpinus) (Batterman and Cook 1981). Fibers were embedded in kidney (41-230 fibers/mg), liver (6-27 fibers/mg), and muscle (1-21 fibers/mg) tissues. However, Keen et al. (unpublished data) did not record asbestos in fish sampled from eastern Lake Superior which has received little influence from taconite tailings containing asbestos dumped into the lake by an iron mining corporation about 60

Table 3. Summary of field investigations of asbestos toxicity to aquatic organisms

Organism	Asbestiform	Fiber Concentration (fibers/liter)	Field Site	Exposure History	Effects	Citations
Salvelinus namaycush	amphibole	50-100 x 10^6	L. Superior Minnesota	litetime (3-4 yrs)	bioaccumulation	Batterman and Cook 1981
Salvelinus namaycush	amphibole	1 x 10^6	L. Superior Michigan	lifetime (4-5 yrs)	bioaccumulation	Batterman and Cook 1981
Salvelinus alpinus	chrysotile	500 x 10^6	Hudson Bay Quebec	lifetime (7-12 yrs)	bioaccumulation	Batterman and Cook 1981
Stizostedion vitreum	??	??	Torch Lake, Michigan	lifetime (8-13 yrs)	mesothelioma	Black et al. 1983
Coregonus clupeaformis	amphibole	??	L. Superior	lifetime	none	Keen et al. unpublished
Salvelinus namaycush	amphibole	??	L. Superior	lifetime	none	Keen et al. unpublished
Osmerus mordax	amphibole	??	L. Superior	lifetime	none	Keen et al. unpublished
Macrobranchium rosenbergii	chrysotile	??	Costa Rica	lifetime (4 months)	lowered production	Bartlett and Enkerlin 1983
Salmo gairdneri	transite	??	Leetowne West Va.	lifetime	mesothelioma	Herman 1985

miles north of Duluth, Minnesota. Two studies (Black et al. 1982; Herman 1985) have observed that fish develop mesotheliomas, a cancer peculiar to asbestos exposure, but in neither study was the presence of asbestos in tissue or water determined analytically.

MECHANISMS OF ASBESTOS TOXICITY

The structural nature of asbestos fibers apparently imparts a surface charge characteristic. Chrysotile is usually positively charged (pH 4-11) and amphiboles are negatively charged. Surface charge characterisitcs and fiber dimensions are believed to influence the interaction of fibers with biological tissues (Light and Wei 1977a, 1977b; Selikoff and Lee 1978).

Asbestos has been shown to have several pathological effects including cytotoxicity, neoplasm and tumor induction, and mutagenicity (Selikoff and Lee 1978; Price-Joans et al. 1980). Chrysotile has been hypothesized to interact with sialic acid groups of cell membrane proteins resulting in cell death (Harington et al. 1975; Depasse 1982). The predominating hypothesis to explain chrysotile asbestos cytotoxicity, first described by Harington et al. (1975), states that the positively charged fiber (imparted by magnesium) causes it to bind to negatively charged sialic acid residues of membrane proteins. The binding would cause

a redistribution of glycoproteins within the membrane leading to abnormal fluxes of Na and K between the cell and its environment. This would result in osmotic imbalance and cell death. Chrysotile has been shown to be more cytotoxic than other asbestiforms, and its ability to interact with epithelial cells is cited as one reason for its importance in co-carcinogenicity (Haugen et al. 1982). Brody et al. (1983) determined asbestos fibers are bound to sialic acid groups through biochemical evidence and that such cells were morphologically distorted and developed unbalanced Na:K ratios with the medium. In contrast, Jaurand et al. (1983) and Pele and Calvert (1983) determined that removal of sialic acid groups from membrane proteins did not completely eliminate asbestos cytotoxicity and other secondary mechanisms must also be involved. A second cytotoxic mechanism involves phagocytosis of fibers by macrophages (Kagan et al. 1983; Warheit 1984). Lysosomes are induced to release cellulytic enzymes resulting in cellular histolysis (Harington et al. 1975).

Dose response relationships among asbestos exposure, cancer incidence and associated diseases remains a volatile topic in asbestos research (Graham 1981). Nicholson et al. (1981, 1982) in epidemiological surveys of workers exposed to asbestos estimate that of 27.5 million people exposed at work from 1940-1979, approximately 8200-9700 excess deaths per year will occur in association with an asbestos-induced

cancer until the year 2000 (approximately 188,000 or 0.6%). Death rates will decline, but remain high, for an additional 30 years afterward. The latency period for cancers to become clinically diagnosable range from 2 to 40 years after exposure. Risk of some cancers (e.g., lung) increase linearly with exposure (Nicholson et al. 1982), except if the person is a smoker, wherein a synergistic relationship is seen. Yet, epidemiological studies are limited in scope and applicability since most do not involve the entire range of potential contibutors to disease (Graham 1981).

Exposure is primarily limited to the upper respiratory tract and lungs, where many fibers become immediately trapped in alveolar interstitial spaces (Brody et al. 1981). However, contamination of drinking water allows a second major portal of entry by affording fibers exposure to the gastrointestinal tract and kidneys. Cook and Olson (1979) showed renal accumulation of amphibole fibers in humans who ingested asbestos tainted water. Radiotracer studies using labelled asbestos fibers have shown that fibers are easily transported to many sites within the body by mobile cells, such as macrophages, and the circulatory system (Cook 1983).

A cancer which has been linked to asbestos exposure, and appears peculiar to it, is mesothelioma, a carcinoma of the pleural and peritoneal mesothelium (Selikoff and Lee 1978; Triol et al. 1984). A concurrent reaction noted by Harington (1974) is fibrogenesis. Fibers become isolated by

macrophages, enveloped in a collagen matrix causing a thickening of the pleura. Reduction in lung capacity and increased rigidity have been described in persons diagnosed with fibrogenesis due to the activity of asbestos and other mineral fibers such as glass and fiberglass (Harington 1974; Heppleston 1984).

THE POTENTIAL OF ENVIRONMENTAL CARCINOGENESIS

Numerous authors have suggested that extrinsic environmental factors play a role in tumor development both in epizootic studies (Brown et al. 1973; Black et al. 1982) and controlled laboratory experiments (Ashley and Halver 1968; Pliss and Khudoley 1975; Black 1984). Monitoring of environmental carcinogensis in fish has only recently been recognized as a method by which fish can act as an indicator of environmental contamination of waterways, both naturally and by man (Stonstegard 1977; Harshbarger et al. 1977; Black et al. 1982). Detection of tumors, neoplastic, and precancerous growths (hyperplasia and metaplasia) requires pathological expertise and knowledge of the harmful agent. Oftentimes physiological processes in the organism are changed concurrently or precede the development of gross pathologies, such as growths or tissue discoloration (Pliss and Khudoley 1975; Lipsky et al. 1977; Klaunig et al. 1978). Reductions in growth, reproductive output, alteration of re-

spiratory function, and changes in swimming behavior have been reported in several studies which implicate suspicion for environmental carcinogens (Ashley and Halver 1968; Black et al. 1982; Belanger et al. 1985). However, most studies fail to investigate these potential correlates.

Fish have received the greatest attention in environmental carcinogensis studies, but other taxa have been used as well. From nearly 1500 diagnosed tumors and neoplastic lesions from the Smithsonian Institute Registry of Tumors in Lower Animals, 45% have been from bony fishes and 20% from mollusks (Harshbarger 1977; Harshbarger et al. 1977). Gill and germinal epithelia and epidermal integuement are most often affected in mollusks. Most of these growths are of an endemic origin, but the role of environmental carcinogenesis and contaminants has been inadequately explored in this group. Among fishes, tumor and neoplastic lesions are most often associated with gill epithelia, liver, and intestinal linings (Harshbarger 1977).

Environmental carcinogensis in walleye (Stizostedion vitreum) and sauger (Stizostedion canadense) occurs in Torch Lake, northern Michigan (Black et al. 1982). Torch Lake has retained 80% of its original volume after receiving copper mining wastes since the turn of the century. One hundred percent of the sauger and 50% of the walleye surveyed possessed neoplasms and some of these were mesotheliomas. A potential carcinogenic role for asbestos was hypothesized for

this study, but the presence of fibers in water or tissue was not determined or attempted. In a second study, Herman (1985) described a fibrous mesothelioma from a female rainbow trout (Salmo gairdneri) reared in a hatchery raceway fed by transite asbestos pipe. As in the previous study, the potential contamination by asbestos was not explored.

STATEMENT OF HYPOTHESIS

The research objectives and general experimental design described below were generated from the following hypothesis: Adverse effects of chrysotile asbestos in fish and shellfish, as measured by tumorgenesis, carcinogenesis, and impaired mechanical and physiological function should increase with duration and magnitude of exposure as levels of asbestos uptake increase.

OBJECTIVES

The objectives of this research investigation are to determine the effects of asbestos on a benthic filter feeder, the Asiatic clam (Corbicula fluminea) and two fish species; one of moderate sensitivity, the fathead minnow (Pimephales promelas) and one of high sensitivity, the Japanese Medaka (Oryzias latipes). The effects of interest were: mortality; impairments in growth, behavior, and swimming performance;

tissue pathology and cell ultrastructure, and fiber uptake in both short (96 hr) and long term (30 to 91 days) exposure regimes.

GENERAL EXPERIMENTAL DESIGN

Asiatic clams: Asiatic clams are well distributed throughout many drainage systems and lakes (McMahon 1982; Scott-Wasilk et al. 1983). The exact taxonomic status of the Asiatic clam introduced to the United States in the 1920s (McMahon 1982) is not clearly understodd (Hillis and Patton 1982). Britton and Morton (1979) originally declared the species to be Corbicula fluminea (Muller 1774). Prior to this the calm also appeared in the literature as Corbicula manilensis. The Corbicula evaluated in this study conforms to the white form (white nacre) of Hillis and Patton (1982). In addition, mollusks have been found to accumulate asbestos fibers by optical microscopy (Halsband et al. 1974). Mollusks have been used as a bioassay test organism and as an in situ field indicator for heavy metals (Graney et al. 1983), organic pesticides (Hartley and Johnston 1983), and radioisotopes (Gardner et al. 1981). The Asiatic clam is a prolific mollusk undergoing reproductive phases twice yearly (Cherry et al. 1984) Also, the clams are easily collected and maintained in the laboratory. A molluscan species was chosen to represent benthic organisms that come into prolonged con-

tact with asbestos. Asbestos has been shown to cycle throughout the water column with peak concentrations occurring during spring and fall turnover in lakes (Cook 1975) and during wet seasons in rivers (McGuire et al. 1982). Significant accumulations in the sediment have been found when asbestos settles out of the water column (McGuire et al. 1982; Hayward 1984; Bagely et al. 1981). Mollusks, like fish, have been shown to be sensitive indicators to potential carcinogens and display tumor pathologies (Harshbarger 1977). In addition, Corbicula has a unique range of behavior patterns which can be used as a functional indicator of stress (Cherry et al. 1980).

Various life history stages of Corbicula were tested. Larvae (<0.5 mm), juveniles (5.2-8.7 mm), and adults (10.3-17.2 mm) were exposed to chrysotile under various regimes. Larvae, primarily benthic veligers, were exposed to chrysotile for 48 hours using a larval capture device designed by Doherty et al. (in review). Reproductively active adults were subjected to 0-10^8 fibers/liter for two weeks and reproductive output and larval mortality were estimated every other day. Juvenile Corbicula, collected during warm and cold months, were exposed to asbestos (0-10^8 fibers/liter) for 30 days and assayed for siphoning activity, shell growth, tissue growth, tissue water content, gill tissue ultrastructure, and chrysotile fiber uptake. Adult Corbicula were exposed to asbestos for 96 hours, with and without food being available,

and for 30 days at 0-10^8 fibers/liter. Siphoning activity, shell growth, tissue growth, tissue water content, gill tissue ultrastructure, and chrysotile fiber uptake were determined for 30-day exposures, and siphoning activity and fiber uptake were analyzed after 96-hour exposures. To validate observations of fiber accumulations observed in laboratory exposed clams, Corbicula from a reservoir in the California Aqueduct System (Hayward 1984) were obtained to evaluate clams exposed for entire lifetimes under real world conditions.

Fathead Minnows: Fathead minnows are a well accepted toxicity test species of the United States Environmental Protection Agency (see Holcombe et al. 1984 for numerous examples). The wide utilization of this species has been due to its moderate sensitivity, the ability to maintain testable populations in a laboratory setting, a relatively wide geographic distribution, and the ease by which it has been adapted for reproductive, chronic toxicity studies (APHA et al. 1981)

Fathead minnows were tested at two life stages: juvenile (15.0-21.2 mm total length) and adult (36.1-47.3 mm total length). Juvenile fish were exposed to chrysotile for 96 hours in static systems (0-10^{12} fibers/liter) and for 30 days in semi-static aquaria (0-10^8 fibers/liter). Mortality and fiber uptake were of interest in the 96-hour tests and mortality, growth, and fiber uptake were determined for the

30-day tests. Adult minnows were exposed and analyzed in a similar manner for 96-hour tests, but 30-day tests were performed in semi-static artificial streams for mortality, growth, swimming performance, and fiber uptake.

Japanese Medaka: The Japanese Medaka is a small oviparous killifish in the family Cyprinodontidae. This species has become a test organism for suspected cancer agents (Klaunig et al. 1984). Its prolific reproductive nature, ease of handling, sensitivity, and rapid maturation period (six months are required to complete one life cycle) makes it an excellent organism for chronic and carcinogenicity studies.

Medaka were reared from a reproductively active population housed in Derring Hall for these studies. Newly hatched larvae were exposed to $0\text{-}10^{10}$ fibers/liter for thirteen weeks in static systems. Larvae were fed a diet of protozoa, brine shrimp flakes, or live Daphnia pulex nenoates depending on the stage of development. Growth (total length) was monitored on weeks 1, 2, 4, 8, and 13; mortality was recorded twice daily, and final condition (weight and length) was determined at the conclusion of the test. Fish were examined for tissue histology, cell ultrastructure, and fiber uptake at one and three months.

SIGNIFICANCE OF THIS RESEARCH

This research provides needed baseline data for the hazards of asbestos exposure to benthic filter-feeders (e.g., the Asiatic clam) and fish (e.g., the Fathead minnow and the Japanese Medaka). The USEPA (1979) reported that based upon accumulated data the potential of ecological effects and bioaccumulation of asbestos was low with "moderate" and "low" confidence, respectively. Demonstration of substantial effects to humans which include asbestosis, fibrogenesis, mesothelioma and co-carcinogenesis with carbon adsorbable compounds (benz-[a]-pyrene), trace metals (nickel, copper, chromium), and gases (radical oxygen, nitrite) cannot be ignored relative to potential aquatic effects.

Various researchers have determined that chrysotile can accumulate in all trophic levels present in aquatic ecosystems under laboratory conditions, and at least for fish, under field conditions. The ability to compare laboratory and field accumulations of chrysotile in Corbicula in this study offers additional potential to assay real-world application to filter feeders. In combination with ecologically important parameters such as growth and behavior, the mechanisms of asbestos toxicity during short and long term exposure can be probed.

The hypothesis of the cytotoxic mechanism described originally by Harington et al. (1975) and researched by numerous

laboratories since are strikingly similar to epidermal vacuolation in fish described by Woodhead et al. (1983) and Belanger et al. (1985). The entry of asbestos into tissue with subsequent necrotic consequences may be studied in vivo instead of by tissue culture or cell isolates. Therefore, the integration of important histological, ultrastructural, and ecological factors with fiber uptake can be explored. If asbestos induces tumors in fish an ideal system may be established as a tool in cancer research since cytotoxicity, fibrogenesis, and asbestos- induced cancers appear to be sequentially linked (Harington 1974; Harington et al. 1975; Triol et al 1984).

Fish are sensitive indicators of environmental carcinogenesis (Stonstegard 1977; Harshbarger 1977). If asbestos is shown to induce tumors over a range of environmentally realistic levels, monitoring for the documented pathologies could be used as an indicator of asbestos pollution. The range of effects already described and attributed to asbestos exposure are more diverse than researchers anticipated and suggest caution in new research. Asbestos levels are rarely monitored, even in regions which are known to be asbestos contaminated (Shear 1981). Nearly all of the areas described in the objectives represent new avenues of research, and will provide new information on the influence of chrysotile asbestos on aquatic life. This will aid in determining if asbestos uptake is of a level high enough to

warrant reevaluation of the discharge of asbestos into aquatic receiving systems as evaluated by federal, state, and industrial groups.

CHAPTER TWO: ADULT CORBICULA

The responses of adult Corbicula sp. to 0-10^8 fibers/liter were evaluated. The levels tested encompassed non-detectable to a common upper level of asbestos concentration of water. Growth, siphoning behavior, gill tissue ultrastructure and fiber uptake of adults were assessed in 96-hr to 30-day tests. Release of larvae and larval mortality were determined in 14-day experiments at the same concentrations.

Growth and siphoning activity are intimately associated and are ecologically important. Effects of asbestos on growth and siphoning may reflect the uptake of chrysotile by selected tissues with a concomittant degradation of tissue. These parameters may provide a framework to explain ecotoxicological effects in terms of fiber accumulation. Larval release patterns by adults and larval mortality were determined since the larval stage of Corbicula may be the most vulnerable to asbestos.

INTRODUCTION

Asbestos is a generic term for a group of intergrading hydrated silicate minerals with fibers having a length to width ratio of 3:1 or greater (Speil and Leineweber 1969). Mineralogically, asbestos occurs in two major forms,

serpentine chrysotile and amphibole. Chrysotile accounts for 95% of the asbestos used industrially as insulation material, fire retardant, and strengthening agent (Levine 1978).

Distribution of asbestos in water, particularly chrysotile, is known worldwide (Millette et al. 1980; Spurny and Schormann 1983), and particle concentrations in excess of 10^6 fibers/liter are common. In a survey of 406 separate water supplies in the United States, Millette et al. (1980) found nearly 20% had greater than 10^6 fibers/liter. Release of asbesots into water occurs via mining of asbestos (Cunningham and Pontefract 1971; Hayward 1984) and other minerals such as iron ore (Cook et al. 1974) and nickel (Langer et al. 1980), use of asbestos-containing materials (Puffer et al. 1980; Williams and Muhlbaier 1982), deposition of atmospherically suspended fibers (Hesse and Hallenbeck 1978; Mizota 1982), and erosion of serpentine parent rock (McGuire et al. 1982; Hayward 1984).

Asbestos has been implicated as a potent carcinogenic and toxicologic agent in mammals by numerous authors. Cook and Olson (1979) reported that amphibole asbestos was eliminated via urine in human subjects who drank asbestos-tainted water. Epidemiological studies of excess cancer risk in populations using asbestos-contaminated waters as a drinking water source have been inconclusive. Some studies (e.g., Sigurdson et al. 1981; Polissar et al. 1982) failed to link asbestos to increased risks of cancer, while others (e.g., Kanarek et al.

1980; Graham 1981) did correlate asbestos to elevated cancers of the pancreas, stomach, esophagus, and mesothelium.

The impact of asbestos on aquatic life has been largely ignored. Halsband (1974) exposed mussels (Mytilus sp.) to high concnetrations of chrysotile (1-10 mg/l) for 10 days which resulted in accumulations of fibers and blocks in intestinal lining tissue. Fibers were not displaced when mussels were replaced in clean water. Batterman and Cook (1981) determined chrysotile burdens in salmonids from Lake Superior and Hudson Bay that had histories of exposure to asbestos. Arctic char (Salvelinus alpinus) from Deception Bay, Canada (an arm of Hudson Bay) had 2.9 and 230.5 fibers/mg in muscle and kidney tissue, respectively, while the Bay had fiber concentrations as high as 6.7×10^8 fibers/liter. Woodhead et al. (1983) exposed mollies (Poecelia formosa) for 6 months to chrysotile and found fish developed epidermal hypertrophy, selective necrosis in kidney cells, and vacuolation in heart cells. Belanger et al. (1985) found that effects of chrysotile on larval coho salmon (Oncorhynchus kisutch) exposed from 40-80 days at 10^6 fibers/liter included lethargic behavior, epidermal hypertrophy, hyperplasia and selective vacuolation near the branchial region, and degradation of the lateral line system. Ecopathological studies on the effects of asbestos in aquatic organisms have not been performed in a systematic fashion, but Black et al. (1982) described mesothelioma in walleye

(Stizostedionvitreum) exposed to potentially asbestos containing copper tailings in Torch Lake, Michigan. Mesothelioma neoplastic lesions are a peculiar carcinoma that is strongly associated with asbestos exposure in mammals (Selikoff and Lee 1978; Triol et al. 1984). Evidence for concern clearly exists for aquatically transmitted asbestos.

This study evaluates short (96-hour) and long-term (30-day) effects of chrysotile asbestos to the Asiatic clam, Corbicula sp. . The exact taxonomic status of the Asiatic clam introduced to the United States in the 1920s (McMahon 1982) is not clearly understood (Hillis and Patton 1982) Britton and Morton (1979) originally declared the species to be Corbicula fluminea (Muller 1774). Prior to this the clam also appeared in the literature as Corbicula manilensis. The Corbicula evaluated in this study conforms to the white form (white nacre of Hillis and Patton (1982)).

Mortality, tissue and shell growth, siphoning behavior, gill tissue pathology, and fiber uptake were assessed for 30-day exposures in the laboratory. Larval clam mortality and release patterns from reproductive adults were assessed in 14-day exposures and mortality, fiber uptake, and siphoning behavior in 96-hour exposures. Mortality was considered to be an unlikely contributor to the analysis of the effects of asbestos on clams because previous researchers (Halsband 1974; Woodhead et al. 1983; Belanger et al. 1985) found acute mortality to be insignificant for mollusks and a variety of

fish species. Siphoning behavior and growth of Corbicula are intimately related and are of considerable ecological and toxicological importance (Haines 1979; Foe and Knight 1985a). Pathologies associated with asbestos exposure are diverse and reflect subtle stress effects. In this study, gill tissue ultrastructure was evaluated since delicate gill tissues would represent one major route of fiber accumulation. Uptake of chrysotile fibers in gill and visceral tissues was determined by transmission electron microscopy (TEM) to integrate with observations on growth and siphoning behavior. Release of larvae by reproductively active adults and larval mortality were investigated at different levels of asbestos exposure. Release of gill incubated veligers was used to corroborate siphoning behavior observations since release would be coincident with the amount of time valves were parted.

MATERIALS AND METHODS

CORBICULA COLLECTION AND HANDLING

Corbicula were selected for these studies for four reasons: (1) it resides in every major river system in the United States (McMahon 1982) and, therefore, is likely to be found within several chrysotile-contaminated waterways; (2) mollusks have been shown previously to accumulate asbestos;

(3) clams are easy to collect and observe in laboratory environments; and (4) known effects of other toxicants on clams allow comparisons.

Adult Corbicula, 12.5-17.00 mm shell length, were collected by dip net from the New River, Virginia, and transported to Virginia Tech. Clams were held in 40 liter aquaria for 7 to 14 days for acclimation to laboratory conditions and fed Chlamydomonas rheinhardti cultured in Bold's Basic medium (Carolina Biological Supply 1978). Algae was added daily to aquaria to achieve a final algal density of 10^6 cells/liter.

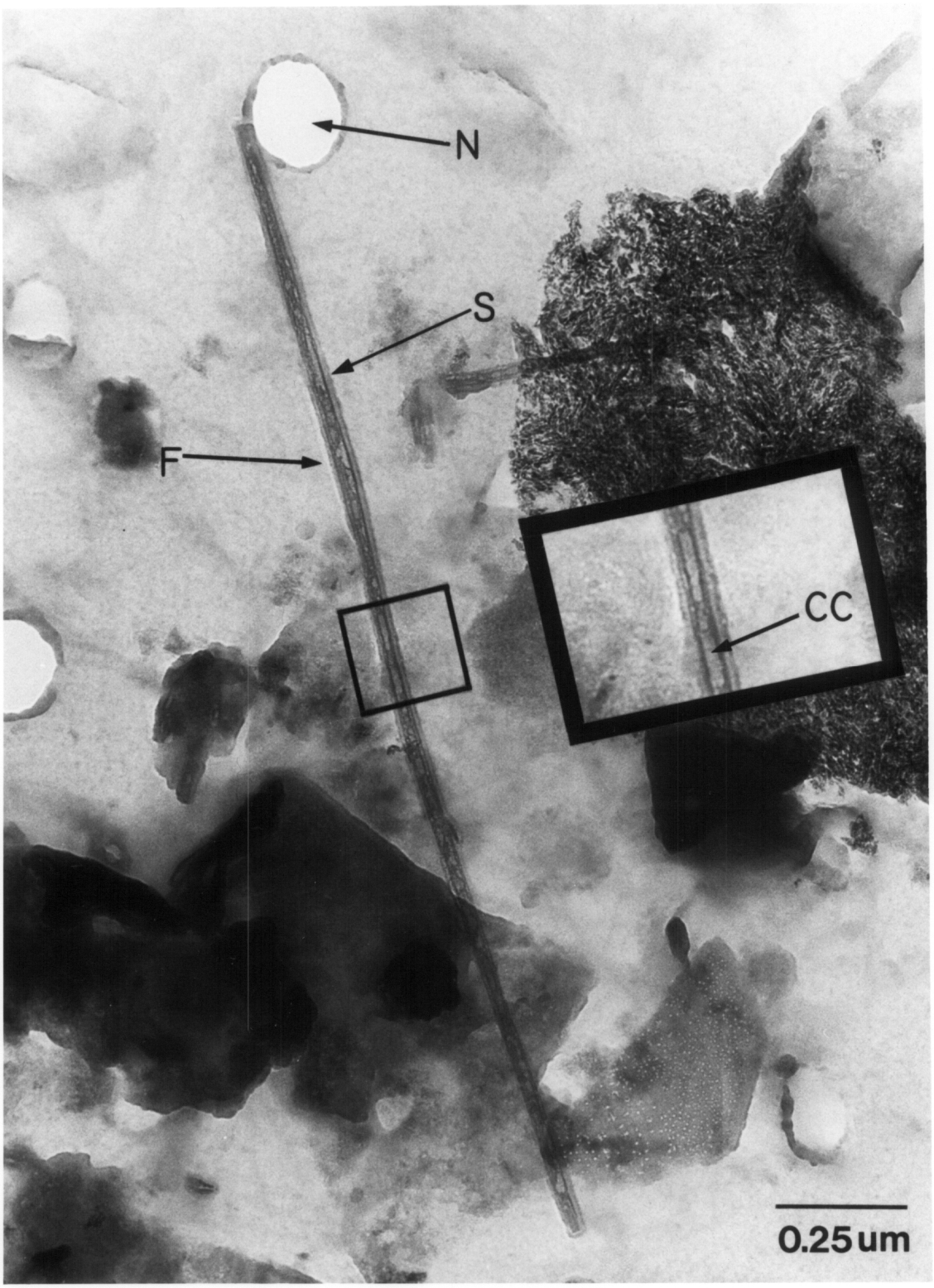
CHRYSOTILE DETERMINATIONS AND PREPARATIONS

Chrysotile was obtained from a commercial supplier as grade-5 chrysotile mined ore and was further prepared for use in these studies. Chrysotile suspensions were prepared by lightly milling 400 mg of asbestos followed by sonicating 500 ml of a 0.060 mg chrysotile/liter stock for 2 hours using a Fisher Ultrasonic Cleaner. Five replicate determinations of the fiber concentrations indicated that the actual concentration would lie between 0.25 to 8.8 x 10^9 fibers/liter by this method.

Fiber identifications and determinations were made by the methods outlined by Anderson and Long (1980). Water samples were collected in asbestos-free nalgene bottles and filtered through 0.20 µm pore polycarbonate filters. After filtering,

samples were carbon-coated, inverted, and placed in a Jaffe-Wick washer for 36 hours on copper TEM grids above filter paper saturated with chloroform. Fiber density estimates were derived by counting three replicate grids of 5 to 10 grid holes per grid (a total of 15 to 30 holes). Background (i.e., control) and blanks were processed simultaneously. Fibers were identified by the characteristic transmission pattern of a central canal and sheath appearance (Fig. 2) using a JEOL JEM 100C Transmission Electron Microscope. Time constraints limited the number of fiber concentration determinations that are possible. Individual analysis, from sample collection to concentration determination by TEM required approximately 2 days for water and 3.5 days for tissue samples. Therefore, water samples from three 96-hour and two to five 30-day experiments were pooled for analysis of realized and nominal concentrations. Actual fiber densities for control, 10^4, 10^5, 10^6, and 10^8 fibers/liter (mean ± SE) were 0 (n = 6), 0 (n = 3), 1.9 ± 0.7 x 10^5 (n = 5), 8.4 ± 4.6 x 10^6 (n = 8), and 7.5 ± 2.1 x 10^8, respectively. Detection limits for all concentrations were within an order of magnitude of the theoretical concentration. Chrysotile fibers were not detected in New River water (n = 5), dechlorinated Blacksburg town water (n = 14), and ultrapurified water (n = 3) used for laboratory purposes.

Figure 2. Chrysotile asbestos fiber (length = 2.141 µm, width = 0.035 µm) isolated from a water sample by the Jaffe-Wick Nuclepore technique. Note the characteristic canal (CC in insert) and sheath (S) of the asbestos fibril. Circular pores (N) from the original polycarbonate filter are 0.2 µm in diameter.


N
S
F
CC
0.25um

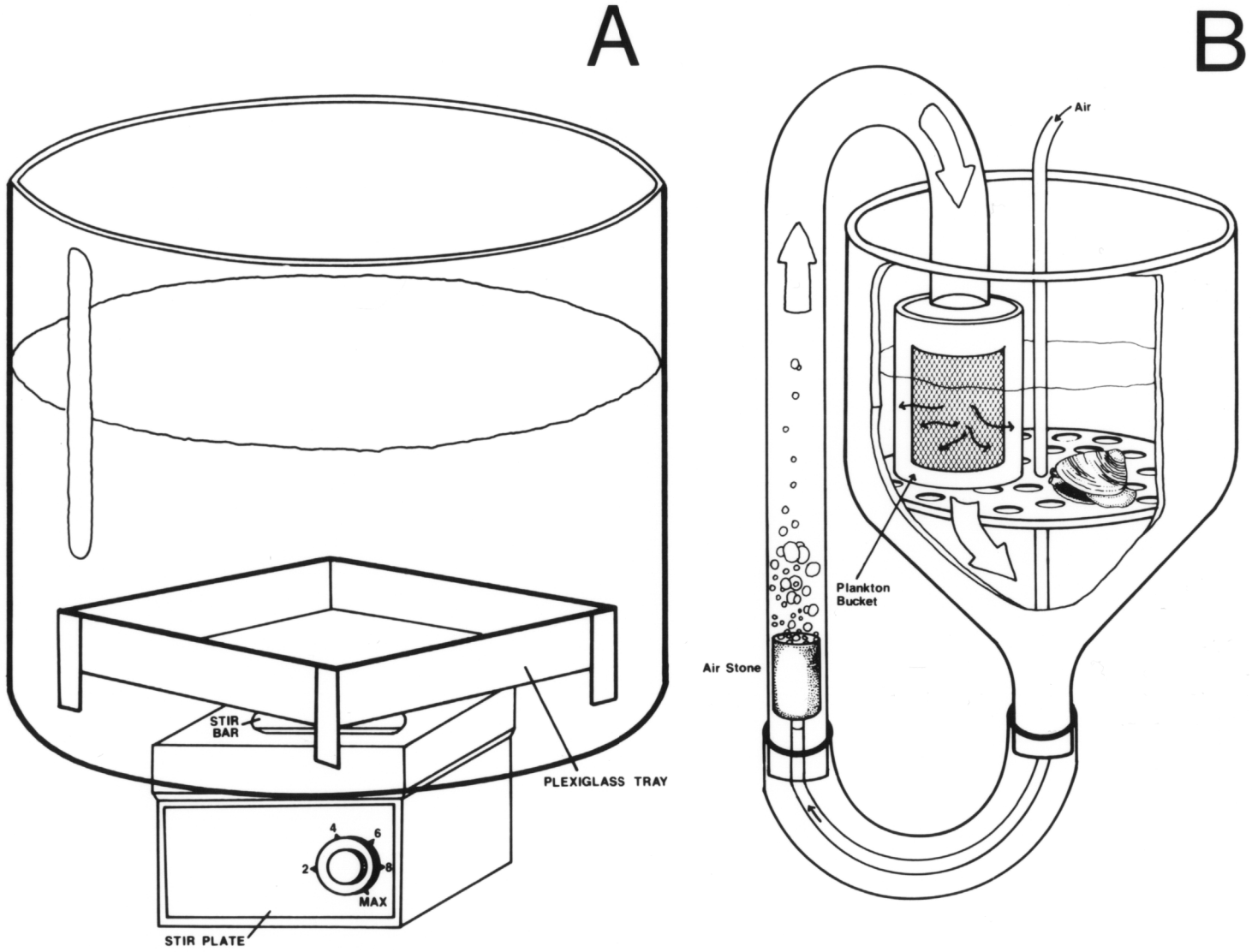
96-HOUR EXPERIMENTS

During the period of May 1983 to August 1984, Corbicula were exposed to suspensions of chrysotile asbestos at 0 (control), 10^2, 10^4, 10^5, 10^6, and 10^8 fibers/liter by placing 10 clams in a raised plexiglass platform in a 15 liter polycarbonate container (Fig. 3). The container was held on a stirplate. Stir bars and aeration kept each container well oxygenated and asbestos suspensions uniform. Water chemistry analysis was performed at the initiation and termination of each experiment. The presence of asbestos fibers did not influence water chemistry parameters. The ranges (given as the mean of the concentration giving the lowest and highest values) were: temperature, 19.7-20.5 C; pH, 6.97-7.58; dissolved oxygen, 8.1-8.6 mg/liter; hardness, 58.6-66.7 mg/liter as $CaCO_3$; specific conductance, 116.6-127.8 umhos/cm^2; NH_3-N, 0.099-0.215 mg/liter; T-PO_4, 4.96-8.03 mg/liter; NO_3-N, 5.32-8.49 mg/liter; SO_4, 16.88-22.39 mg/liter; and chloride, 8.25-12.98 mg/liter. Anomalously high nutrient levels are a result of the feeding clams algae cultured in nutrient media (described below). Two sets of experiments were performed: 96-hour with (n = 7) and without (n = 5) food added to the exposure system.

Siphoning behavior was observed at 0, 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 hours by counting the number of clams in each container with parted valves. Experiments were performed in

Figure 3. A. Exposure system used for asbestos experiments (96-hour and 30-day) that employed a raised plexiglass tray above a stir bar used to keep asbestos in suspension.

B. Device used to capture larval *Corbicula* during 14-day exposures to evaluate mortality and number of larvae released. The plankton bucket was inserted on sample days.


A
B
STIR BAR
PLEXIGLASS TRAY
4
6
2
8
MAX
STIR PLATE
Air
Plankton Bucket
Air Stone

an isolated room having a 16L/8D lighting schedule. Clams were housed in a corner at the farthest distance from the door (approximately 8 m) to eliminate disturbances prior to observations. For experiments with food offered, clams were observed two hours after algae was added to the chamber (approximately 10:30 a.m. on days 2 through 4 of each experiment).

The infiltration of asbestos into clam tissue was evaluated at the end of 96-hour experiments by the method used by Batterman and Cook (1981) as modified by Patel-Mandelik (1981). Tissue samples were excised and placed in porcelain dishes that were previously sonicated in an acid bath. Each dish was tightly covered with aluminum foil, and the tissue was ashed at 500 C for 8 hours. The ash was then suspended in 6M HCl, filtered, and otherwise treated as a water sample in preparation for TEM analysis.

30-DAY EXPERIMENTS

Corbicula were exposed to chrysotile for 30 days (n = 5) at the same concentrations used in 96-hour experiments during the peiod of July 1983 to August 1984. Experiments where algae were offered for 96-hour exposures were extended to 30 days to evaluate the longer term effects on siphoning, growth, pathology, and fiber uptake. After the initial 24-hour period, clams were observed once daily 2 hours after

feeding for siphoning activity. Growth of Corbicula was determined by measuring each individual at the beginning and end of each experiment for shell growth by vernier calipers (± 0.025 mm precision) and weight (shell plus viscera) by a Mettler PC 440 microanalytical digital balance (± 0.0005 g precision). The condition of clams was evaluated at the termination of the experiments dissecting out visceral and gill tissue, determining wet weight, and drying the sample at 90 C to constant weight (generally 48 hours). Percent tissue water was calculated by subtracting dry weight from wet weight and dividing the difference by the wet weight. Heath (1984) used this index successfully for bluegill sunfish exposed to copper and it may represent a generalized response of many organisms to a wide variety of toxicants.

Infiltration of asbestos into clam tissues was evaluated in two ways for these experiments. First, fiber burdens in gill and viscera were analyzed at the conclusion of each test using the method described previously. Fiber dimensions in visceral and gill tissue were investigated in two clams exposed to 10^8 fibers/liter for 30 days. Length, width, and aspect ratios of fibers were determined from projected micrographs measured by Vernier calipers to evaluate the relationship of water to tissue distributions. Second, gill tissue was evalauated for cell ultrastructure by TEM. Tissue was carefully dissected from clams in a bath of 5% glutaraldehyde buffered with 0.2M phosphate buffer. Tissues

were fixed for 24 hours in buffered glutaraldehyde, post-fixed in phosphate buffered 0.1% osmium tetraoxide, dehydrated through a graded ethanol series, and flat embedded in Polybed 812 resin. Blocks were cut using an American Optical Ultracut microtome with a diamond knife. Thin sections were stained with lead citrate and uranyl acetate and viewed using a JEOL JEM 100C TEM. Micrographs of gill tissue were reconstructed in composites to analyze entire gill lamellae by planimetric techniques (Sorenson and Bauer 1984). For water chemistry in 30-day exposures, the ranges (given as the mean of the concentration with the lowest and highest values) were: temperature, 19.2-19.5 C; pH, 7.15-7.29; hardness, 67.0-100.0 mg/liter as $CaCO_3$; alkalinity, 41.2-56.2 mg/liter as $CaCO_3$; specific conductance, 109.8-131.2 umhos/cm^2; NH_3-N, 0.204-0.261 mg/liter; T-PO_4, 14.16-21.83 mg/liter; NO_3-N, 13.61-17.78 mg/liter; SO_4, 18.80-20.59 mg/liter; chloride, 12.17-14.93 mg/liter.

LARVAL RELEASE AND MORTALITY

During the spring reproductive season, larval release patterns of Corbicula adults (15.5-17.2 mm) were tested during 14-day exposures to asbestos. Clams were held in 2.3 liter Buchner funnels designed to accomodate the collection of larvae, primarily as veligers (Fig. 3B). A U-shaped tube was fitted to the bottom stem of the funnel and in air stone was

inserted. A steady stream of air forced water up a column that was directed back into the funnel. At this point, 65 µm mesh plankton buckets caught larvae circulating in the water column while allowing water to remain free-flowing. Replicate containers were established at 0, 10^2, 10^4, 10^6, and 10^8 fibers/liter with 10 adults per container. Larval release and mortality were monitored every other day during the 2-week period of June 11 to June 25, 1984. The plankton bucket was inserted into the chamber for 2 hours on the day of collection allowing sufficient time for 10 complete overturns of water in the system. A concentrate of veligers and trochophores was subsampled three times from each plankton bucket, and the number of larvae present were counted by using a Sedgewick-Rafter counting chamber (1.0 ml total volume). Larval mortality was noted by counting the number of clams with and without mobility. Each larva was observed for at least one minute. The three subsamples were averaged to determine total larvae released and percent mortality in each container. At the conclusion of the 14-day period, the adults in each chamber were sacrificed, and the tissue was excised and dried. Numbers of larvae collected on each day were scaled to numbers released per milligram of total adult tissue dry weight in each container to account for differences in size among the adults.

STATISTICAL ANALYSIS OF THE RESULTS

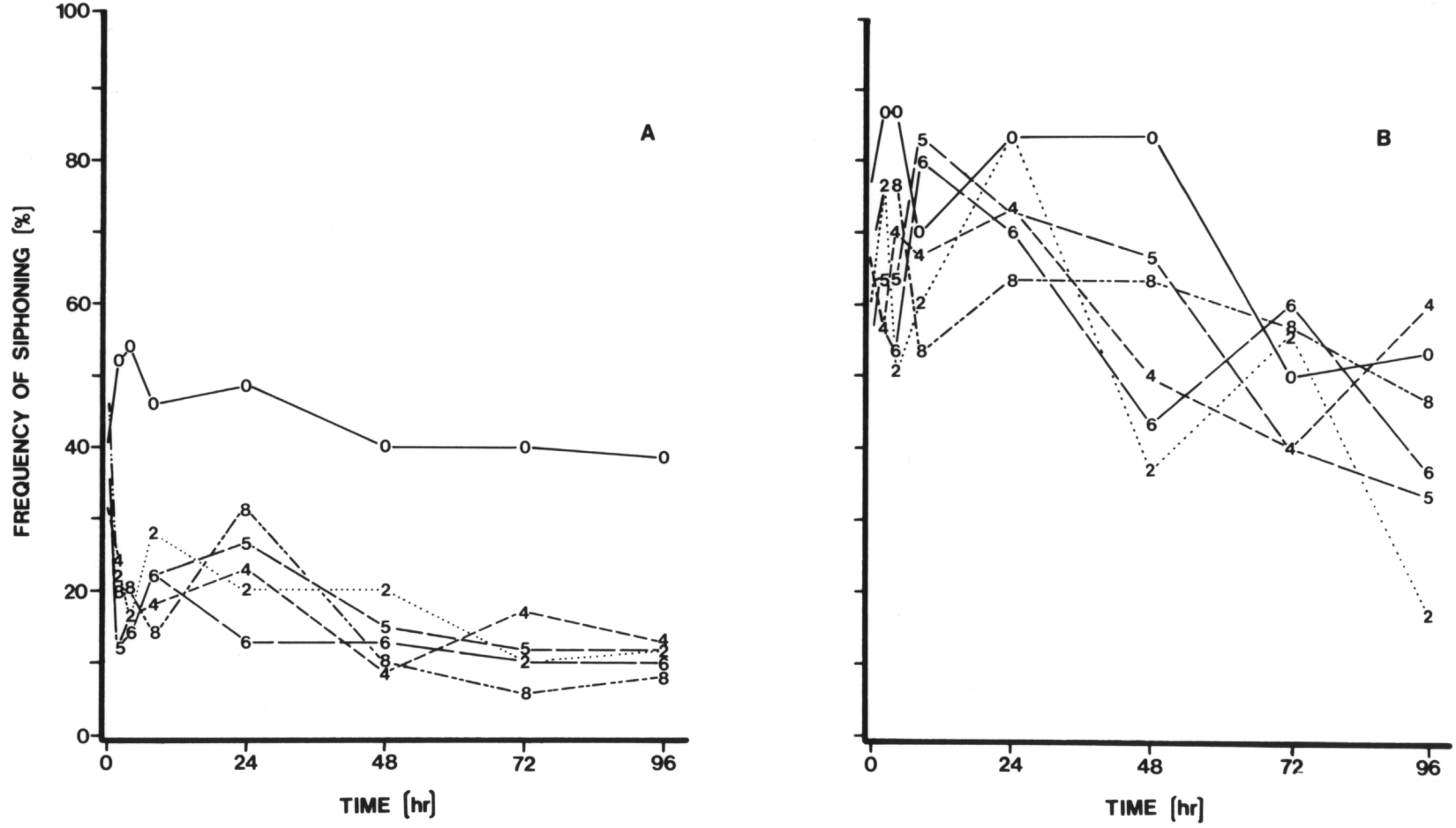
Nonparametric statistical techniques were applied in all analyses to ensure uniformity of interpretation because some, but not all, data were not normally distributed. A one-way analysis of variance rank-analogue, the Kruskal-Wallis test, was applied for one-way layout data (Hollander and Wolfe 1973). This included observations of siphoning behavior at each observation time for all experiments, clam growth and condition data for 30-day exposures, fiber dimension analysis for 30-day exposures, and larval clam mortality and release patterns from 14-day tests. If significant differences were indicated by the Kruskal-Wallis Test ($\alpha = 0.05$), a rank-like least significant differences procedure was used to determine which means were significantly different. In all comparisons of means, standard errors were calculated. In cases of two-sample data (e.g., planimetric analysis of gill tissue), Wilcoxon's Rank Sum Test was used to test differences between means.

RESULTS

96-HOUR EXPOSURES WITHOUT FOOD

Corbicula reduced siphoning activity upon exposure to

Figure 4. Mean frequency of siphoning during 96-hour experiments with no food (A) and with food (B). 0, 10^2, 10^4, 10^5, 10^6, and 10^8 fibers per liter are denoted by 0, 2, 4, 5, 6, and 8, respectively.


A
B
FREQUENCY OF SIPHONING [%]
TIME [hr]
0
20
40
60
80
100
0
24
48
72
96

chrysotile (Fig. 4A). Siphoning activity was depressed quickly (in 8 hours), and all asbestos exposures had nearly equal effect in reducing siphoning activity. By 48 hours, all asbestos exposed groups siphoned significantly less than controls ($\chi^2 = 12.215$; $p < 0.05$); by 72 hours, all asbestos exposed Corbicula siphoned with similar frequency at one-third the level of control activity ($\chi^2 = 17.375$, $p < 0.05$).

Chrysotile fibers were not oberved in any Corbicula. exposed for 96-hours without food in either gill or viscera (Table 4). Detection limits were calculated by assuming one fiber was present in the sample (Anderson and Long 1980). Generally, less than 30 fibers/mg were found.

96-HOUR EXPOSURES WITH FOOD

When Corbicula were offered Chlamydomonas, they siphoned more often in all exposures relative to non-feeding experiments (Fig. 4B). Siphoning frequency was not suppressed as observed in asbestos-treated clams in non-feeding experiments, and no significant differences were found in these experiments ($\chi^2 = 2.328$; $p > 0.05$ at 96 hours). Lower siphoning activity in all treatments by 72-96 hours was probably due to satiation as substantial amounts of pseudo-feces were observed in the plexiglass trays.

Chrysotile fibers were not found in control and 10^4 fibers/liter exposed clams at the conclusion of the 96 hour

Table 4. Chrysotile asbestos accumulations in adult Corbicula exposed in the laboratory (G = gill tissue , V = visceral tissue, W = whole clam homogenate).

Treatment (fibers/ liter)	Experiment type	Tissue	n	Fibers/mg dry weight (mean ± SE)	Detection limit (range)
Control	96-hr no food	G	6	0	15.2-23.2
		V	6	0	16.0-35.1
10^4		G	5	0	17.1-29.5
		V	5	0	18.3-22.0
10^8		G	3	0	29.4-33.4
		V	3	0	20.0-28.7
Control	96-hr with food	W	3	0	16.9-25.8
10^4		W	3	0	21.5-29.8
10^8		W	3	69.1±17.1	29.5-38.2
Control	30-day	G	3	0	18.7-22.3
		V	3	0	14.2-18.6
10^4		G	3	0	19.1-39.2
		V	3	0	30.0-32.0
10^8		G	3	147.3±52.6	18.7-32.4
		V	3	903.7±122.9	29.2-49.4

observation period using whole clam homogenates. At 10^8 fibers/liter, an average of 69.1 fibers/mg was found (Table 4), which was approximately two times above the detection limit.

30-DAY EXPOSURES

Siphoning activity was depressed upon exposure to asbestos as observed in five 30-day experiments (Fig. 5). Control and asbestos-exposed clams siphoned approximately 60% and 40-50% of the time, respectively. Control siphoning was signficantly greater than all asbestos-exposed groups, which among themselves were essentially equal.

During these same 30-day experiemnts, Corbicula grew most in control chambers, with an average increase of 0.105 ± 0.034 mm shell length (Fig. 5). Controls were signifcantly greater than asbestos-exposed groups of 10^4, 10^5, 10^6, and 10^8 fibers/liter, of which some decreased in shell length. A similar, but statistically not significant, trend was apparent in weight gain and final dry weight (Table 5). Weight gain was confounded by an apparent change in percent tissue water. Clams receiving no asbestos in the water had significantly lower tissue water than asbestos-treated clams. The increases in tissue water, although slight (1-2%), may account for no observable differences in final wet weights of Corbicula receiving asbestos exposure (Table 5).

Figure 5. Siphoning activity and growth of Corbicula during 30-day exposures to chrysotile. For siphoning data, the Kruskal-Wallis Test indicated significnat differences between groups (n=100 for all treatments $\chi^2 = 21.589$ with 5 d.f., $p < 0.005$). Means with the same letter are not significantly different ($\alpha = 0.05$). For shell growth data the Kruskal-Wallis Test also indicated significant differences between groups (n = 30 for all treatments, $\chi^2 = 23.843$ with 5 d.f., $p < 0.005$). Means with the same letter are not significantly different ($\alpha = 0.05$).

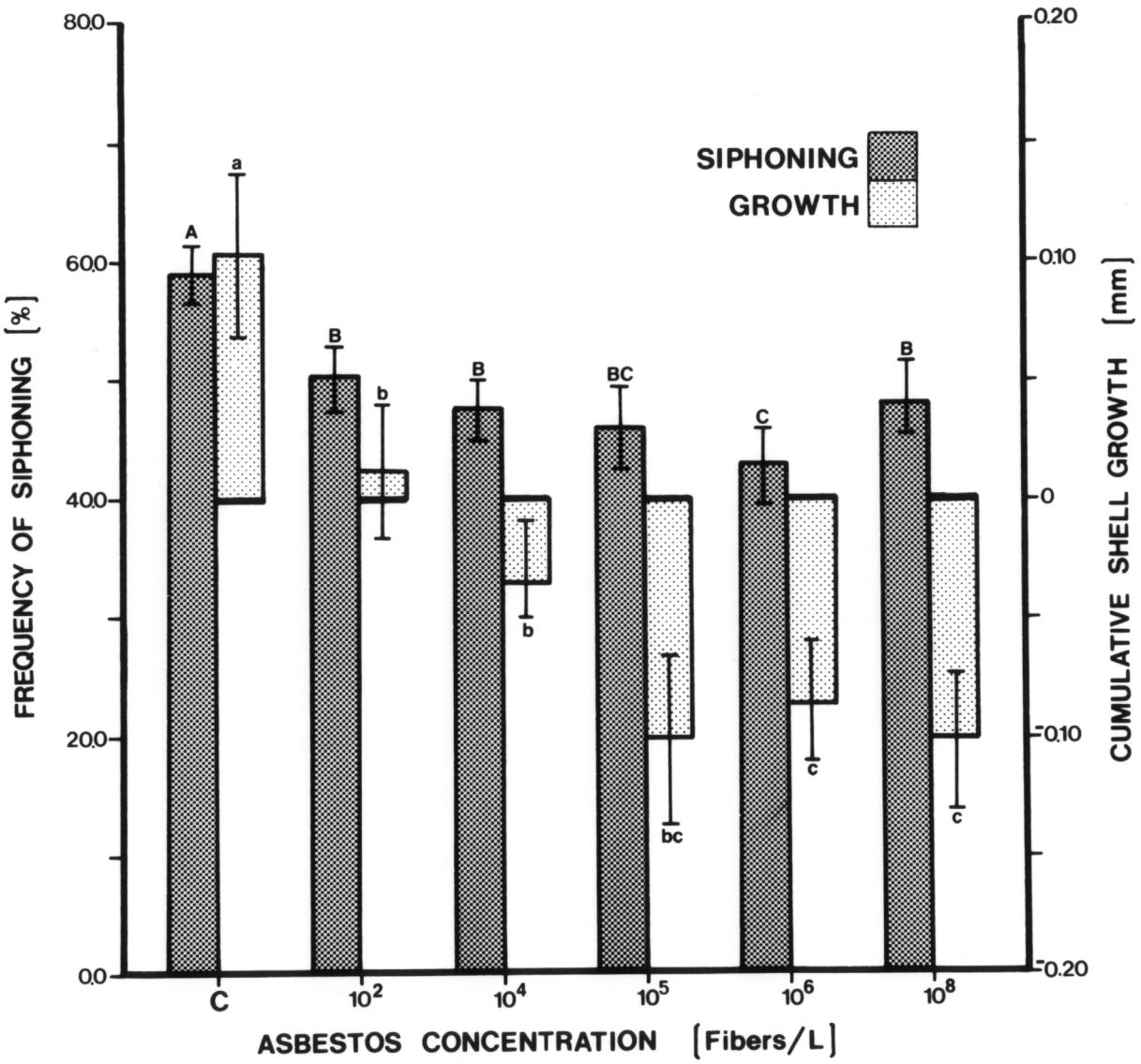

80.0
60.0
40.0
20.0
0.0
0.20
0.10
0
0.10
0.20
SIPHONING
GROWTH
FREQUENCY OF SIPHONING [%]
CUMULATIVE SHELL GROWTH [mm]
A
a
B
b
B
b
BC
bc
C
c
B
c
C
10^2
10^4
10^5
10^6
10^8
ASBESTOS CONCENTRATION [Fibers/L]

Table 5. Weight gain and final condition (wet weight, dry weight, and tissue water) for Corbicula exposed to chrysotile for 30 d. Sample sizes are indicated below the mean and standard error. Means that are not significantly different as indicated by the Kruskal-Wallis Test, are denoted by the same letter ($\alpha = 0.05$).

Parameter evaluated	Asbestos concentration (fibers/L)						Kruskal-Wallis Test (p-value)
	0	10^2	10^4	10^5	10^6	10^8	
Weight gain (g)	0.0165±0.0088 (30)	0.0076±0.0075 (30)	0.0063±0.100 (30)	0.0060±0.0099 (30)	0.0052±0.0112 (30)	-0.0002±0.0103 (30)	0.8979 (>0.95)
Wet weight (g)	0.3043±0.0194 (14)	0.2755±0.0187 (20)	0.2831±0.0214 (18)	0.2998±0.0184 (20)	0.3133±0.0246 (15)	0.3256±0.0156 (13)	2.5535 (>0.75)
Dry weight (g)	0.0423±0.0038 (14)	0.0349±0.0032 (20)	0.0376±0.0038 (18)	0.0356±0.0029 (20)	0.0383±0.0045 (15)	0.405±0.0035 (13)	4.2528 (>0.50)
Tissue water (%)	86.10±0.34[a] (14)	87.33±0.42[b] (20)	86.74±0.91[ab] (18)	88.29±0.36[b] (20)	87.81±0.63[b] (15)	87.56±0.39[b] (13)	12.4164 (>0.05)

After 30-day exposure to 10^8 fibers/liter, clams accumulated approximately 150 fibers/mg in gill and 900 fibers/mg in visceral tissues (Table 4). However, no accumulation was apparent in 10^4 fibers/liter, a concentration which was below detection in water.

Measurements of fiber dimensions from two clams exposed at 10^8 fibers/liter revealed that smaller fibers were present in gill and visceral tissues than water. Fibers suspended in water (W) averaged 6.53 ± 2.34 µm long and 0.67 ± 0.11 µm wide (n = 100); while in gill tissue (G), fibers were 1.21 ± 0.96 µm long and 0.05 ± 0.03 µm wide; in visceral tissue (V), fibers were 3.94 ± 2.10 µm long and 0.48 ± 0.32 µm wide. The Kruskal-Wallis Test indicated that for length G < V < W (χ^2= 21.41; $p < 0.005$) and for width G = V < W ($\chi^2 = 17.29$; $p < 0.005$). The mean aspect ratio, length/width, was 9.7 for W, 24.2 for G, and 8.3 for V.

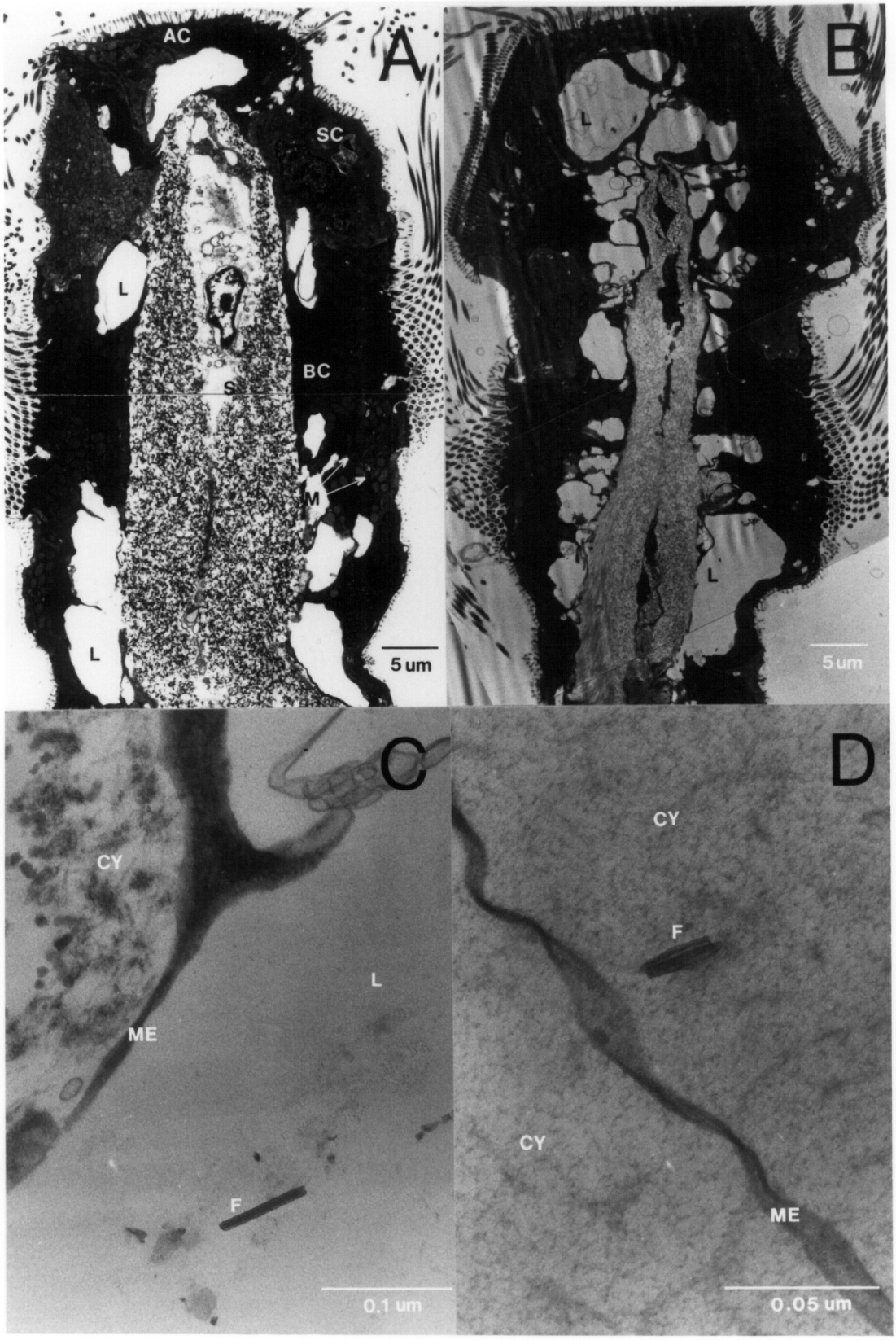
Since asbestos was not accumulated in clams exposed to 10^4 fibers/liter, only control and 10^8 fibers/liter gill tissue were analyzed by TEM. In gill tissue of controls, the central support cell is surrounded by unciliated secretory cells and ciliated cells located apically and basally on the lamella (Fig. 6). Open locules, which are apparently fluid-filled, are evenly dispersed along the support cell (Fig. 6A). Locules were 14.01 ± 1.96% of the total surface area of the lamella as measured by planimetry (n = 5). An average of 21.4 ± 4.8 locules were observed in each gill lamella. Gill tissue

Figure 6. A. Gill lamella from a control clam held in the laboratory for 30 days. Locules (L) are interspersed among basal (BC) and apical (AC) ciliated cells and secretory cells (SC). Ciliated cells are mitochondria-rich (M). A central support cell (S) lies inside the peripheral cells and locules.

B. Gill lamella from a clam exposed to 10^8 fibers/liter chrysotile for 30 days. Locules appear with significantly greater frequency and occupied greater surface area than controls.

C. Chrysotile fibers (F) were found most often in gill locules. No fibers were observed in direct association with cell membranes (ME).

D. Two fibers were found in the cytoplasm (CY) of ctenidial cells.


A
AC
SC
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BC
S
M
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B
L
L
5 um
C
CY
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ME
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0.1 um
D
CY
F
CY
ME
0.05 um

from clams exposed to chrysoitle for 30 days possessed significantly more locules in each lamella, 25.7 ± 3.2 (Wilcoxon Rank Sum = 48.0; $p < 0.005$), and locules occuppied signif- greater surface area of the lamella, 26.7 ± 2.2 (Wilcoxon Rank Sum = 51.0; $p < 0.001$) (Fig. 6B). Eleven asbestos fibers were observed embedded in gill tissue. Eight fibers (72%) were in locules, apparently not embedded in a tissue matrix (Fig. 6C). Two fibers were located in connective ctenidial tissue (Fig. 6D), and one fiber was depositied in an apical ciliated cell. At no time were chrysotile fibers found in association with cellular isolating mechanisms (e.g., vacuoles, collagen or macrophages).

LARVAL RELEASE PATTERNS

Control gravid clams released larvae at the rate of 175-800 veligers/adult every two days. Larvae released by adults experiencing asbestos exposure fell as low as 10/adult every two days in 10^8 fibers/liter. On an adult dry weight basis, the numbers released in control, 10^2, 10^4, 10^6, and 10^8 fibers/liter were 29.23 ± 10.15, 14.43 ± 6.21, 9.03 ± 4.19, 5.97 ± 5.28, and 7.58 ± 2.65, respectively. The Kruskal-Wallis Test indicated that the release of control > 10^2 > 10^4 > 10^6 = 10^8 fibers/liter (Chi-square = 29.735; $p < 0.005$). During this same period, the percent mortality of control, 10^2, 10^4, 10^6, 10^8 fibers/liter treated groups was

11.26 ± 2.19, 18.26 ± 7.15, 25.13 ± 6.14, 26.69 ± 9.77, 27.60 ± 8.73, respectively. The Kruskal-Wallis Test indicated that mortality of control < 10^2 = 10^4 = 10^6 < 10^8.

DISCUSSION

Chrysotile asbestos has been shown to reduce Corbicula siphoning activity in this study. Reductions in siphoning were 10 to 20% of control activity in all asbestos exposures over a 30-day period. This resulted in significant reductions in shell growth, and the same trend existed for weight gain over the 30-day period. All levels of asbestos had the same inhibitory effect. These observations are consistent with studies by Foe and Knight (1985a) who fed clams a diet of algae and small particulate inorganic sediment (98% was smaller than 64 µm and the modal size was 14 µm) in varying concentrations. Growth was significantly depressed in groups fed particulates and algae than algae alone, perhaps due to the inability of clams to distinguish between organic and inorganic matter. In this study, when no food was offered to clams in 96-hour experiments, clams rapidly reduced siphoning activity in asbestos exposures to one-third the level of controls. However, when food was offered, this inhibitory effect was overriden and siphoning frequency remained unaltered. Controls exhibited shell and tissue growth, and our findings are similar to those of Dauble et al. (1985) and Foe

and Knight (1985b) who investigated nutritional needs of Corbicula. Dauble et al. (1985) determined that approximately 1000 cells/ml (summer phytoplankton density) gave adequate growth while 350 cells/ml (winter phytoplankton density) did not using flow-through Columbia River water (Washington state). Foe and Knight (1985b) found that tri-algal diets of green algae (Chlamydomonas, Chlorella, and Ankitrodesmus) gave superior growth compared to di-algal formulations at 1 x 10^6 cells/ml in 10 liter aquaria with 18 clams per aquarium. In this study (10 clams/15 liters), the effective algal concentration per clam was 1.5 x 10^6 cells/day, whereas Foe and Knight (1985b) used 5.5 x 10^8 cells/day. In comparison, both these values are at or above the upper limit of river phytoplankton densities (Hynes 1970; Cohen et al. 1984), and it is apparent that more information on the nutritional and ecological needs of Corbicula need to be investigated in the laboratory relative to growth potential in freshwater rivers.

Corbicula is an efficient accumulator of chrysotile asbestos. Uptake is relatively low in short-term, 96-hour experiments, but is well above the level of detection at 10^8 fibers/liter of exposure and is consistent with 30-day fiber burden observations. The body burdens reported in this study upon exposure to 10^8 fibers/liter for 30 days represent the greatest body body concentration known for this duration of exposure. In 30-day tests, the uptake of fibers also inhib-

ited growth and suppressed siphoning activity. Kidneys from Arctic char that experienced 3-4 years of exposure to 10^8 fibers/liter had 230 fibers/mg. Patel-Mandelik (1981) reported that human subjects with lifetime exposures to asbestos developed kidney tumors from 9500 fibers/mg in kidney tissue. Halsband (1974) reported that mussels exposed to chrysotile accumulated fibers and blocks of asbestos that they were unable to depurate. In the context of these observations combined with our study, it appears likely that clams may bioaccumulate fibers through a lifetime of exposure. Lauth and Schurr (1983, 1984) exposed a phytoflagellate, Cryptomonas erosa, to chrysotile. Algal cells deposited fibers internally and adhered externally; therefore, it is conceivable that food sources may represent another vector for fiber uptake in suspension feeding bivalves.

Evidence for pathological and toxicological effects of chrysotile to Corbicula, in addition to inhibition of growth and siphoning, is presented in this study. Tissue water content of clams exposed to chrysotile for 30 days increased relative to controls. This is consistent with electron microscopy observations of gill tissue where fluid-filled spaces were significantly increased in number and size upon exposure to 10^8 fibers/liter relative to controls. Fibers were also isolated in locules more frequently than in other gill regions. The size distribution of asbestos fibers in water versus clam tissues in asbestos-stressed clams indicate

that small fibers were taken up most easily. Small, thin particulates have been shown to be more biologically interactive than large blocky particulates (Cook et al. 1982; Hesterberg and Barrett 1984). Intracellular distributions, phagocytosis, transport and clearance of fibers are believed to be correlated with fiber size with small, high aspect ratio fibers being most mobile (Selikoff and Lee 1978; Cook 1983). Fibers in visceral tissue were intermediate in size between water and gills, probably due to the presence of some larger fibers that were not yet embedded in the visceral area but may have been in the process of being moving through the gastrointestinal tract of the clam.

Mortality of adults was not observed in 96-hour experiments and was not significant in 30-day tests. Belanger et al. (1985) demonstrated that mortality for fish was not greatly enhanced by asbestos exposure in 2 to 3-month tests at 10^6 fibers/liter, but that pathology and behavioral effects were evident and were better indicators of subtle, potentially chronic effects. In this study, asbestos influenced larval clam survivorship. Asbestos exposure increased mortality by 7 to 17% in exposures of 10^2 to 10^8 fibers/liter relative to controls. The effect on actual recruitment to Corbicula populations is difficult to infer from this data since no published studies exist on natural survivorship of veliger Corbicula. Stewart and Schurr (1980) demonstrated larval Artemia salina had 20% mortality above baseline con-

trol mortality after 24-hour exposures to 10^7 fibers/liter chrysotile asbestos. Further evidence of chrysotile effects on sensitive or critical life stages of aquatic organisms is needed.

To date, chrysotile asbestos has not been reported as a significant water quality problem for aquatic life. Amphibole asbestos is a well documented contaminant of Lake Superior but is disregarded in contaminant programs of the Great Lakes (Shear 1981). This aspect is probably due to the fact that the majority of toxicity information generated for bivalves and fish has been from acute exposure type with mortality as the end point. Effects of asbestos on Corbicula at levels known to occur in the environment that we present here include growth inhibition, alteration of siphoning behavior, gill tissue degradation, and reduced condition of clams with a concomittant uptake of chrysotile fibers. This study imparts two final caveats. First, clams may become useful indicators of asbestos contamination since clams are efficient accumulators of fibers. Lifetime exposures, even at non-detectable concentrations (e.g., $< 10^5$ fibers/liter) in water, may conceivably result in detectable tissue concentrations. This result is not suprising for bivalves as accumulation and indicator potential has been demonstrated for organic compounds (Hartely and Johnston 1983) and heavy metals (Graney et al. 1983). Second and more important, this and recent studies by Woodhead et al. (1983), Lauth and

Schurr (1983, 1984), and Belanger et al. (1985a) dictate that a review of the effects of asbestos on aquatic life is needed. The previously "low confidence" in the bioaccumulation data base for asbestos by aquatic organisms (USEPA 1979) is now moderately confident that bioaccumulation is possible at all trophic levels. Recent programs instituted by the USEPA in California include establishing a coastal monitoring program for asbestos in mussels (Brian Melzian, USEPA, Region IX, 215 Freemont St., San Fransisco, California 94105, personal communication). Caution is warranted as industrial use and environmental distribution of chrysotile continues to expand and the potential impact by asbestos is increased to humans, fish, and shellfish.

CHAPTER THREE: JUVENILE CORBICULA

The responses of juvenile Corbicula collected during summer and winter seasons to 0-10^8 fibers/liter of chrysotile were evaluated. Growth, siphoning behavior, gill tissue ultrastructure, tissue water content and fiber uptake of clams were assessed during both seasons in 30-day tests.

The uptake and resulting toxicity of chrysotile may be modified due to the conditions of clams during winter and summer when allocations to shell and weight growth can differ. As with adult Corbicula, siphoning activity and growth are ecologically important, and effects on one should be mirrored by effects on the other. Tissue water content was included as a tissue condition index to support gill tissue ultrastructure and fiber uptake observations.

Juvenile Corbicula may be intermediate in sensitivity to asbestos exposure between adult and larval life stages. The determination of effects of chrysotile to these major stages of Corbicula life history can provide a more complete analysis of the influence of asbestos on mollusks than previous studies

INTRODUCTION

The effects of asbestos upon aquatic organisms have received little attention since the discovery of asbestos contamination in Lake Superior in the early 1970's (Cook et al. 1974) and the subsequent documentation of widespread contamination in the United States and abroad (Millette et al. 1980; Mizota 1982; Spurny and Schormann 1983). Spurny and Schormann (1983) demonstrated in 18 water supplies tested in the Federal Republic of Germany that seven contained 0.9 x 10^6 fibers/liter or greater. Millette et al (1980) compiled data from 406 water sources in the United States and found that 73 (18%) were above 10^6 fibers/liter. In regions of Canada where asbestos is mined, as in Quebec, concentrations in water are 10^7-10^8 fibers/liter (Cunningham and Pontefract 1971; Batterman and Cook 1981).

The effects of chrysotile asbestos on aquatic organisms are of particular importance since this asbestiform accounts for 95% of the asbestos used industrially. Most asbestos utilized in the United State is imported and approximately 96% comes from Canada (Levine 1978). Halsband (1974) exposed mussels (Mytilus sp.) to 10-100 mg/liter of chrysotile and found fibers and asbestos blocks embedded in intestinal lining tissue. Once exposure was relieved, fibers were not displaced. Batterman and Cook (1981) surveyed salmonids (Arctic char, Salvelinus alpinus, and lake trout, Salvelinus

namaycush) that were sampled in regions of high chrysotile asbestos contmaination (in excess of 10^8 fibers/liter). Tissue levels of chrysotile were 2.9-5.4 and 6.5-230.5 fibers/mg dry weight for kidney and liver, respectively. Woodhead et al. (1983) exposed Amazon mollies (Poecilia formosa) to chrysotile and found epidermal hypertrophy and necrosis of kidney, heart, and epidermal tissues. Similarly, Belanger et al. (1985) exposed larval coho salmon (Oncorhynchus kisutch) and juvenile green sunfish (Lepomis cyanellus) to 10^6 fibers/liter for 40-80 days and described epidermal hypertrophy superimposed on hyperplasia, necrotic epidermis, lateral line degradation, and lesions near the branchial region. Lateral line abnormalities were associated with a loss of swimminig and orientational ability. Although few studies on asbestos effects on aquatic organisms are available, Black et al. (1982) and Herman (1985) describe walleye (Stizostedion vitreum) and rainbow trout (Salmo gairdneri) as possessing mesothelioma neoplasms, a tumor peculiar to asbestos exposure in mammals. The case for asbestos influence is strengthened in wallleye as these fish were exposed to copper mine tailings that could contain amphibole asbestos (Black et al. 1982). Effects of chrysotile asbestos on microcrustacea (Artemia salina) included 20% excess mortality at 10^7 fibers/liter (Stewart and Schurr 1980). Lauth and Schurr (1983, 1984) documented Cryptomonas erosa, a

phytoflagellate, to accumulate chrysotile intracellularly and adhere extracellularly.

Long-term chronic effects on juvenile Corbicula sp., the Asiatic clam, were investigated to assess fiber uptake and behavioral and growth responses. Recent studies by Dauble et al. (1985) and Foe and Knight (1985) have shown the importance of initial organism condition, test conditions, and diet quality on growth of Corbicula in the laboratory. Clams are weakened during winter conditions in North America due to reduced internal metabolic reserves, food resources, and cold winter temperatures (Dauble et al. 1985). As a consequence of the initial condition of clams collected at different times of the year, the response from exposure to a toxic substance may be vastly different in the laboratory. Evaluation of the effects of chrysotile to Corbicula must necessarily include this aspect of the ecology of the organism.

MATERIALS AND METHODS

COLLECTION AND HOLDING

Juvenile Corbicula (5.2-8.6 mm shell length) were collected from the New River Virginia, by dip net adjacent to an industrial pumphouse station (Celanese Fibers Corporation, Narrows, Virginia). Juvenile clams were sorted from adults

and sediment in the field and returned to Virginia Tech where they were acclimated to constant temperature laboratory conditions for 7 days in 40 liter aquaria. Tests during both seasons were performed at the same laboratory temperature in accordance with Standard Methods (APHA et al. 1981) and standard toxicity protocols. Four collections were made in warm water conditons at 17-23 C (7 June and 16 August 1983, and 12 May and 2 July 1984), and two collections were made in cold water at 8-12 C (17 December 1983 and 18 February 1984).

ASBESTOS EXPOSURE AND GROWTH

Clams were exposed to 0, 10^2, 10^4, 10^5, 10^6, 10^8 fibers/liter chrysotile asbestos in 4 liter aquaria situated above a magnetic stirrer that kept asbestos in suspension. Asbestos stocks were prepared by lightly milling 400 mg of asbestos, followed by sonicating 500 ml of a 0.060 mg/liter chrysotile stock for 2 hours with a Fisher Ultrasonic cleaner. Groups of 10 clams were placed in a raised plexiglass platform of 315 cm^2 surface area in each tank. Growth was monitored during the 30-day exposure by weighing and measuring (total shell length) each clam using a micro-analytical electronic Metler PC 440 balance (± 0.0005 g precision) and Vernier calipers (± 0.025 mm), respectively. Clams were fed diet of 1000 cells/ml of green algae

(Chlamydomonas rheinhardti) cultured in Bold's Basic Medium (Carolina Biological Supply 1978) each day. Two hours after feeding (approximatley 10:00 AM), the number of clams in each chamber with valves parted were counted as an indication of siphoning activity.

TISSUE CONDITION, ULTRASTRUCTURE, AND FIBER ANALYSIS

At the conclusion of the 30 day exposure, clams were sacrificed for three types of analyses: tissue water content, body burdens of asbestos fibers, and gill ultrastructure. Tissue water content was determined by dissecting the visceral mass, weighing the tissue, drying at 90 C for 48 hours, and reweighing the dried tissue. Tissue water content was calculated by substracting wet from dry weight and dividing by wet weight. Asbestos fibers in tissue were analyzed by the methods described by Batterman and Cook (1981) and Patel-Mandlik (1981). Gill and visceral tissue were dissected, dried for 48 hours at 90 C, and then ashed at 500 C for 8 hours. Ash was suspended in 6M HCl, filtered through a 0.2 µm pore polycarbonate Nuclepore filter, and carbon coated with a Ladd Vacuum Evaporator. Small (2 mm^2) pieces of coated filters were cut and inverted onto 200 mesh brass Transmission Electron Microscope (TEM) grids. The grid and filter preparation was transferred to a Jaffe-Wick washer and placed above several filter papers saturated in chloroform

for 36 hours. This technique allows asbestos fibers present in tissue to be permanently embedded in a carbon matrix suitable for TEM (Fig. 7). Three replicate grids and 5 to 10 holes per grid were viewed for each sample at 20,000 X using a JEOL JEM 100C TEM. For in-depth analysis of fiber size distributions in gill and visceral tissues, three clams exposed to 10^8 fibers/liter were used. Micrographs were taken of the first 15-25 fibers encountered and subsequently measured for length, width, and aspect ratio.

Ultrastructural analysis of gill tissue was performed on clams exposed from collections in warm and cold water conditions. Gill tissue was excised intact from clams exposed to 0 and 10^8 fibers/liter and preserved in 5% glutaraldehyde in 0.2M phosphate buffer at pH 7.2. Gills were post-fixed in 0.1% osmium tetraoxide, series alcohol dehydrated, infiltrated with propylene oxide, and flat embedded in Polybed 812 resin. Tissue was thin-sectioned with a diamond knife on an American Optical Ultracut Microtome. Sections were stained with lead citrate and uranyl acetate and viewed at 3300X with a JEOL JEM 100C TEM to reconstruct composite photomicrographs of entire gill lamellae. Gill lamellae were analyzed planimetrically to quantitate structural differences between groups in the manner of Sorenson and Bauer (1984).

Water chemistry analyses were performed on days 0 and 30 of each experiment (Table 6). Nutrients (total phosphate, nitrate, sulfate, and chloride) were analyzed by column ion

Figure 7. Chrysotile fibers isolated from water (A) and Corbicula gill tissue (B) by the nuclepore technique. Asbestos fibers were identified by characteristic central canal and tubular sheath described by Anderson and Long (1980).

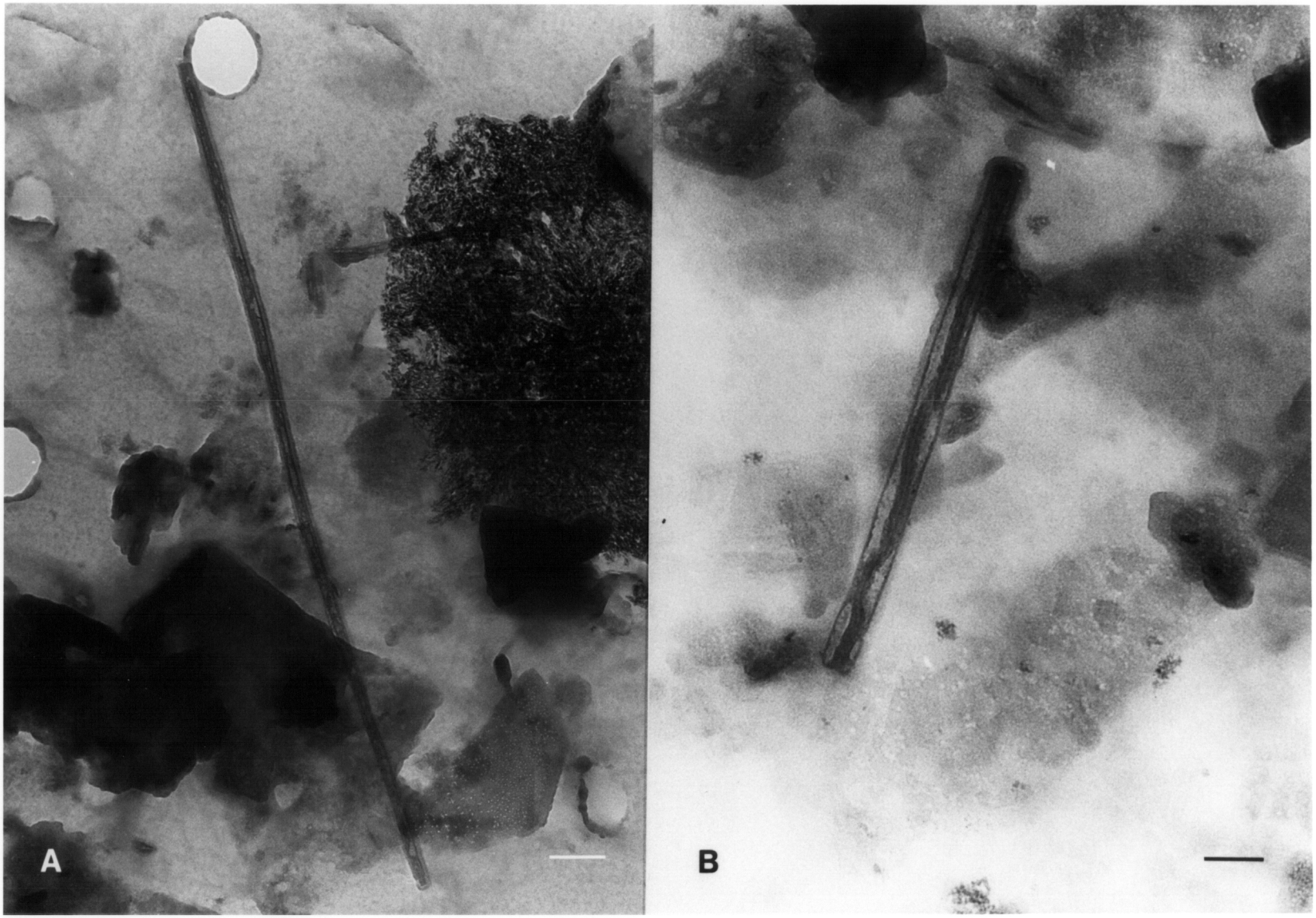

A
B

Table 6. Asbestos fiber concentration and water chemistry analyses for winter and summer exposed juvenile Corbicula. Mean ± SE are given above the sample size.

Parameter	Chrysotile concentration (fibers/liter)						
	Initial	0	10^2	10^4	10^5	10^6	10^8
Chrysotile concentration (fibers/liter)	-	BDL* (9)	BDL (3)	BDL (2)	5.7×10^5 (2)	1.3×10^7 (4)	2.1×10^8 (3)
	Winter						
Temp (°C)	20.0 ± 0 (2)	20.2 ± 0.35 (2)	20.5 ± 0 (2)	20.2 ± 0.35 (2)	20.5 ± 0.7 (2)	20.2 ± 0.35 (2)	20.2 ± 0.35 (2)
pH	6.95 ± 0.07 (2)	7.0 ± 0.14 (2)	6.85 ± 0.07 (2)	7.23 ± 0.04 (2)	7.07 ± 0.04 (2)	7.00 ± 0.07 (2)	7.02 ± 0.18 (2)
Hardness (mg/liter) as $CaCO_3$)	65.0 ± 3.5 (2)	65.0 ± 7.0 (2)	72.5 ± 3.5 (2)	75.0 ± 0 (2)	65.0 ± 0 (2)	60.0 ± 0 (2)	60.0 ± 3.5 (2)
Alkalinity (mg/liter) as $CaCO_3$)	42.0 ± 3.6 (2)	44.5 ± 6.4 (2)	46.5 ± 0.7 (2)	48.5 ± 2.1 (2)	43.0 ± 2.8 (2)	44.5 ± 2.1 (2)	43.5 ± 2.1 (2)
Conductivity (umhos/cm^2)	148.0 ± 11.3 (2)	147.5 ± 3.5 (2)	150.0 ± 9.9 (2)	152.0 ± 7.1 (2)	157.0 ± 12.7 (2)	156.5 ± 4.9 (2)	147.0 ± 2.8 (2)
Ammonia (mg/liter)	0.038 ± 0.032 (2)	0.403 ± 0.110 (2)	0.178 ± 0.073 (2)	0.142 ± 0.062 (2)	0.387 ± 0.142 (2)	0.263 ± 0.095 (2)	0.143 ± 0.079 (2)
T-PO_4 (mg/liter)	-	19.98 ± 5.52 (2)	17.21 ± 6.54 (2)	16.15 ± 10.73 (2)	22.31 ± 7.51 (2)	16.75 ± 43.9 (2)	16.11 ± 8.47 (2)

Table 6. Continued

Parameter	Chrysotile concentration (fibers/liter)						
	Initial	0	10^2	10^4	10^5	10^6	10^8
NO_3 (mg/liter)	-	10.13 ± 8.36 (2)	8.75 ± 8.67 (2)	7.76 ± 4.90 (2)	9.75 ± 4.81 (2)	14.12 ± 5.38 (2)	19.71 ± 9.50 (2)
SO_4 (mg/liter)	-	26.63 ± 12.33 (2)	23.49 ± 8.02 (2)	21.02 ± 5.94 (2)	26.19 ± 10.24 (2)	16.50 ± 3.09 (2)	19.019 ± 0.94 (2)
Cl (mg/liter)	-	10.84 ± 1.69 (2)	8.28 ± 2.75 (2)	11.00 ± 3.38 (2)	12.06 ± 3.31 (2)	8.61 ± 1.82 (2)	15.29 ± 7.57 (2)
	Summer						
Temp (°C)	20.2 ± 0.3 (2)	20.4 ± 0.5 (4)	20.3 ± 0.3 (4)	20.3 ± 0.3 (4)	20.3 ± 0.3 (4)	20.3 ± 0.3 (4)	20.3 ± 0.3 (4)
pH	7.38 ± 0.62 (4)	7.48 ± 0.31 (4)	7.71 ± 0.48 (4)	7.60 ± 0.35 (4)	7.46 ± 0.18 (4)	7.55 ± 0.25 (4)	7.61 ± 0.37 (4)
Hardness (mg/liter as $CaCO_3$)	72.0 ± 3.8 (4)	77.5 ± 10.6 (4)	72.5 ± 10.6 (4)	72.5 ± 8.6 (4)	65.0 ± 4.1 (4)	68.7 ± 6.3 (4)	66.3 ± 7.5 (4)
Alkalinity (mg/liter as $CaCO_3$)	40.0 ± 2.8 (4)	42.0 ± 4.2 (4)	43.2 ± 2.2 (4)	40.5 ± 4.9 (4)	43.3 ± 1.4 (4)	39.2 ± 3.8 (4)	41.0 ± 3.5 (4)
Conductivity (umhos/cm^2)	-	153.0 ± 5.6 (2)	156.0 ± 4.2 (2)	158.5 ± 14.8 (2)	154.5 ± 7.8 (2)	159.5 ± 13.4 (2)	156.5 ± 8.9 (2)
Ammonia (mg/liter)	0.040 ± 0.009 (2)	0.211 ± 0.114 (4)	0.199 ± 149 (4)	0.142 ± 0.107 (4)	0.141 ± 0.078 (4)	0.286 ± 0.223 (4)	0.232 ± 0.095 (4)

Table 6. Continued

Parameter	Chrysotile concentration (fibers/liter)						
	Initial	0	10^2	10^4	10^5	10^6	10^8
$T\text{-}PO_4$ (mg/liter)	-	17.39 ± 10.79 (4)	16.15 ± 7.47 (4)	14.25 ± 7.8 (4)	15.74 ± 11.36 (4)	16.30 ± 11.24 (4)	16.25 ± 12.44 (4)
NO_3 (mg/liter)	-	15.25 ± 9.04 (4)	17.02 ± 12.74 (4)	16.02 ± 10.71 (4)	13.55 ± 7.09 (4)	13.64 ± 8.85 (4)	14.70 ± 10.08 (4)
SO_4 (mg/liter)	-	16.31 ± 4.06 (4)	20.49 ± 7.41 (4)	19.19 ± 5.64 (4)	21.21 ± 5.69 (4)	18.92 ± 7.50 (4)	16.53 ± 6.69 (4)
Cl (mg/liter)	-	15.27 ± 7.39 (4)	16.87 ± 8.68 (4)	16.06 ± 8.51 (4)	17.01 ± 7.83 (4)	17.48 ± 9.96 (4)	17.76 ± 10.25 (4)

*BDL = below detection limits

chromotography with a Dionex Ion chromotagraph, pH by a Fisher Model 650 pH meter, ammonia by the Nesslerization method, alkalinity and hardness by titration (APHA et al. 1981). Asbestos fiber concentrations in water were determined by the TEM method described above except that water samples were directly filtered onto Nuclepore filters. Background and blanks were processed simultaneously.

STATISTICAL ANALYSIS

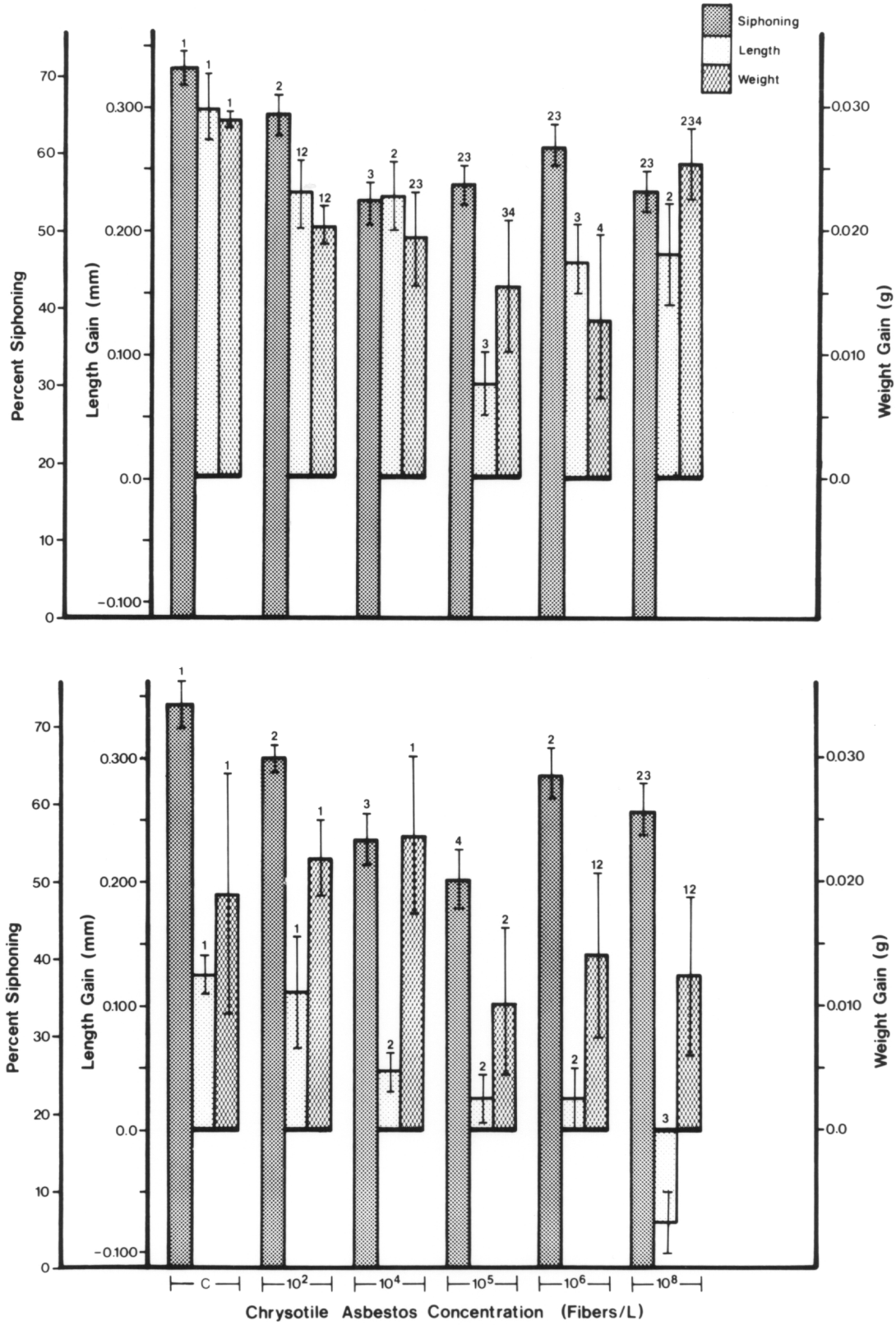
Nonparametric statistical techniques were applied in all analyses. The one-way analysis of variance rank-analogue, the Kruskal-Wallis Test, was used for one-way layout data (Hollander and Wolfe 1973), i.e., tissue and shell growth, tissue conditon, siphoning activity, and fiber dimension analysis. If significant differences were indicated (α = 0.05), a rank-like Least Signficant Differences Procedure was used to determine the relationships between groups. In cases of two sample data, (i.e., planimetric analysis of gill tissue), Wilcoxon's Rank Sum Test was used to test differences between groups (Hollander and Wolfe 1973).

RESULTS

SIPHONING ACTIVITY AND GROWTH

Siphoning activity was significantly depressed in all asbestos exposures relative to controls in winter and all exposures except 10^2 fibers/liter in summer ($p < 0.05$) (Fig. 8) Controls in summer had a mean siphoning frequency of 70.8%, while clams exposed to chrysotile ranged from 53.1% (10^4 fibers/liter) to 64.7% (10^2 fibers/liter). Controls in winter had a mean siphoning frequency of 73.8%, while clams exposed to chrysotile ranged from 50.3% (10^5 fibers/liter) to 65.8% (10^2 fibers/liter). Shell and tissue growth were reduced in accordance with lower siphoning in asbestos exposed clams. Clams exposed in the summer had significantly less shell and tissue growth at 10^4 fibers/liter and above compared to controls ($p < 0.05$). Clams exposed to 10^4 fibers/liter and above in winter had significantly reduced shell growth; however, tissue growth was only significantly altered at 10^5 fibers/liter compared to controls. The relative shell growth:tissue growth (mm/mg) was greater in summer than winter tests, and the ratio tended to be greater in clams exposed to no or low asbestos concentrations. The ratio of shell:tissue growth of clams exposed in the summer were 0.0135, 0.0102, 0.0138, 0.0048, 0.0130, and 0.0077 for control, 10^2, 10^4, 10^5, 10^6, and 10^8 fibers/liter, respectively.

Figure 8. Siphoning activity and growth (tissue and shell) of Corbicula collected and tested during the summer (A) and winter (B). Means are indicated by the height of the bar ± 1SE. The Kruskal-Wallis statistics for summer siphoning, shell growth, and tissue growth were 25.581, 18.978, and 13.659, respectively, and for winter were 25.655, 48.857, and 12.135, respectively (all $p < 0.05$). Significantly different groups are indicated by different numbers above the error bar.


Siphoning
Length
Weight
Percent Siphoning
Length Gain (mm)
Weight Gain (g)
70
60
50
40
30
20
10
0
0.300
0.200
0.100
0.0
-0.100
0.030
0.020
0.010
0.0
C
10^2
10^4
10^5
10^6
10^8
Chrysotile Asbestos Concentration (Fibers/L)

During winter, the ratios were (given in the same order), 0.0064, 0.0053, 0.0016, 0.0018, 0.0013, and -0.0058.

The final condition of clam tissue (wet and dry weight) at the conclusion of the 30-day tests in both seasons was not significantly different between groups (Table 7). The ranges for dry and wet weight for summer were 0.0662-0.0780 g and 0.0111-0.0136 g, and for winter were 0.0588-0.0651 g and 0.0090-0.0114 g. The simultaneous comparison of dry and wet weight presented in tissue water content revealed significantly less water present in control (81.32%) and 10^2 fibers/liter groups (80.75%) compared to 82.98-85.70% at 10^5-10^8 fibers/liter in summer. In winter, control (82.71%), 10^2 (81.80%), and 10^4 (82.90%) fibers/liter groups were signifcantly lower than higher asbestos exposures (84.01-85.62% at 10^5- 10^8 fibers/liter).

MORTALITY AND FIBER ACCUMULATION

Few Corbicula died during both series of experiments. No deaths were recorded at concentrations below 10^8 fibers/liter. Two of 120 (1.7%) died at 10^8 fibers/liter in summer, and three of 60 (5%) died at the same concentration in winter.

Fibers were accumulated in gill and visceral tissues in clams exposed to 10^8 fibers/liter for 30 days (Fig. 7). Control and 10^4 fibers/liter groups were below detection in both

Table 7. Tissue condition analyses (mean ± SE) for juvenile Corbicula exposed to chrysotile asbestos for 30 days in the laboratory. Sample sizes are n = 15 for summer and n = 10 for winter condition Corbicula. Means with the same letter are not significantly different ($\alpha = 0.05$).

Group	Parameter	Asbestos concentrations (fiber l/liter)						
		0	10^2	10^4	10^5	10^6	10^8	Kruskal-Wallis (p-value)
Summer	Wet weight (g)	0.0731 ± 0.0240	0.0690 ± 0.0301	0.0745 ± 0.0280	0.0662 ± 0.199	0.0780 ± 0.0323	0.0688 ± 0.0309	2.3142 (p>0.900)
	Dry weight (g)	0.0136 ± 0.0016	0.0133 ± 0.0021	0.0123 ± 0.0014	0.0133 ± 0.0009	0.0111 ± 0.0018	0.0111 ± 0.0010	6.9177 (p>0.250)
	Tissue water content (%)	81.32^a ± 2.64	80.75^a ± 3.78	83.45^b ± 7.31	82.98^b ± 1.94	85.70^c ± 2.06	83.88^b ± 1.04	12.750 (p<0.025)
Winter	Wet weight (g)	0.0599 ± 0.0190	0.0625 ± 0.0287	0.0651 ± 0.0313	0.0588 ± 0.0214	0.0630 ± 0.0215	0.0600 ± 0.0199	1.795 (p>0.950)
	Dry weight (g)	0.0103 ± 0.0011	0.0114 ± 0.0015	0.0111 ± 0.0017	0.0091 ± 0.0010	0.0101 ± 0.0009	0.0090 ± 0.0011	5.355 (p>0.250)
	Tissue water content (%)	82.71^a ± 3.15	81.80^a ± 2.60	82.90^a ± 1.74	84.63^b ± 2.78	84.01^b ± 2.00	85.62^b ± 1.95	14.216 (p<0.025)

seasons. Fiber burdens were approximately 10 times greater in viscera (1100 fibers/mg) than gill (150 fibers/mg) tissue (Table 8). Analysis of fiber size distributions in summer exposed clams indicated smaller fibers were present in gill compared to viscera (Table 9). Aspect ratios (length:width) were relatively homogeneous with a few exceptions (e.g., Clam C viscera). Visceral tissue contained fibers intermediate in size relative to gills and water. The greater density of fibers and their size distributions in visceral tissue samples suggest that many of these fibers may not have been embedded in the tissue per se, but solely present in the gastrointestinal lumen.

The primary ultrastructural response of Corbicula gill tissue upon exposure to asbestos was an increase in the size and surface area of locules in the gill (Fig. 9). Control clams in winter and summer possessed gill lamellae in which locules accounted for 16.7 ± 4.2 and 14.7 ± 3.1% of the total lamellae surface area respectively and 10^8 fibers/liter exposed clams in winter and summer had 27.6 ± 7.2 and 23.1 ± 3.5%, respectively. In both winter and summer, asbestos exposed clams were significantly greater (Rank Sum = 85, $p < 0.05$ in winter; Rank Sum = 71, $p < 0.05$ in summer). Representative planimetric outlines of gill lamellae from control (A and B) and 10^8 fibers/liter exposed clams (C and D) are given in Fig. 9. Other cellular responses such as

Table 8. Asbestos fiber burdens in clams exposed to chrysotile for 30 days during two seasons.

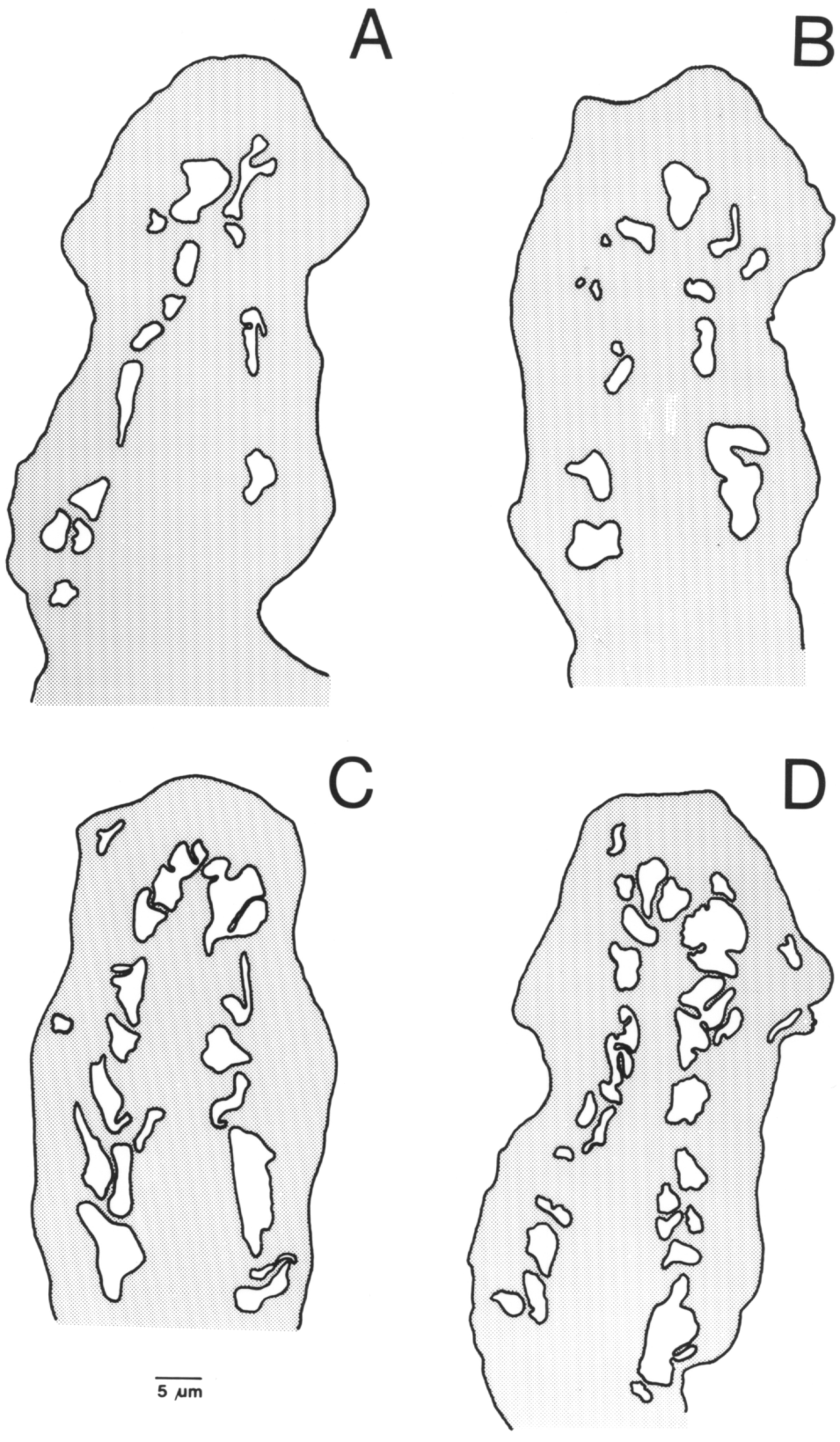
Season	Asbestos Exposure (fibers/ liter)	Tissue	n	Fibers/mg dry weight mean ± SE	Detection Limit (range)
Summer	Control	gill	2	BDL[1]	28.0-62.1
		viscera	2	BDL	39.3-81.5
	10^4	gill	3	BDL	30.5-71.3
		viscera	3	BDL	40.6-53.1
	10^8	gill	4	147.5 ± 30.9	83.1-100.2
		viscera	4	1127.4 ± 190.2	105.1-181.1
Winter	Control	gill	2	BDL	29.0-47.5
		viscera	2	BDL	21.8-39.7
	10^4	gill	2	BDL	61.4-88.2
		viscera	2	BDL	42.4-59.6
	10^8	gill	4	132.1 ± 36.4	67.4-93.2
		viscera	4	1055.1 ± 235.9	112.4-170.4

[1]BDL = below detection limit

Table 9. Comparison of fiber size distributions (mean ± SE) in water, gill tissue, and visceral tissue of Corbicula exposed to 10^8 fibers/liter for 30 days in the summer. The Kruskal-Wallis statistic was 37.614, 24.218, and 14.729 for length, width and aspect ratio, respectively. Means with the same letter are not significantly different ($\alpha = 0.05$).

Fiber source	n	Length (um)	Width (um)	Aspect ratio (length:width)
Water	100	6.45 ± 0.239^{a}	0.57 ± 0.009^{b}	11.3 ± 0.6^{b}
Gill (Clam A)	16	0.749 ± 0.081^{b}	0.079 ± 0.009^{b}	9.5 ± 2.8^{b}
Gill (Clam B)	19	0.832 ± 0.091^{b}	0.099 ± 0.012^{b}	8.4 ± 1.9^{ab}
Gill (Clam C)	24	0.715 ± 0.060^{b}	0.059 ± 0.009^{b}	12.1 ± 1.8^{b}
Viscera (Clam A)	21	2.751 ± 0.252^{c}	0.313 ± 0.037^{ac}	8.8 ± 0.7^{ab}
Viscera (Clam B)	17	3.319 ± 0.172^{c}	0.292 ± 0.008^{c}	11.4 ± 0.6^{b}
Viscera (Clam C)	15	1.977 ± 0.315^{c}	0.338 ± 0.029^{ac}	5.9 ± 0.9^{a}

Figure 9. Planimetric outlines of Corbicula gill lamellae after 30 days in the laboratory. Controls (A and B) had few, small locules as compared to clams exposed to 10^8 fibers/liter (C and D).


A
B
C
D
5 µm

fibrogenesis, phagocytosis, hypertrophy, or hyperplasia, were not observed in association with asbestos fibers.

DISCUSSION

SIPHONING AND GROWTH

Asbestos is ascribed here to reduce siphoning activity and subsequently reduce growth (shell and tissue weight) of clams collected during both summer and winter seasons and then tested under standard laboratory conditions. However, the energy allocated to shell vs tissue growth shows seasonal and dose dependence. During winter, when clams were in an emaciated condition initially, more energy was allocated to tissue rather than shell growth when compared to summer. Russel-Hunter et al. (1984) described shifts in snail growth allocations (Halisoma trivolis and Lymnaea palustris) during winter with some examples of tissue degrowth under extreme winter conditions. Kat (1982) described shell decalcification (i.e., shell degrowth) as a major cause of Corbicula mortalities during exposure to acidic pH of 5.6 in natural populations. During winter, some clams (e.g., 10^8 fibers/liter group) gained weight while losing shell length in this study; however, mortality was negligible (5%) and more likely associated with reduced siphoning activity. Dauble et al. (1985) tested growth patterns of Corbicula collected during winter

and summer and reared at different temperatures and reported growth patterns similar to that reported here. The daily food allocation to each exposure chamber (1000 cells/ml in our study) was equal to that required by clams in summer to elicit significant growth (Dauble et al. 1985). The inclusion of seasonal characterisitcs to toxicity studies using Corbicula is warranted for proper evaluation of the potential effects of any toxicant.

FIBER ACCUMULATIONS

Fiber accumulations were high in visceral and gill tissue after 30 days of exposure to 10^8 fibers/liter (Table 9). Fiber burdens are greater than those documented in the literature for fish exposed for their lifetimes to 10^8 fibers/liter (230 fibers/mg in Arctic char kidney) (Batterman and Cook 1981). Fiber accumulations in gills are reflected in deteriorated gill tissue and greater tissue water content in asbestos exposed clams (Fig. 9). Increase in locule surface area has potential osmoregulatory and oxygen transfer efficiency implications for asbestos stressed clams. Growth responses described earlier may also be attributed in part to gill tissue alterations.

Size distribution of fibers in water vs gill vs viscera suggested that small fibers were absorbed preferentially and that some fibers present in the viscera were not necessarily

embedded in situ. Small fibers of high aspect ratios are considered the most biologically interactive (Selikoff and Lee 1978). Isolation mechanisms against fibers, such as fibrogenesis or phagocytosis of fibers, were not observed in clam tissues, which suggests that fibers could pose long-term, chronic problems at the cellular level. Halsband (1974) found that mussels (Mytilus sp.) exposed to asbestos did not depurate fibers and asbestos blocks from intestinal lining tissue.

The resulting impact of asbestos on Corbicula populations in the field deserves inquiry. Corbicula were impaired at concentrations above 10^4 fibers/liter in both seasons in this study. This is considered the lower limit of detection by present technologies. Corbicula are residents of every major drainiage system in the continental United States (McMahon 1982), some of which have high asbestos content (Hayward 1984). Due to the high bioaccumulatory potential of asbestos in clams, they may prove to be an ideal indicator species for this contaminant. Graney et al. (1983, 1984) and Hartley and Johnston (1983) showed Corbicula to be an efficient accumulator of heavy metals and organic pesticides, respectively. The widespread distribution of this mollusk could allow a systematic survey of asbestos contamination in waterways across the United States and abroad. The full life cycle uptake of a non-depuratable substance such as chrysotile would allow evaluation of waterways where asbestos is below

instrumentational detection (e.g., < 10^5 fibers/liter) and a more comprehensive analysis and understanding of the potential influences of asbestos on aquatic life.

CHAPTER FOUR: FIELD-LABORATORY COMPARISONS

Fiber accumulation in adult Corbicula exposed to chrysotile for 30 days in the laboratory were compared to adult Corbicula exposed to 10^9 fibers/liter under natural conditions in Lake Silverwood, California. Corbicula were collected from California in the fall of 1983 and winter of 1984. Accumulations and bioconcentration factors of gill and visceral tissues and whole clam homogenates were determined for all three groups to determine whether laboratory exposures would mimic field exposures, an essential requisite for the employment of Corbicula as an in situ biomonitor.

INTRODUCTION

Chrysotile asbestos has been found in water throughout the United States as a result of mining activities (Batterman and Cook 1981; Cook et al. 1974), deposition of atmospherically suspended asbestos (Cunningham and Pontefract 1971; Hesse and Hallenbeck 1978; Mizota 1982), and non-point asbestos emissions (Hesse and Hallenbeck 1978; Puffer et al. 1980; Millette et al. 1980; Ase et al. 1982). Millette et al (1980, 1983) determined that of 406 water supplies surveyed in the United States and its territories, 18% were in excess of 10^6 fibers/liter.

Numerous studies have investigated the relationship between asbestos in water and excess cancer risks to populations so exposed. Kanarek et al. (1980) and Conforti et al. (1981) determined that populations in the highly contaminated San Francisco Bay Area had elevated risks of pancreatic, peritoneal, and gastrointestinal cancers. Other studies of the Duluth, Minnesota region, another site with highly contaminated water (> 10^6 fibers/liter), to have elevated risk of cancers of the pancreas only (Sigurdson et al. 1981). Studies in the contaminated Puget Sound Region found no correlation between asbestos in drinking water and elevated cancer risk; however, Kanarek (1983) points out that small sample sizes associated with smaller populations (of Duluth and Puget Sound) lower the sensitivity of these epidemiological studies.

Few studies have investigated effects of chrysotile in aquatic systems. The USEPA (1979) suggested that asbestos would not bioaccumulate in aquatic organisms, but that the confidence of the statement relative to the amount of data collected was low. Batterman and Cook (1981) and Belanger et al. (1985a) found fish to accumulate chrysotile in the laboratory and field. Long-term exposures to chrysotile in fish result in lesions of the epidermis, heart, lateral line, and kidney (Woodhead et al. 1983; Belanger et al. 1985a). Belanger et al. (1985a) found that pathologic stress was associated with impaired swimming and rheotaxic ability.

Halsband (1974) and Belanger et al. (1985b) studied effects of asbestos on mussels (Mytilus sp.) and Asiatic clams (Corbicula sp.), respectively. Halsband (1974) determined that mussels accumulated asbestos fibers and asbestos blocks in intestinal lining tissue. Fibers remained embedded after mussels were removed to clean water. Belanger et al. (1985b) found Corbicula to accumulate fibers in gill and visceral tissue after 30-day exposures to 10^8 fibers/liter and that accumulations were associated with deteriorated gill tissue, reduced growth, and impaired siphoning activity.

Corbicula has been touted as a potential biomonitor for heavy metals (Graney et al. 1983) and organic compounds (Hartely and Johnston 1983) and has been employed in acute and chronic toxicity studies (Rodgers et al. 1980; Graney et al. 1983, 1984; Harrison et al. 1984). The wide distribution of Corbicula throughout North America, its ease of collection, sedentary nature, relative hardiness, and ability to maintain in the laboratory make it an ideal indicator organism (Graney et al. 1983; McMahon 1982).

In 1982, chrysotile asbestos was discovered in the California Aqueduct System (CAS), primarily due to natural erosion of serpentine parent rock, although the region is also heavily mined for asbestos (McGuire et al. 1982). The discovery of 10^6-10^8 fibers/liter resulted in various state agencies investigating methods of removal. McGuire et al. (1983) describe the removal of chrysotile by present water

treatment technology, and reductions by a factor of 10^4 was obtained on one occassion. Hayward (1984) investigated the contaminated region further and found ambient levels in the Aqueduct System were highest after storms which eventually contributed to the load of asbestos in the sediment. This region was considered ideal for determining the potential use of Corbicula as an in situ asbestos biomonitor. Clams have intimate contact with asbestos-laden sediment, filter contaminated water, and possibly ingest algae which accumulate fibers (Lauth and Schurr 1983, 1984) and, therefore, integrate all possible modes of uptake by aquatic species. Belanger et al. (1985b) determined Corbicula to be an efficient accumulator of asbestos in the laboratory and Eng (1979) documented it's population dynamics in the California Aqueduct System.

This study was undertaken to address two objectives: (1) to document the total body and tissue (gill and visceral mass) burdens of chrysotile in the field during two periods of the year in the CAS; and (2) to relate results from long-term laboratory exposures to field determinations under real-world conditions.

MATERIALS AND METHODS

COLLECTION OF CLAMS

Field collections of *Corbicula* sp. exposed for their lifetimes to asbestos in the CAS at Lake Silverwood, California were made at a depth of 45 m on 7 October 1983 and 2 February 1984. Clams ranged from 17.2-25.65 mm total shell length.

Clams used in laboratory experiments were collected at 1.0-1.5 m depth in the New River, Virginia during May, 1983 to August 1984. A complete description of the exposure methodology can be found in Belanger et al. (1985b) and are briefly summarized here. Clams were acclimated for 7 to 14 days in aquaria prior to exposure and were fed 10^6 cells/liter of *Chlamydononas rheinhardti* throughout the acclimation and testing period. *Corbicula* were exposed to 0 to 10^8 fibers/liter of chrysotile for 30-days in circular, 15 liter polycarbonate jars. A stir bar above a magnetic stirrer and an air stone kept fibers in suspension. Ten clams were placed in each chamber in a raised plexiglass holder.

COLLECTION OF TISSUE FOR ANALYSIS

Clams from California were preserved in 10% formalin in the field for transport to VPI & SU. After arrival at the laboratory, clams were measured for shell length and dissected. Some clams were prepared for analysis using the entire visceral mass and gills (hereafter referred to as a whole clam homogenate). Others had gill tissue and viscera excised separately.

Clams exposed to 0, 10^4, and 10^8 fibers/liter in the laboratory were killed and dissected after 30 days of exposure. Clams were measured for shell length and dissected as described above.

ASBESTOS DETERMINATIONS.

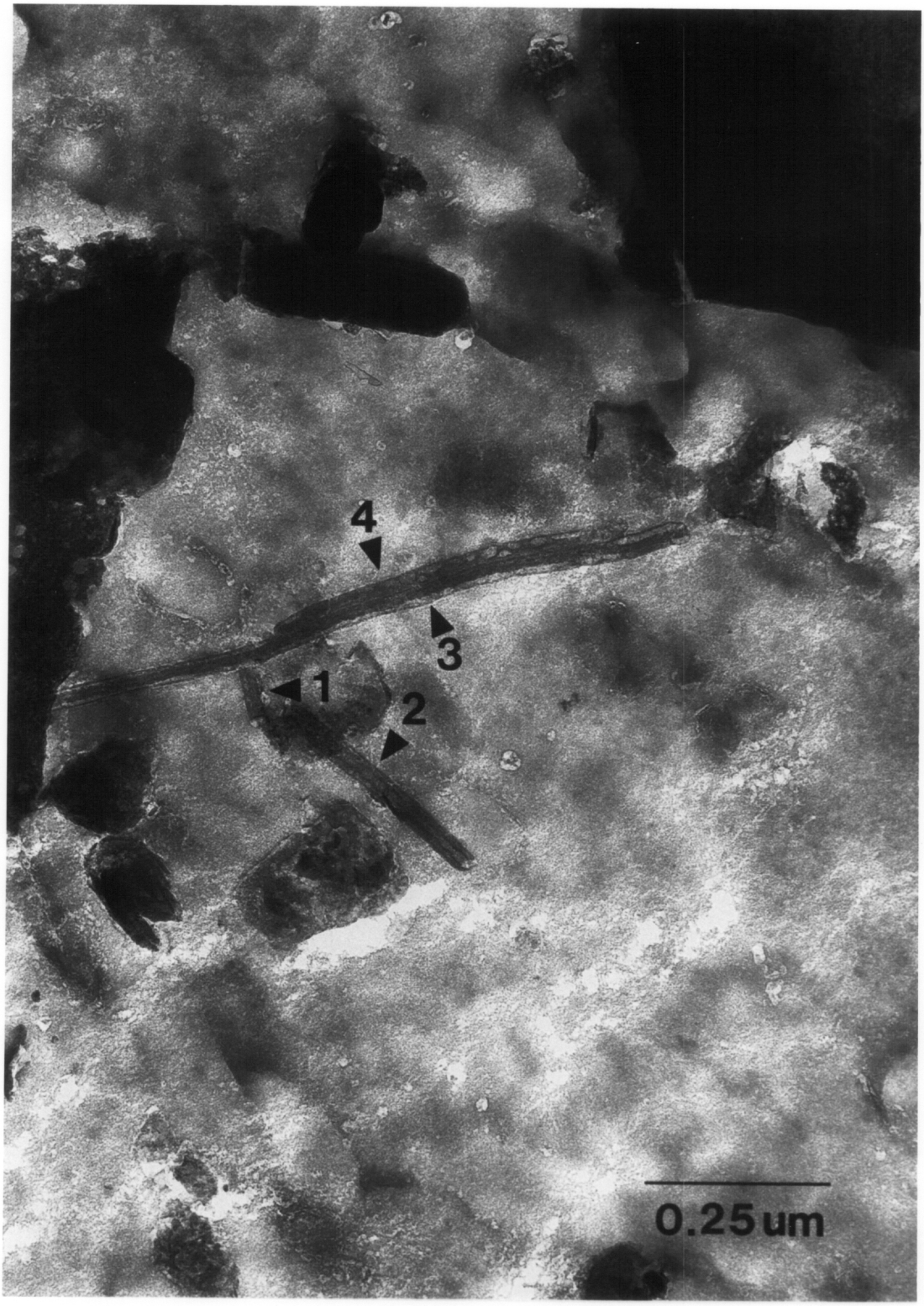
Chrysotile was obtained as grade-5 milled ore from a commercial supplier for laboratory experiments. Suspensions of asbesots were prepared by lightly milling 400 mg of chrysotile followed by sonicating 500 ml of a 0.060 mg/liter stock for two hours in a Fisher Ultrasonic Cleaner.

Fiber identifications and determinations were made by the methods outlined by Anderson and Long (1980). Water samples were collected in asbestos-free Nalgene bottles and filtered through 0.2 um pore polycarbonate filters. After filtering, samples were carbon-coated, inverted, and placed in a Jaffe-

Wick washer for 36 hours on brass TEM grids above chloroform saturated filter papers. Fiber density estimates were derived by counting three replicate grids of 5 to 10 grid holes per grid (a total of 15 to 30 holes). Background (control) and blanks were processed simultaneously. Fibers were identified by the characteristic transmission pattern of a central canal and sheath appearance (Fig. 10) using a JEOL JEM 100C Electron Microscope (TEM). Control samples (n = 14) were never asbestos contaminated. The 10^4 fibers/liter exposure was below detection (n = 3, detection limit was 2.99-3.24 x 10^4 fibers/liter) and the realized concentration at the nominal 10^8 fibers/liter was 7.52 x 10^7 fibers/liter (n = 8, detection limit was 3.24-4.50 x 10^4 fibers/liter).

The presence of asbestos in clam tissue was evaluated at the conclusion of the 30-day laboratory exposures at 0, 10^4, and 10^8 fibers/liter and in clams collected from Lake Silverwood, California on 7 October 1983 and 2 February 1984. Gill and visceral tissue, and whole clam homogenates were analyzed by the methods given in Batterman and Cook (1981) and Patel-Mandlik (1981). Tissues were placed in porcelain dishes previously sonicated in an acid bath. Each dish was tightly covered with aluminum foil, and the tissue was ashed at 500 C for 8 hours. The ash was then resuspended in 6M HCl and otherwise treated as a water sample in preparation for TEM analysis. Fibers which could not be positively identified as chrysotile asbestos (occurring exclusively in the

Figure 10. Chrysotile asbestos fibers isolated from an adult Asiatic clam exposed to chrysotile asbestos in Lake Silverwood, California.


4
3
1
2
0.25 um

field exposed clams) by the characteristic transmission pattern were analyzed separately as non-chrysotile fibers.

Fiber dimensions were determined from visceral and gill samples from laboratory and field-exposed Corbicula. Length, width, and aspect ratios (length:width ratio) of fibers were determined from projected micrographs measured by Vernier calipers to evaluate the relationship of water to tissue distributions.

Bioconcentration factors (BCF), the proportion of tissue to water asbestos content, were calculated for laboratory and field exposed clams. Published waterborne and sediment concentrations from Lake Silverwood (McGuire et al. 1982, 1983; Hayward 1984) were used to compare field with laboratory results.

STATISTICAL ANALYSES.

Fiber concentrations and size distributions in tissue (gill, viscera, and whole clam homogenates) and bioconcentration data were compared for laboratory and field-exposed Corbicula by the one-way analysis of variance rank-analogue, the Kruskal-Wallis Test (Hollander and Wolfe 1973). If significant differences were indicated ($\alpha = 0.05$) a rank-like Least Significant Differences procedure was used to determine which means were significantly different.

RESULTS

FIBER ACCUMULATIONS

Corbicula accumulated significant amounts of chrysotile by 30 days of exposure to 10^8 fibers/liter. Gill tissue contained approximately 150 fibers/mg, visceral tissue 900 fibers/mg, and whole clam homogenates 1000 fibers/mg (Table 10). All concentrations were an order of magnitude above the detection limits (18.7-71.7 fibers/mg). Chrysotile was not found in control and 10^4 fibers/liter exposures. Gill tissue accumulations were significantly lower than visceral and whole clam homogenates ($p < 0.05$).

Clams exposed to 10^9 fibers/liter in Lake Silverwood had suprisingly high fiber burdens. Gill tissue contained 710.3 and 865.2 fibers/mg in the 2 February 1984 and 7 October 1983 samples, respectively. Visceral tissues contained approximately 10^5 fibers/mg from both samples. Whole clam homogenates had approximately 2×10^7 and 6×10^6 fibers/mg for 7 October and 2 February samples. In both instances gill accumulations were significantly lower than visceral accumulations which were significantly lower than total body burdens ($p < 0.05$).

Table 10. Chrysotile asbestos accumulations in Corbicula exposed in the laboratory and field. G = gill tissue, V = visceral tissue, and W = whole clam homogenates. Means ± SE are given. Significantly different means are indicated by different letters.

Site	Asbestos Concentration (Fibers/Liter)	Tissue	n	Chrysotile fibers/mg dry weight	Non-chrysotile fibers/mg dry weight	Detection limit range in fibers/mg dry weight
Laboratory	Control	G	3	0	0	18.7-22.3
		V	3	0	0	14.2-18.6
		W	3	0	0	29.3-37.5
	10^4	G	3	0	0	19.1-39.2
		V	3	0	0	30.0-32.0
		W	3	0	0	39.2-48.6
	10^8	G	3	147.3 ± 52.6^a	0	18.7-32.4
		V	3	903.7 ± 122.9^b	0	29.2-49.4
		W	3	996.3 ± 199.2^b	0	46.4-71.7
Field (7 Oct 1983)		G	3	865.2 ± 379.1^a	62.3 ± 62.3	168.0-459.1
		V	3	$4.63 \pm 1.75 \times 10^{5b}$	0	1795.0-2541.6
		W	7	$2.38 \pm 1.09 \times 10^{7c}$	0	$3.15\text{-}16.7 \times 10^3$
Field (2 Feb 1984)		G	3	710.3 ± 166.1^a	0	145.0-387.0
		V	3	$2.92 \pm 1.27 \times 10^{5b}$	0	349.0-2010.0
		W	3	$6.49 \pm 0.89 \times 10^{6c}$	$2.52 \pm 2.72 \times 10^4$	$1.29\text{-}23.4 \times 10^4$

FIBER SIZE DISTRIBUTIONS

Two clams which accumulated asbestos in the laboratory and field were selected to determine the size distributions of chrysotile in clam tissue. Gill tissue accumulated significantly smaller asbestos fibers (in terms of length) than visceral tissue in both the laboratory and field (Table 11). Fiber widths were consistently smaller in gill tissue. Aspect ratios (the proportion of length to width) were greater in the field than the laboratory. Water-borne asbestos in the laboratory averaged 6.53 µm in length, 0.67 µm in width, and had an aspect ratio of 9.4 (n = 100). In the CAS, McGuire et al. (1982) reported that approximately 50% of the suspended chrysotile was between 0.5-1.0 µm in length and that most fiber widths from the same samples were in the range of 0.05-0.09 µm.

BIOCONCENTRATION FACTORS

Bioconcentration factors (BCF) for laboratory-exposed clams after 30 days exposure to 10^8 fibers/liter were 0.308 for gill, 1.89 for viscera, and 1.91 for whole clam homogenates (Table 12 and Appendix A) BCF values were not calculated for sediment since water was the only potential route of exposure in these experiments. From field-exposed

Table 11. Fiber size distributions in gill and visceral tissue in clams exposed to chrysotile asbestos in the laboratory and field. Means (± SE) which are not significantly different are indicated by the same letter ($\alpha = 0.05$).

Exposure	Tissue	n[a]	Fiber Length (um)	Fiber Width (um)	Aspect Ratio
Laboratory	Gill	50	1.21 ± 0.96[a]	0.05 ± 0.03[a]	24.2 ± 16.6[b]
	Viscera	50	3.62 ± 2.00[c]	0.21 ± 0.14[c]	17.2 ± 10.4[a]
Field	Gill	44	1.09 ± 0.39[a]	0.06 ± 0.06[a]	18.1 ± 3.7[a]
	Viscera	68	2.99 ± 4.95[b]	0.07 ± 0.05[b]	42.2 ± 10.5[c]

[a]For laboratory and field analyses of size distributions, two clams were pooled to yield the total number of fibers (n) analyzed.

Table 12. Bioconcentration factors (BCF) for field and laboratory exposed Corbicula based upon the mean observed fiber counts from gill and visceral tissues and whole clam homogenates. See Appendix A for calculations.

Site	Tissue	BCF	
		Sediment	Water
Laboratory	Gill	-	0.308
	Viscera	-	1.89
	Whole Clam	-	1.91
Field 7 Oct 1983	Gill	1.35×10^{-4}	0.19
	Viscera	0.72	102
	Whole Clam	3.68	5222
Field 2 Feb 1984	Gill	1.11×10^{-4}	0.16
	Viscera	0.35	64.9
	Whole Clam	1.02	1442

clams, BCF values for gill, viscera, and whole clams ranged from 0.16-0.19, 64.9-101, and 1442-5222, respectively.

To relate 30-day experimentally determined uptake to field results, laboratory accumulations were extrapolated to lifetime body burdens under two major assumptions: (1) asbestos, once accumulated in tissue, is not depurated (according to Halsband (1974) mollusks did not depurate asbestos once embedded in intestinal lining tissue); and (2) accumulations do not vary greatly between early life stages (e.g., juveniles) and later life stages (adults) (Belanger et al., in review, have shown that young Corbicula, 5.2-8.6 mm shell length, accumulated chrysotile in gill and visceral tissues on the same order of magnitude as those reported here). Since clams from the CAS were in their second (or possibly third) year of development, the laboratory data were empirically extrapolated to 24 months. Under these conditions, a clam exposed to 10^8 fibers/liter for 2 years would be predicted to contain 3500 fibers/mg in gill, 2.2×10^4 fibers/mg in viscera, and 2.4×10^4 fibers/mg in whole clam honogenates. These would yield BCF values of 7.3, 45, and 50, respectively.

DISCUSSION

FIBER ACCUMULATIONS

Corbicula accumulated chrysotile asbestos to an extent greater than that known for any aquatic organism researched to date. Tissue burdens in viscera of clams exposed to 10^8 fibers/liter approached 1000 fibers/mg and are 4 times greater than those reported by Batterman and Cook (1981) from Arctic char kidney during 3-4 years of exposure to 10^8 fibers/liter of chrysotile. In field-exposed clams, visceral burdens were approximately 10^5 fibers/mg and gill burdens were 700-850 fibers/mg. Halsband (1974) documented by histological techniques that *Mytilus* sp. did not depurate chrysotile asbestos after exposures of 10-100 mg/liter and that fibers and blocks of asbestos remianed embedded in intestinal lining tissue. This may be one reason why field and laboratory exposures resulted in high body burdens. In addition, field-exposed *Corbicula* were subjected to 4.5×10^9 fibers/liter, 20 times greater than in the laboratory.

Some differences were observed in clams collected from Lake Silverwood in October and February, even though variability around the observed mean values were large. Whole clam homogenate asbestos levels in February were 27% of those found in October, visceral burdens were 63% of October values, and gill burdens were 82% of October values. October

is generally a more wet season than February in California which results in higher erosion and asbestos levels at that time (McGuire et al. 1982; Hayward 1984). Lower body burdens in mid to late winter may in large part be a function of lower exposure concentrations at that time. The large reduction in whole clam homogenate burdens (which include free fibers in the gastrointestinal tract) would be expected. The more conservative and apparently real reductions in tissue burdens is evidence for some removal of fibers from clam tissues contrary to Halsband (1974). Belanger et al. (1985b) showed accumulated chrysotile fibers in open locules of the clam gill which could be a mechanism for internal transport of fibers. However, isolation mechanisms, such as fibrogenesis, phagocytosis, or asbestos-body formations, were not observed in Corbicula gill tissue.

FIBER SIZE DISTRIBUTIONS

In laboratory exposed clams, smaller fibers were taken up by gill and visceral tissues than were available in the water. Fibers in tissue were characterized by possessing aspect ratios that were 2 to 3 times greater than water-borne fibers. Slightly larger fibers were present in viscera than gills (Table 11).

Under field conditions gill tissue also had smaller fibers (which were similar in length and width to water-borne

asbestos reported by McGuire et al. (1982)). Visceral tissue contained fibers which were larger than the majority of asbestos in the CAS. Perhaps this is related to larger fibers settling out quickly from the water (McGuire et al. 1982) with subsequent uptake from sediment and near the sediment water interface. The large variability associated with visceral tissue fiber sizes stems from a number of very small (0.1-0.3 um in length) fibers being present resulting in a left-skewed distribution. The aspect ratio of asbestos in California clams, double that of laboratory exposed Corbicula is a result of the overall smaller nature of environmentally distributed chrysotile. The laboratory preparation does not adequately mimic the field asbestos fiber distribution in the CAS.

Several researchers have suggested that small fibers are most biologically interactive (Selikoff and Lee 1978; Cook et al. 1982; Cook 1983; Hesterberg and Barrett 1984). The observations reported here support the thesis that small, high aspect ratio fibers easily penetrate biological tissue. Harington et al. (1975) suggested that the active uptake mechanism functions by the attraction of negatively charged sialic acid groups of membrane proteins to the magnesium of chrysotile fibers. This results in an attraction of membrane proteins to the fibers at physiological pH and weaken the cell's ability to maintain a proper Na:K balance. Ionic im-

balances result in fiber penetration and cellulytic action (Brody et al. 1983).

BCF'S AND LABORATORY EXTRAPOLATION

Bioconcentration of chrysotile by Corbicula is modest compared to heavy metal exposure (Graney et al. 1983, 1984) or organochlorine compounds (Hartley and Johnston 1983). Waterborne BCF's are relatively conservative in this study even though concentrations in water in the CAS can fluctuate up to 10 times throughout a one year period (McGuire et al. 1982).

Laboratory data would have predicted gill burdens 44 times higher than observed in the field (exposed at 10^9 fibers/liter), visceral burdens of .06 times the field observed values, and whole body burdens only 0.02 of field observations. Given that the laboratory exposure concentrations were 5% of the Lake Silverwood levels this would account for lower predicted visceral and whole clam homogenate concentrations; however, the greater predictions for gills is anomolous and cannot be explained by the present study.

UTILITY OF CORBICULA AS A BIOMONITOR

Corbicula accumulated greater amounts of chrysotile than any aquatic animal researched thus far. The Arctic char

kidney BCF (calculated from data by Batterman and Cook 1981) was approximately 0.0001, whereas for Corbicula viscera the BCF was as high as 102 in field-exposed clams. BCF's of whole clam homogenates, which were as high as 5200, may be useful to demonstrate short-term fluctuations in environemntal asbestos levels.

The USEPA (1979) suggested that aquatic life would not (or had low potential to) bioaccumulate of chrysotile. A review of point and non-point discharges of asbestos may be advised. Thus far, algae (Lauth and Schurr 1983, 1984), macrophytes (Pfister 1980), microcrustacea (Stewart and Schurr 1980), mollusks (Halsband 1974; Belanger et al. 1985b), and fish (Batterman and Cook 1981; Woodhead et al. 1983; Belanger et al. 1985a) accumulate chrysotile in field and laboratory environments. Of these, mollusks have the greatest potential for use as an in situ biomonitor for chrysotile, due to the sedentary lifestyle of mollusks, relatively long life span, ubiquitous nature, ease of collection, and manipulability in the laboratory (Graney et al. 1983; Belanger et al. 1985b).

Further research is necessary to define the correlation of environmental asbestos exposure and tissue uptake of chrysotile in systems which undergo cyclic changes in waterborne asbestos (Cook 1975; McGuire et al. 1982) and in a variety of waterways containing different levels of asbestos (to below detection of 10^4 fibers/liter). Under

these conditions the utility of Corbicula as a biomonitor for chrysotile can be systematically determined.

CHAPTER FIVE: FATHEAD MINNOWS

Fathead minnows are an accepted toxicity test species and have gained popularity as the species of choice in industrial permitting of effluent discharges into aquatic receiving systems. The responses of juvenile and adult minnows to chrysotile asbestos were evaluated at 0-10^8 fibers/liter for 30-days. Analyses of growth, mortality, swimming performance and infiltration of fibers into gill, liver, kidney, and muscle tissues were performed. Two life stages were analyzed since fish are often more sensitive in early life stages, but the adult is used most often for acute toxicity tests. Swimming performance was used as a benchmark challenge test to determine the effect of chrysotile on fish stamina and the efficacy of the adult exposure system (a modified artificial stream). Growth is often a sensitive indicator of long-term stress in fish and is a popular toxicity endpoint. Fiber accumulations in a variety of tissues were used as an indicator of which tissue would be most sensitive to asbestos exposure

INTRODUCTION

Chrysotile asbestos is a fibrous serpentine mineral with a length to width ratio of three to one or greater.

Asbestiform minerals, as a group, are hydrated silicates (Speil and Leineweber 1969). Chrysotile is mined in various serpetine rich areas of the United States (Levine 1978; Puffer et al. 1980; McGuire et al. 1982) and is used industrially as a strengthening agent in cement products, fire retardant, and dispersing agent (Levine 1978). It occurs incidentally in other mined ores (e.g., copper, chromium, iron, and nickel) and talc (Cook et al. 1974; Rohl and Langer 1974; Langer et al. 1980). Because of its wide use in industrial societies, chrysotile has been dispersed into aquatic systems by mining (Batterman and Cook 1981; McGuire et al. 1982; Hayward 1984), rainfall (Cunningham and Pontefrtact 1971; Hesse and Hallenbeck 1978; Mizota 1982), non-point anthropogenic use (e.g., cars and fugitive dust emissions from road surfaces) (Ase et al. 1982; Williams and Muhlbaier 1982), and natural erosion of serpentine parent material (Hayward 1984). As a result, Millette et al. (1980) found 20% of United States and its territories water supplies contaminated at or above 1 x 10^6 fibers/liter; approximately 9% were contaminated above 10^8 fibers/liter. Cook et al. (1974) and Hayward (1984) described waters which were contaminated above 10^9 fibers/liter.

Few investigations have been conducted on the effects of chrysotile to fish. Batterman and Cook (1981) determined chrysotile burdens in salmonids from Lake Superior and near Hudson Bay that had histories of exposure to asbestos.

Arctic char (Salvelinus alpinus) from Deception Bay, Canada (an arm of Hudson Bay) had 2.9 and 230.5 fibers/mg in muscle and kidney tissue, respectively, while the Bay had fiber concentrations as high as 6.7×10^8 fibers/liter. Woodhead et al. (1983) exposed Poecilia formosa for 6 months to chrysotile and found fish developed epithelial hypertrophy, selective necrosis in kidney cells, and vacuolation of heart cells. Belanger et al. (1985a) correlated chrysotile uptake in larval coho salmon (Oncorhynchus kisutch) exposed for 40-80 days at 10^6 fibers/liter with pathologic (epidermal vacuolation with hypertrophy superimosed on hyperplasia, and lateral line degradation) and behavioral (reduced rheotaxic ability and lethargy) effects. Definitive relationships between environmental exposure to asbestos and fish pathologies have not been made, but two studies have suggested such a relationship exists. Black et al. (1982) described mesothelioma in walleye (Stizostedion vitreum) exposed to copper tailings (which may contain asbestos fibers) in Torch Lake, Michigan. Mesothelioma neoplasms are a peculiar carcinoma that is strongly associated with asbestos exposure in mammals (Selikoff and Lee 1978; Triol et al. 1984). Herman (1985) found rainbow trout (Salmo gairdneri) to develop mesothelioma in a trout hatchery fed water through a transite pipe. Unfortunately, water-borne asbestos was not evaluated in either study.

The objectives of this study were to relate ecologically important parameters, such as organismal growth, mortality, and swimming performance to asbestos exposure and fiber accumulations during long-term exposure to asbestos.

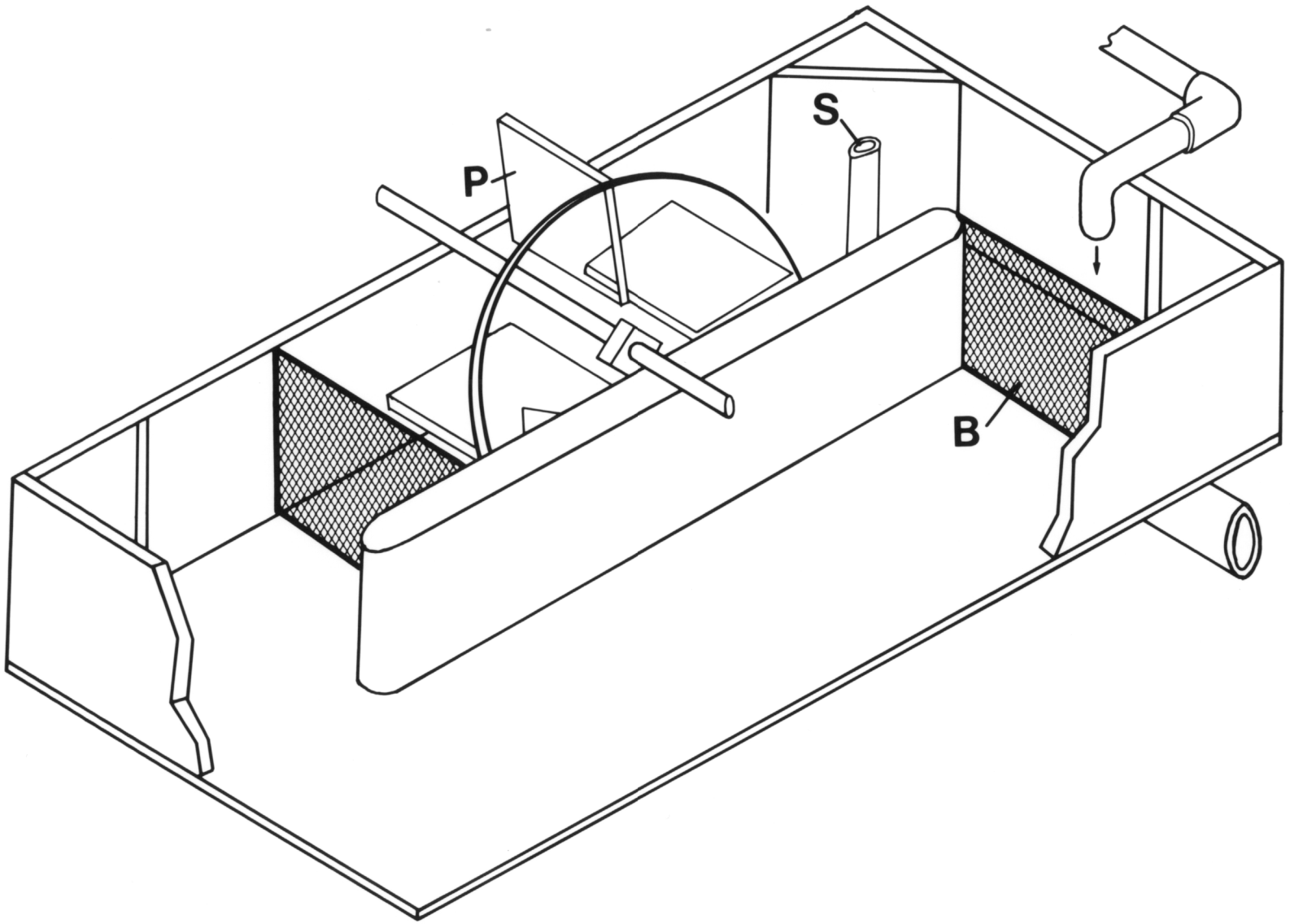
MATERIALS AND METHODS

Fathead minnows (Pimephales promelas) were purchased from Kurtz Fish Hatchery (Elverson, Pennsylvania) on 9 January (juveniles) and 15 January 1985 (adults). The fathead minnow is a standard toxicity test species utilized by the USEPA and is of moderate sensitivity (Holcombe et al. 1984), maintains well in the laboratory (APHA et al. 1981), and has a wide ecological and geographical distribition east of the Rocky Mountains (Eddy and Underhill 1976).

LONG-TERM TESTS

Prior to testing, minnows were acclimated to a 14L:10D light cycle at the Ecosystem Simulation Laboratory at Virginia Tech, fed Tetramin to satiation daily, and maintained in 200 gallon flow-through streams to the final test temperature . Fifteen adult minnows per stream (36.1-47.3 mm TL) were exposed in replicate to 0, 10^4, 10^6, and 10^8 fibers/liter for 30 days in semi-static oval artificial stream systems (Fig. 11) from 6 February to 2 March 1985.

Figure 11. Artificial stream system (modified from Farris et al., In Review) used for exposing adult minnows to chrysotile asbestos. Nylon mesh screen (B) was used to keep fish from the paddle wheel (P) area. A standpipe (P) was located in the rear of the stream.


S
P
B

Streams were constructed of plexiglass and were 20-liters in volume. Water was circulated in the streams by a paddle-wheel design and was renewed completely only on days 10 and 20 to remove excess wastes. Adjustments for evaporation were made daily. Artifical stream systems such as these increase the environmental realism of the test and reduce stress to natural stream-dwelling organisms (Belanger et al. 1985b; Farris et al. In Review). Nylon mesh screen (2 mm^2) was inserted at the forward edge of the paddle wheel and before the drain pipe to eliminate potential damage to fish by the revolving wheel. Water chemistry was determined on days 0, 10, 20, and 30 by Standard Methods (APHA et al. 1981) (Table 13). Fish were checked for mortality twice daily.

Juvenile minnows (15.0-21.2 mm) were exposed to 0, 10^4, 10^6, and 10^8 fibers/liter chrysotile in 30-liter aquaria (two replicates per concentration with 10 fish in each system) equipped with a Dyna-flo circulator without charcoal or floss to allow particles of asbestos to pass through the system (Fig. 12). A commercially available ammonia absorbant, Ammo-Chips, was held in the circulator in a plankton bucket cod-end (65 µm) to scavenge excess ammonia. Water was changed on the fifteenth day of the test, and water chemistry was monitored on days 0, 15, and 30. Fish were checked for mortality twice daily (Table 13).

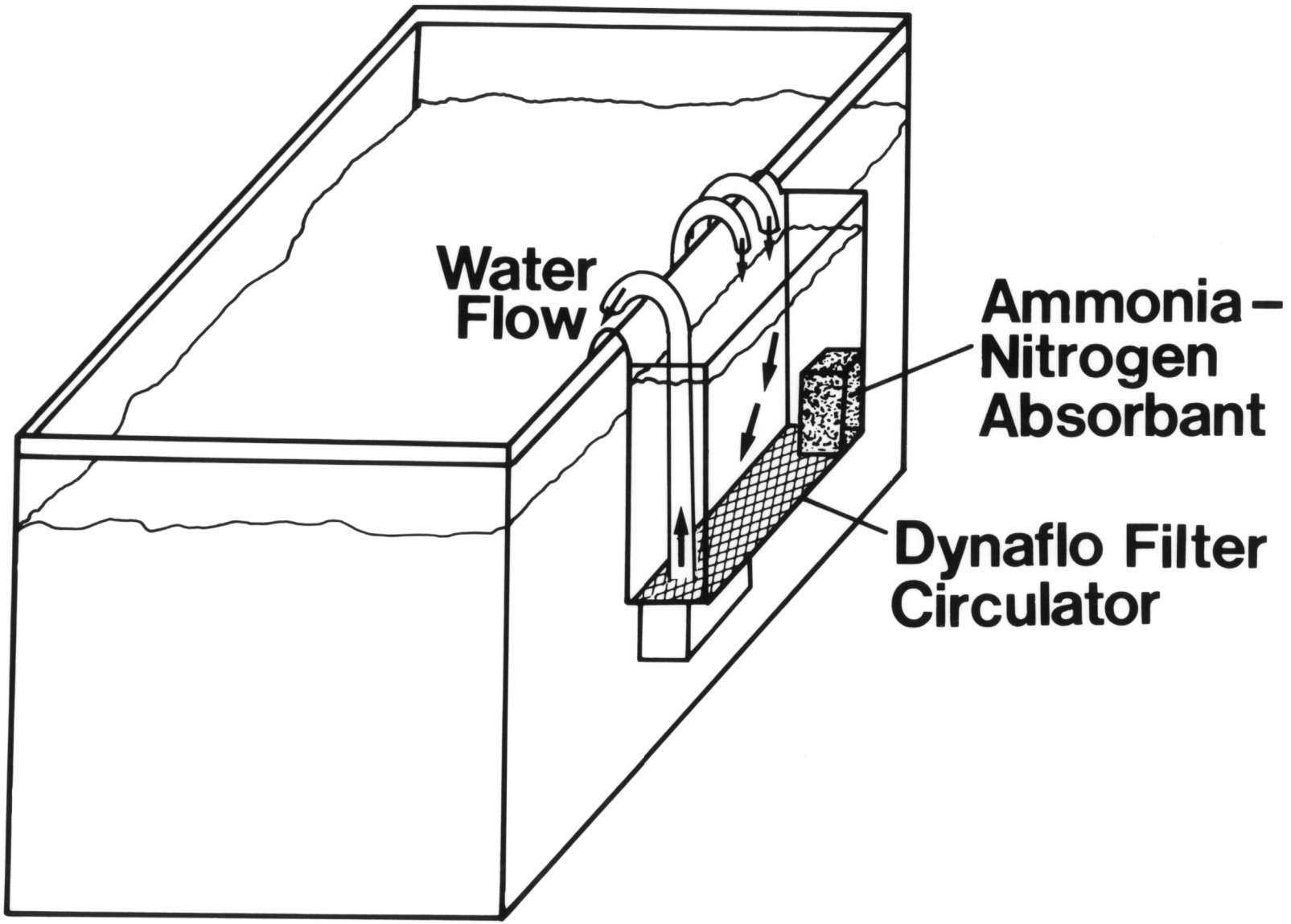
Table 13. Selected water chemistry parameters measured during 30-day fathead minnow (Pimephales promelas) experiments. Means ± SE are given. n = 8 for adults, n = 6 for juveniles

	Parameter	Asbestos Concentration (Fibers/liter)			
		0	10^4	10^6	10^8
Adults	Chrysotile Concentration (Fibers/liter)	0[1]	0[1]	$7.2 \pm 1.9 \times 10^5$	$6.1 \pm 0.7 \times 10^8$
	Temperature (°C)	15.25 ± 1.17	15.75 ± 0.97	15.25 ± 1.17	15.20 ± 0.44
	pH	8.08 ± 0.03	8.26 ± 0.02	8.16 ± 0.06	8.17 ± 0.01
	Dissolved Oxygen (mg/Liter)	9.5 ± 0.3	9.0 ± 0.1	9.2 ± 0.2	9.4 ± 0.5
	Hardness (mg/Liter as $CaCO_3$)	85.0 ± 3.5	92.5 ± 1.1	86.25 ± 4.8	90.0 ± 5.0
	Alkalinity (mg/Liter as $CaCO_3$)	52.1 ± 7.2	64.2 ± 4.7	64.2 ± 1.3	55.5 ± 2.4
	NH_3-N (mg/Liter)	0.766 ± 0.255	0.527 ± 0.199	0.642 ± 0.210	0.436 ± 0.179
Juveniles					
	Temperature (°C)	20.4 ± 0.1	20.4 ± 0.1	20.4 ± 0.1	20.4 ± 0.1
	pH	8.30 ± 0.02	8.32 ± 0.01	8.15 ± 0.3	8.25 ± 0.01
	Dissolved Oxygen (mg/Liter)	8.7 ± 0.4	8.9 ± 1.0	8.5 ± 0.1	8.5 ± 0.2

Table 13. Continued

Parameter	Asbestos Concentration (Fibers/Liter)			
	0	10^4	10^6	10^8
Hardness (mg/Liter as $CaCO_3$)	91.2 ± 1.7	105.0 ± 2.5	100.1 ± 7.5	79.5 ± 6.2
Alkalinity (mg/Liter as $CaCO_3$)	60.7 ± 0.4	59.0 ± 0.5	44.5 ± 7.25	53.2 ± 6.4
NH_3-N (mg/Liter)	0.091 ± 0.025	0.083 ± 0.037	0.081 ± 0.019	.082 ± 0.022

Figure 12. Modified aquarium system used to expose juvenile fathead minnows to chrysotile asbestos. A Dynaflo water circulator kept dissolved oxygen high and circulated water and asbestos. An ammonia absorbant was used to reduce water ammonia content.


Water
Flow
Ammonia –
Nitrogen
Absorbant
Dynaflo Filter
Circulator

SHORT-TERM (96-HR) EXPOSURES

Adult and juvenile fathead minnows were exposed to chrysotile for 96-hours in 15 liter polycarbonate jars to determine short-term effects on fish. Replicate containers of 0, 10^4, 10^6, 10^8, 10^{10}, and 10^{12} fibers/liter were used with ten fish per container. Water chemistry was analyzed at the beginning and end of the exposure (Table 14). Containers were checked for mortality twice daily.

ASBESTOS DETERMINATIONS

Chrysotile was obtained from a commercial supplier as grade-5 chrysotile mined ore and was further processed for use in these studies. Chrysotile suspensions were prepared by lightly milling 400 mg of asbestos followed by sonicating 500 ml of a 0.060 mg chrysotile/liter stock for 2 hours using a Fisher Ultrasonic Cleaner. Five replicate determinations of the fiber concentrations indicated that the actual concentration would lie between 2.5×10^8 to 8.8×10^9 fibers/liter by this method.

Fiber identification and determinations were made by the methods outlined by Anderson and Long (1980). Water samples were collected in asbestos-free nalgene bottles and filtered through 0.2 um pore polycarbonate Nuclepore filters. After filtering samples were carbon-coated, inverted, and placed

Table 14. Selected water chemistry parameters measured during 96-hr fathead minnow bioassays. Means ± S.E. are given (n = 2)

Parameter	Asbestos Concentration (Fibers/l)					
	0	10^4	10^6	10^8	10^{10}	10^{12}
Adults						
Temperature (°C)	18.7 ± 0	18.7 ± 0	18.7 ± 0	18.7 ± 0	18.7 ± 0	18.7 ± 0
pH	8.2 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
Dissolved Oxygen (mg/Liter)	9.0 ± 0.4	-	-	-	-	9.3 ± 0.7
Hardness (mg/Liter as $CaCO_3$)	72.5 ± 2.5	70.0 ± 0	62.5 ± 2.5	65.0 ± 0	70.0 ± 2.5	65.0 ± 0
Alkalinity (mg/Liter as $CaCO_3$)	50 ± 0	47.0 ± 2.0	49.0 ± 0	45.5 ± 0.5	43.0 ± 1.0	46.0 ± 4.0
NH_3-N (mg/Liter)	1.307 ± 0.076	1.293 ± 0.165	1.115 ± 0.039	0.760 ± 0.014	0.885 ± 0.053	0.68 ± 0.03
Juveniles						
Temperature (°C)	16.3 ± 0	16.3 ± 0	16.3 ± 0	16.3 ± 0	16.3 ± 0	16.3 ± 0
pH	7.5 ± 0.1	7.55 ± 0.5	7.55 ± 0.5	7.5 ± 0.1	7.5 ± 0	7.75 ± 0.08
Dissolved Oxygen (mg/Liter)	9.6 ± 0.1	-	-	-	-	9.4 ± 0.3

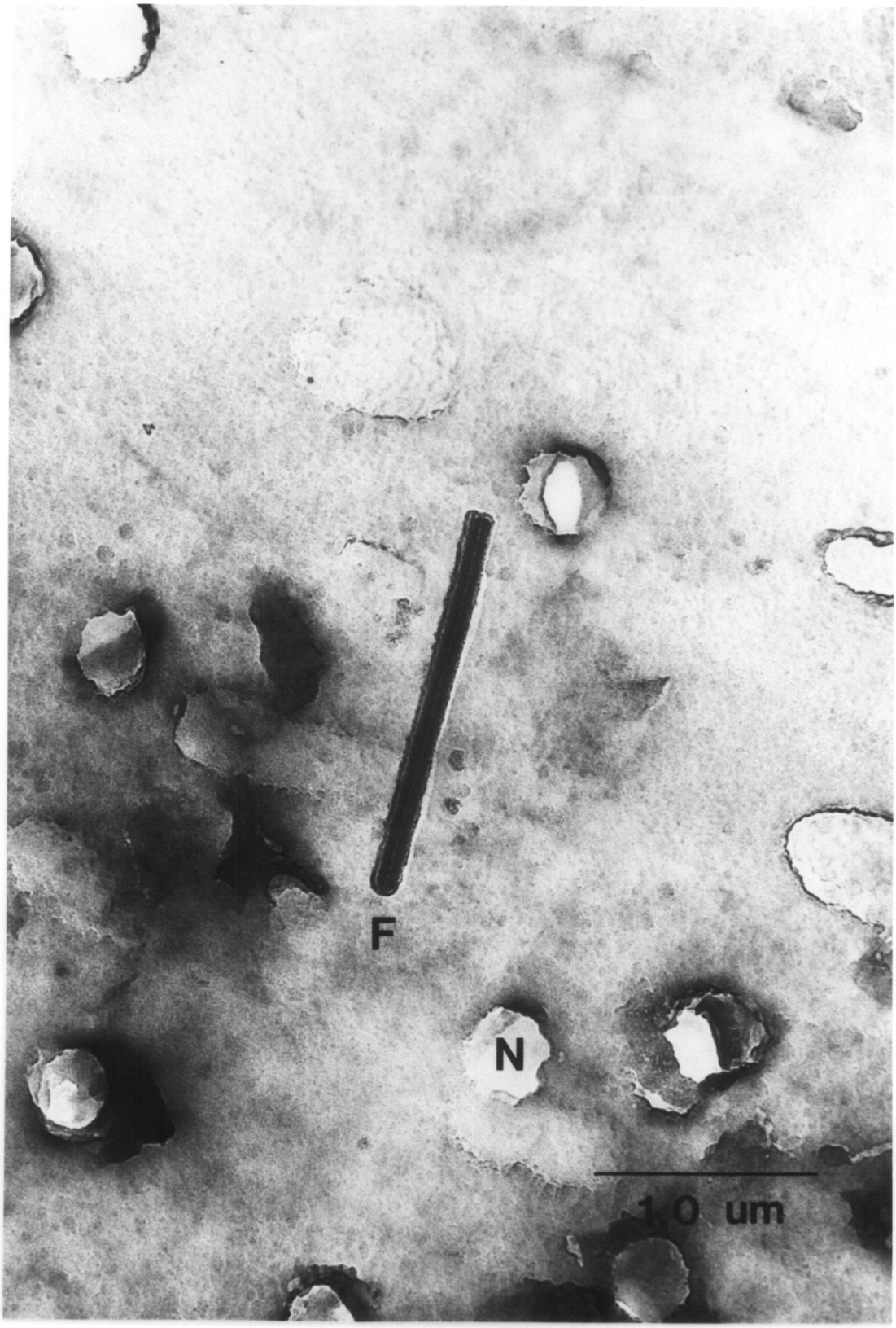
Table 14. Continued

Parameter	Asbestos Concentration (Fibers/1)					
	0	10^4	10^6	10^8	10^{10}	10^{12}
Hardness (mg/Liter as $CaCO_3$)	60.0 ± 5.0	57.5 ± 2.5	60.0 ± 0	55.0 ± 0	60.0 ± 0	60.0 ± 0
Alkalinity (mg/Liter as $CaCo_3$)	29.8 ± 3.2	34.2 ± 0.2	34.2 ± 0.2	34.0 ± 0	34.0 ± 0	34.7 ± 0.7
NH_3-N (mg/Liter)	0.853 ± 0.002	0.585 ± 0.106	0.656 ± 0.033	0.549 ± 0.019	0.622 ± 0.009	0.63 ± 0.01

in a Jaffe-Wick washer for 36 hours on brass TEM grids above filter paper saturated with chloroform. Fiber density estimates were derived by counting three replicate grids of 5 to 10 grid holes per grid (a total of 15 to 30 holes). Background (i.e., control) and blanks were processed simultaneously. Fibers were identified by the characteristic transmission pattern of a central canal and sheath appearnace (Fig. 13) using a JEOL JEM 100C Transmission Electron Microscope (TEM). Water samples from 96-hour and 30-day tests were pooled for analysis of realized and nominal concentrations (Table 13).

Uptake of chrysotile into fish tissues was evaluated by TEM. Juvenile and adult fish exposed for 96 hours and 30 days were dissected in buffered formalin (pH 7.2). Gill, liver, lateral muscle, and kidney samples were removed, weighed wet, dried at 90 C for 48 hours, and prepared for analysis by methods given by Batterman and Cook (1981) and Patel-Mandlik (1981). Dried tissue was placed in acid washed porcelain dishes and ashed at 500 C for 8 hours. After ashing, the remaining organic matter was digested for two minutes in 6M HCl. The suspension was filtered and processed in a manner identical to water samples.

Figure 13. Chrysotile asbestos fiber from a juvenile fathead minnow kidney after exposure to 10^8 fibers/liter for 30 days.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10 um

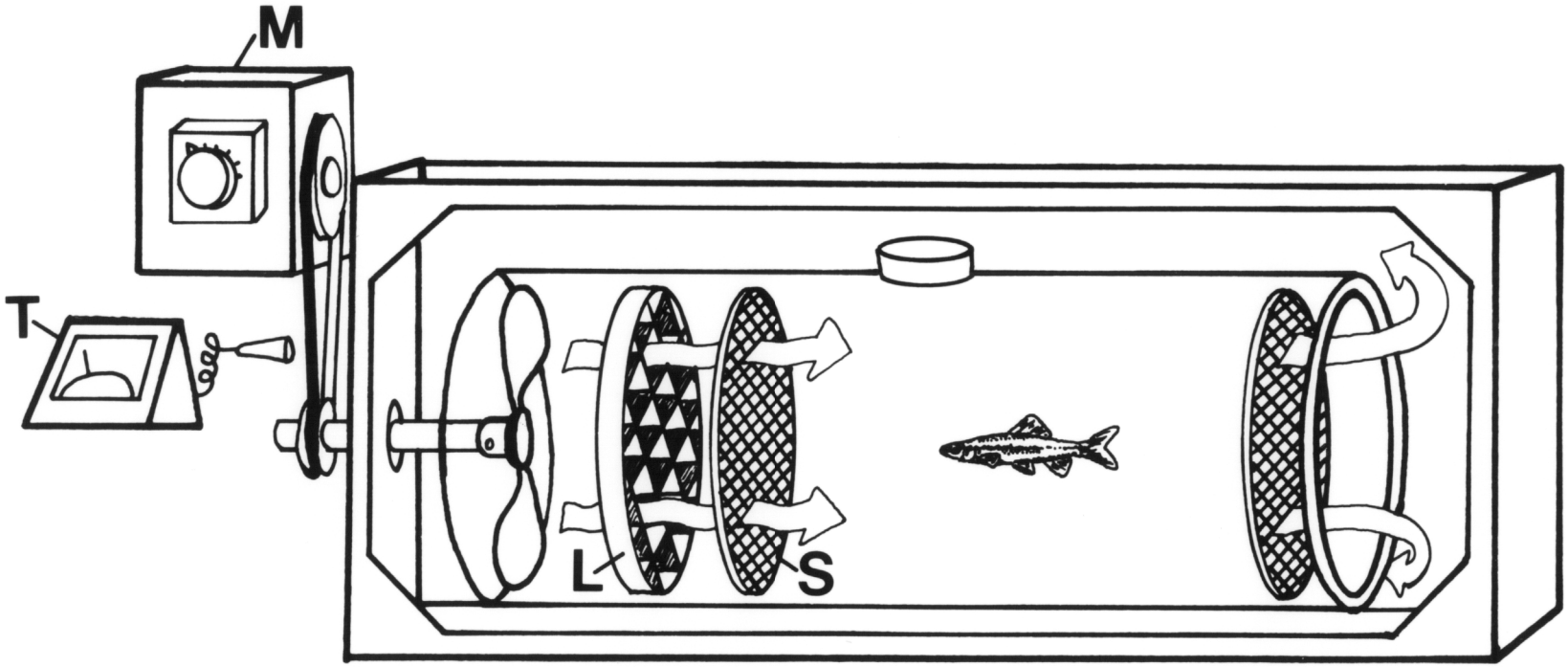
CONDITION OF FISH

Length and weight (hereafter referred to as condition) of adult and juvenile fathead minnows was determined at the termination of 30 day artificial stream and modified aquaria exposures, respectively. Individual fish were weighed wet by a Metler PC-440 electronic analytical balance (± 0.0005 g precision) and measured for total length by vernier calipers (± 0.025 mm precision).

SWIMMING PERFORMANCE

Adult minnows were subjected to a challenge stress test at conclusion of the 30-day exposure. Swimming performance of individual fish was determined using the chamber in Fig. 14. A motor driven propellor generated a 90 cm/sec current (monitored by a tachometer) through a 12.5 cm diameter, 45.0 cm long tunnel. A laminar flow screen evened out the current and removed dead spaces where fish could relax. Fish surviving the 30-day test were sequentially tested and timed to exhaustion. Total distance traversed in cm and body lengths (normalized distance traversed taking into account individual size differences) was calculated for each fish. At the beginning of the 30-day asbestos exposure period several fish were evaluated (unexposed) to provide a reference point for initial organism vigor.

Figure 14. Swimming performance apparatus used for adult minnows exposed to chrysotile for 30 days. A laminar flow screen (L) evened out turbulence. A tachometer (T) was used to measure the current speed generated by the motor-driven (M) propellor.


M
T
L
S

STATISTICAL ANALYSIS

All data are presented as mean ± 1 SE. For comparisons of final organism condition, swimming performance and asbestos accumulation, a rank-like analysis of variance procedure, the Kruskal-Wallis Test was used to compare treatments. If sig-differences were indicated, a rank-like Least Signifcant Differences procedure, was used to determine which means were significantly different ($\alpha = 0.05$) (Hollander and Wolfe 1973). Correlations of water chemistry (particularly ammonia) with asbestos exposure levels were made using Kendall's Rank Correlation Procedure.

RESULTS

CONDITION AFTER 30-DAY TESTS AND MORTALITY

Adult fathead minnows exposed to chrysotile asbestos were not significantly different from controls with respect to total length or weight at the end of the test (Table 15). Control fish had a mean length and weight of 49.9 mm and 1.065 g, respectively; whereas, minnows exposed to 10^8 fibers/liter were 50.1 mm and 1.118 g on the average.

Juvenile minnows exposed to asbestos exhibited signif-icantly reduced weight compared to controls after 30 days (Table 16). Controls (0.253 g) were 28% heavier than fish

Table 15. Final condition (mean ± SE) of adult fathead minnows upon exposure to chrysotile asbestos for 30 days in artificial stream systems. Significantly different means are indicated by different letters (α = 0.05).

Asbestos Concentration (fibers/liter)	n	Total Length (mm)	Kruskal-Wallis (p-value)	Total Weight (g)	Kruskal-Wallis (p-value)
0	24	49.9 ± 0.7		1.065 ± 0.052	
10^4	26	49.5 ± 0.5		1.029 ± 0.045	
10^6	25	50.1 ± 0.4		1.089 ± 0.037	
10^8	25	50.1 ± 0.6	2.791 ($p > 0.25$)	1.118 ± 0.039	2.410 ($p > 0.50$)

Table 16. Final condition (mean ± SE) of juvenile fathead minnows upon exposure to chrysotile asbestos for 30 days in modified aquaria. Significantly different means are indicated by different letters ($\alpha = 0.05$).

Asbestos Concentration (fibers/liter)	n	Total Length (mm)	Kruskal-Wallis (p-value)	Total Weight (g)	Kruskal-Wallis (p-value)
0	19	28.4 ± 0.7		0.253 ± 0.028[a]	
10^4	20	28.1 ± 0.6		0.196 ± 0.019[ab]	
10^6	20	27.1 ± 0.6		0.166 ± 0.017 [b]	
10^8	20	27.8 ± 0.4	2.783 ($p < 0.25$)	0.181 ± 0.017 [b]	9.114 ($p < 0.025$)

exposed to 10^8 fibers/liter (0.181 g). Controls were not significantly different from 10^4 fibers/liter exposed fish. Signifcant differences were not found with respect to fish length, although exposed fish (e.g., 10^8 fibers/liter) were slightly smaller.

Differential mortality between treatments was not observed in adult (Table 15) and juvenile (Table 16) tests. Adult mortality was greatest in control groups (20%) and lowest in 10^4 fibers/liter (13%). Juvenile fish did not suffer any mortality except for one control fish.

SWIMMING PERFORMANCE

Adult minnows subjected to a swimming performance challenge test after the exposure period did not exhibit any significant differences (total time and total body lengths) with respect to asbestos exposure (Table 17). Controls did perform better than other groups (i.e., swam 11% more body lengths than 10^8 fibers/liter groups and withstanding the current 5% longer than 10^6 fibers/liter groups before exhaustion). A large amount of variability was present for all exposures. The values for controls (596.1 sec and 1082.5 body lengths) represent substantial reductions from reference fish (total length 47.2 ± 0.7 cm, n = 4) values who did not fatigue after 60 min (3600 sec) for a total body lengths traversed of approximately 6860.

Table 17. Swimming performance (total time and total body lengths) of adult fathead minnows subjected to a constant 90 cm/sec current speed after a 30-day exposure to chrysotile asbestos. Significantly different means (± SE) are indicated by different letters (α = 0.05).

Asbestos Concentration (fibers/liter)	n	Time (sec)	Kruskal-Wallis (p-value)	Body Lengths (cm)	Kruskal-Wallis (p-value)
0	24	596.1 ± 82.3		1082.5 ± 149.3	
10^4	24	411.6 ± 134.9		948.0 ± 226.9	
10^6	25	563.4 ± 124.9		931.2 ± 237.8	
10^8	25	528.3 ± 126.1	2.310 ($p < 0.50$)	958.8 ± 223.4	2.300 ($p < 0.50$)

96-HOUR BIOASSAYS

Fish exposed to asbestos up to 10^{12} fibers/liter did not suffer acute toxicity in either juvenile or adult minnow assays. Some fish (< 10 %) were darkened in coloration by test's end but did not exhibit behavioral stress symptoms such as gulping for air or lethargy.

AMMONIA - ASBESTOS CORRELATIONS

Water chemistry was not altered in the presence of asbestos with the exception of unionized ammonia (Tables 13, 14, 18). In 96-hour tests, ammonia was significantly correlated with nominal asbestos concentrations. At high concentrations of asbestos, ammonia levels were low (up to one-half of control concentrations) and, therefore, negatively correlated with asbestos presence ($r = -0.83$, $p < 0.05$) for adults; $r = -0.71$, $p < 0.05$ for juveniles). After pooling all data for 30-day tests a similar result was achieved for adult exposures ($r = -0.73$, $p < 0.05$). However, the presence of ammonia-scavenging additives in juvenile minnow exposure systems reduced unionized ammonia 10-fold and the correlation with asbestos concentration was not significant.

Table 18. Total Ammonia (NH_3-N in mg/liter) from water sampled during 96 hour and 30 day tests with adult and juvenile fathead minnows. Samples during 30 day tests were taken at 10, 20, and 30 days for adults and 15 and 30 days for juveniles.

Group	Asbestos Concentration	96-hr NH_3-N (mg/liter)	Kendall's Rank Correlation Coefficient (p-value)	30-day NH_3-N (mg/liter)	Kendall's Rank Correlation Coefficient (p-value)
Adult	0	1.307 ± 0.076		0.978 ± 0.169	
	10^4	1.293 ± 0.165		0.978 ± 0.141	
	10^6	1.115 ± 0.039		0.812 ± 0.184	
	10^8	0.760 ± 0.014		0.538 ± 0.112	r = -0.73 ($p < 0.05$)
	10^{10}	0.885 ± 0.053		---	
	10^{12}	0.680 ± 0.035	r = -0.83 ($p < 0.05$)	---	
Juvenile	0	0.853 ± 0.002		0.081 ± 0.006	
	10^4	0.585 ± 0.106		0.069 ± 0.005	
	10^6	0.656 ± 0.033		0.067 ± 0.003	
	10^8	0.549 ± 0.019		0.073 ± 0.004	r = -0.17 ($p > 0.05$)
	10^{10}	0.622 ± 0.009		---	
	10^{12}	0.633 ± 0.014	r = -0.71 ($p < 0.05$)	---	

TISSUE BURDENS OF CHRYSOTILE

Chrysotile was not found at or above the limits of detection in either 96-hour adults or juvenile static tests (Table 19). One exception was one of three adult minnows exposed to 10^{12} fibers/liter (159 fibers/mg in kidney tissue).

In thirty day exposures chrysotile was detected in liver and kidney tissue for juveniles and adults. One of two adults had concentrations above detection in kidney (357.5 fibers/mg) and liver (154.9 fibers/mg). Both juveniles which were analyzed possessed significant burdens in liver (117.2 fibers/mg with a high of 121.0 fibers/mg) and kidney (385.9 fibers/mg with a high of 404.2 fibers/mg). At no time were gills or muscle determined to be above detection.

DISCUSSION

CONDITION, SWIMMING PERFORMANCE, MORTALITY

The response of juvenile and adult fathead minnows to chrysotile asbestos were very similar with juveniles being slightly more sensitive. Exposures to higher levels of chrysotile (10^{6}-10^{8} fibers/liter) for 30 days resulted in significantly lower condition relative to weight; however, mortality was not affected and differences in total length were not found. Woltering (1984) in a comprehensive litera-

Table 19. Tissue burdens of chrysotile asbestos in fathead minnows after 96-hour and 30-day exposures. Mean ± SE are given, n = 2 for all samples.

Life Stage	Exposure Length	Asbestos Concentration (fibers/liter)	Tissue	Fiber Burden (fibers/mg)	Detection Limit (fibers/mg)
Adult	96-hr	0	gill	0	37.8-46.4
			liver	0	38.7-27.5
			muscle	0	50.5-77.5
			kidney	0	51.6-55.3
		10^6	gill	0	48.4-89.4
			liver	0	37.4-43.3
			muscle	0	36.3-83.0
			kidney	0	43.0-193.6
		10^{12}	gill	0	57.2-69.1
			liver	0	38.1-38.4
			muscle	0	41.3-59.7
			kidney	53.0±91.8[a]	105.6-145.0
	30-day	0	gill	0	44.1-23.2
			liver	0	68.3-166.0
			muscle	0	85.4-88.0
			kidney	0	105.6-258.0
		10^4	gill	0	47.3-48.5
			liver	0	34.6-21.5
			muscle	0	51.4-72.6
			kidney	0	116.2-89.3
		10^8	gill	0	35.7-54.1
			liver	77.4±77.4[b]	68.2-77.4
			muscle	0	44.7-64.5
			kidney	178.8±178.8[c]	178.8-232.4
Juvenile	96-hr	0	gill	0	25.5-39.2
			liver	0	40.7-71.1
			muscle	0	39.0-92.3
			kidney	0	34.0-182.2
		10^6	gill	0	58.3-85.1
			liver	0	24.1-63.0
			muscle	0	44.5-57.4
			kidney	0	124.6-174.1

Table 19. Continued

Life Stage	Exposure Length	Asbestos Concentration (fibers/liter)	Tissue	Fiber Burden (fibers/mg)	Detection Limit (fibers/mg)
		10^{12}	gill	0	65.9-111.9
			liver	0	20.5-68.9
			muscle	0	27.9-53.5
			kidney	0	88.0-101.3
	30-day	0	gill	0	26.3-69.1
			liver	0	45.2-100.3
			muscle	0	88.0-154.2
			kidney	0	73.9-296.7
		10^{4}	gill	0	27.2-42.2
			liver	0	57.6-58.8
			muscle	0	37.2-42.3
			kidney	0	131.2-151.4
		10^{8}	gill	0	50.9-55.1
			liver	117.2±5.4[d]	56.5-60.5
			muscle	0	65.7-132.9
			kidney	38.59±25.8[e]	183.8-202.1

[a]one fish was above detection at 159 fibers/mg

[b]one fish was above detection at 155 fibers/mg

[c]one fish was above detection at 358 fibers/mg

[d]two fish were above detection at 113 and 121 fibers/mg

[e]two fish were above detection at 368 and 404 fibers/mg

ture survey of 173 toxic substances concluded that growth and mortality responses of larval fish were comparable and both were more sensitive than adult growth, survival, and reprod- In a comparison of different life stages of rainbow trout (Salmo gairdneri) to nickel, Nebecker (1985) found juveniles to be 10-20% more resistant than larval fish in acute tests. Therefore, it is not suprising that growth responses of fish employed in these studies was not greatly altered. The ultimate effect of chrysotile on fish growth may be best explored in younger fish. For example, if chrysotile does influence growth rate and robustness (as is suggested in juvenile fathead minnows) delayed maturation to reproductive age may be observed and result in lowered reproductive output.

Chrysotile exposure did not reduce the swimming performance of adult fish, although controls withstood the 90 cm/sec current longer and swam more body lengths than any other group tested after the exposure period. However, the marked reduction in vigor found in all groups including controls relative to reference fish suggests that testing conditions were sub-optimal for adult minnows. High concentrations of ammonia (0.6-0.8 mg/liter) probably influenced fish health, but are below the 96-hour LC50 of 13.2 mg/L for fathead minnows (API 1980). In other studies which evaluated the behavioral effects of chrysotile to fish Belanger et al. (1985) found that larval coho salmon and juvenile green

sunfish exposed to 10^6 chrysotile fibers/liter for 40-80 days had reduced rheotaxic and swimming ability and were significantly more susceptible to anasthesia. Fibers were found in epidermal tissues and larval salmon developed hypertrophy and hyperplasia of the epidermis. Reductions in swimming ability were attributed to deterioration of the lateral line and surrounding tissue.

AMMONIA CORRELATIONS AND FIBER ACCUMULATION

The presence of asbestos was significantly negatively correlated with total ammonia in the three tests which did not utilize an ammonia scavenging agent: 96-hour adult minnow bioassays ($r = -0.83$), 96-hour juvenile minnow bioassays ($r = -0.71$), and 30-day adult minnow tests ($r = -0.73$). The lack of ammonia in the waters of asbestos-exposed groups could be attributed to any of several sources such as reduced overall activity and reduced excretion, reduced ammonia excreting ability of gills in asbestos-exposed fish, or alteration of kidney function which impaired the ability of fish to excrete ammonia. Woodhead et al. (1983) found that chronic exposures of chrysotile at 1-10 mg/liter to *Poecilia formosa* disrupted the integrity of kidney tissue by selective necrosis, and reduction in tubule diameters from swollen kidney cells. In this study juvenile and adult fish accumulated significant amounts of chrysotile in kidney tissue (178-386 fibers/mg

with a maximum of 404 fibers/mg). Batterman and Cook (1981) reported that of kidney, liver, and muscle tissue, kidney tissue had the greatest burden at 230.5 fibers/mg in arctic char exposed to 10^8 fibers/liter of chrysotile for 3-4 years. It appears likely that reduced ammonia in the water during asbestos exposure to fish is correlated to accumulated fibers and integrity of the kidney, especially in light of the lack of asbestos in fish gill tissue (Table 19).

Cook and Olson (1979) found that humans who ingested water contaminated with amphibole asbestos eliminated fibers in urine for up to 10 days until levels fell below detection. Prolonged contact with kidney tissue and deposition in situ was considered to be one likely contributor to kidney cancers in men occupationally exposed to asbestos (Patel-Mandlik 1981). Epidemiolgical studies also suggest kidneys are particularly susceptible to cancers caused by asbestos (Kanarek et al. 1980; Sigurdson et al. 1981).

In conclusion, the present study suggests that fish exposed to chrysotile at the juvenile and adult life stages do not suffer acute toxicity, but that growth may be affected especially in younger fish. Accumulated asbestos fibers in the kidney may be mostly or entirely responsible for observations of reduced total ammonia in the water during asbestos exposure. Up to 400 fibers/mg were detected in juvenile minnow kidney tissue which are of the same order of magnitude as values reported by other researchers. Earlier life stages

need to be researched to determine effects on the most sensitive life stages.

CHAPTER SIX: JAPANESE MEDAKA

The responses of egg, larval, and juvenile Japanese Medaka to 0, 10^4, 10^6, 10^8, and 10^{10} fibers/liter of chrysotile were evaluated. Medaka are sensitive to tumorgenic compounds and have been used as a model test organism to study the ontogeny of liver cancers. In this study ecologically and toxicologically relevenat parameters (growth and mortality of larval-juvenile fish) are related to histopathology and asbestos fiber uptake. The embryonic stage of development was assayed for hatchability and time to hatch.

Fish have proven in previous research to be sensitive to amphibole and chrysotile asbestos. The pathologies that have been described, however, have not been related to an established chronic toxicity endpoint (e.g., growth) or bioaccumulation of asbestos fibers. This research was undertaken to bridge these gaps.

INTRODUCTION

Chrysotile asbestos is a hydrated silicate of the serpentine mineral family. Fibrous chrysotile has a length to width ratio of 3:1 or greater and occurs in many regions of the world (Speil and Leineweber 1969). Chrysotile accounts for 95% of all asbestos utilized in the United States

of which 96% is imported from Canada (Levine 1978). Chrysotile has been found in 45.8% of 406 water supplies in the United States and its territories and 18% were 10^6 fibers/liter or greater in concentration (Millette et al. 1980). The known sources of contamination are varied and include erosion and decomposition of asbestos-containing materials (Millette et al. 1980; Williams and Muhlbaier 1982), asbestos mining wastes (Batterman and Cook 1981; Hayward 1984), wastes from metal ores which contain asbestos as a contaminant (Cook et al. 1974), aerial deposition of atmospherically suspended asbestos (Cunningham and Pontefract 1971; Hesse and Hallenbeck 1978; Mizota 1982), and natural erosion of parent rock (McGuire et al. 1982; 1983; Polissar et al. 1982).

Reports on the effects and accumulation of chrysotile to aquatic life have been rare. Most of the published studies have assessed effects on fish. Batterman and Cook (1981) reported chrysotile accumulations in arctic char (Salvelinus alpinus) environmentally exposed to 6.7 x 10^8 fibers/liter of chrysotile in Deception Bay, Canada (an arm of Hudson Bay). Up to 230.5 fibers/mg were found in kidney tissue. Woodhead et al. (1983) exposed Poecelia formosa to 0.1-10 mg/liter chrysotile for 6 months and found fish developed epithelial hypertrophy, selective necrosis and swelling of kidney cells, and vacuolation of heart cells. Belanger et al. (1985a) found that larval coho salmon (Oncorhynchus

kisutch) exposed to 3.0 x 10^6 fibers/liter of chrysotile for 40 days lost orientational and swimming ability. The lateral line was found to be degraded in structure and severe hypertrophy superimposed on hyperplasia was evident in epidermal tissues. The expression of laboratory determined pathologies in fish exposed to chrysotile in the field have not been investigated, but two studies suggested that asbestos could affect some pathologies under field conditions. Black et al. (1982) described mesothelioma neoplasms in walleye (Stizostedion vitreum) exposed to copper tailings in Torch Lake, Michigan. Mesothelioma is a carcinoma that is unique to mineral fiber exposure in mammals (Selikoff and Lee 1978; Triol et al. 1984). Secondly, Herman (1985) found rainbow trout (Salmo gairdneri) developed mesothelioma in a trout hatchery fed water through a transite pipe. Unfortunately, in neither study was water evaluated for asbestos content.

Belanger et al. (1985a) compared responses of larval coho salmon and green sunfish (Lepomis cyanellus) during asbestos exposure and determined that the former species at the earlier life stage was more sensitive to asbestos exposure. Woltering (1984) compiled data for nearly 200 toxicants whcih evaluated early life stage responses. He found that larval growth and mortality were equally sensitive and that adult growth, survival, and reproduction were less sensitive indicators of stress than either larval parameter. Egg

hatchability was variable in sensitivity and was considered to be the result of differences in egg composition of the different species evaluated.

The objectives of this study were to evaluate the effects of chrysotile asbestos to Japanese Medaka (Oryzias latipes) eggs and larvae by assessing: (1) egg hatchability, (2) egg survival, (3) larval/juvenile growth, (4) larval/juvenile survival, (5) uptake of chrysotile fibers by fish, and (6) cell ultrastructure and tissue histology of young fish.

MATERIALS AND METHODS.

SPECIES CHOICE

The Japanese Medaka is a freshwater cyprinodontid killifish native to rice fields of China and Japan. Klaunig et al. (1984) explored the use of Medaka in testing the carcinogenicity of organic compounds. The species has a high reproductive capacity under controlled breeding conditions and can yield 20-50 eggs per female per day. Young fish mature rapidly and are very sensitive to liver-specific carcinogens.

BREEDING AND REARING OF FISH

Male and female Medaka (25-35 mm total length) were obtained from Carolina Biological Supply (Burlington, North Carolina) and held in a 110 liter aquarium. Fish were acclimated for two weeks to a 14L:10D light cycle, and cyclic temperature regime (maximum 24.5 C at 10:00 am - 9:00 pm, minimum 20.5 C at 7:30 am when the lights came on) to initiate and sustain daily breeding activity. Adults were fed Tetramin daily (at 7:30 am) and Daphnia pulex every other day.

Medaka eggs gather posterior to the female's vent where they are fertilized by courting male fish (Kirchen and West 1976). Females with greater than five eggs were removed from the aquarium by dip net and curved forceps were used to harvest eggs from the female. Eggs were placed in embryo-rearing solution (Table 20) with methylene blue dye. The dye is useful for combating disease and allows identification of dead eggs whcih take up the stain.

After eggs were harvested, they were sorted and teased apart by forceps. Medaka eggs possess chorionic filaments which are used for attachment to submerged vegetation under natural conditions. Groups of ten eggs were placed in 25 ml of rearing solution in a 100 by 15 mm petri dish until hatching. Preliminary data indicated that hatching would take three weeks at 20 C (20.7 ± 4.8 days, n = 432).

Table 20. Embryo rearing solution for Japanese Medaka (Oryzias latipes).

Compound	Percent
NaCl	15
KCl	0.3
$CaCl_2 \cdot 2H_2O$	0.4
$MgSO_4 \cdot 7H_2O$	1.63
H_2O distilled	(1 liter)
Methylene blue	(4 drops/liter)

Newly hatched larvae were placed in 4 liter aquaria in groups of 15 and fed protozoan infusoria (dominated by Mona, Anthophysis, Oikomonas, Rhynchomonas, and Bodo sp.) twice daily. Older larvae (10-14 mm) were fed Daphnia neonates and brine shrimp flakes. Rearing aquaria water was changed weekly.

PREPARTION OF ASBESTOS.

Grade-5 chrysotile mined ore was obtained from a commercial supplier and suspensions were prepared by lightly milling 400 mg of asbestos, followed by sonicating 500 ml of a 0.060 mg chrysotile/liter stock for two hours using a Fisher Ultrasonic Cleaner. Five replicate determinations of the fiber concentrations indicated that the actual concentration would lie between 3.7×10^8 to 4.3×10^9 fibers/liter.

Fiber identification and determinations were made by the methods of Anderson and Long (1980). Water samples were collected in asbestos-free nalgene bottles and filtered through 0.2 µm pore polycarbonate filters. The samples were carbon-coated, inverted, and placed in a Jaffe-Wick washer for 36 hours on brass TEM grids above filter paper saturated with chloroform. Fiber density estimates were derived by counting three replicate grids of 5 to 10 grid holes per grid (a total of 15 to 30 holes). Background and blanks were processed simultaneously. Fibers were identified by the

characteristic transmission pattern of a central canal and sheath appearance using a JEOL JEM 100C Transmission Electron Microscope (TEM). Four water samples were pooled for analysis of realized chrysotile concentrations at 0, 10^4, 10^6, 10^8, and 10^{10} (Table 21). For all detectable concentrations the realized concentrations were within an order of magnitude of the theoretical concentrations. Chrysotile fibers were not detected in Blacksburg town water (n = 3) and ultrapurified water (n = 2) used for laboratory purposes.

EGG EXPOSURES

Newly fertilized eggs in groups of ten were exposed to chrysotile at 0, 10^2, 10^4, 10^6, 10^8, and 10^{10} fibers/liter in petri dishes and aquaria and assayed for mortality and days to hatch. For petri dish exposures, an aliquot of 25 ml of embryo rearing solution was measured into each dish. An appropriate dilution from a stock concentrate was micropippetted into the appropriate dishes. For aquaria exposures, individual stocks were prepared as described in the section above, and each container was dosed accordingly. Eggs which died in the first 30 minutes of exposure were assumed to be unviable and, therefore, unrelated to asbestos exposure. Rearing medium was changed weekly until all individuals hatched. Each container was checked daily for newly hatched fish and deaths.

Table 21. Selected water chemistry parameters monitored during the 13 week larval Medaka experiment. Means ± SE are given. Sample sizes are 21, 21, 16, 17, and 10 for control to 10^8 fibers/liter, respectively.

Parameter	Asbestos Concentration (fibers/liter)				
	0	10^4	10^6	10^8	10^{10}
Asbestos Concentration[1] (fibers/liter)	0	0	5.1 ± 2.8 x 10^6	4.7 ± 3.2x10^8	7.6 ± 8.1x10^{10}
Temperature (°C)	19.8 ± 0.3	19.7 ± 0.4	19.7 ± 0.4	20.0 ± 0.5	19.8 ± 0.3
pH	8.10 ± 0.03	8.15 ± 0.01	8.02 ± 0.05	8.33 ± 0.07	8.15 ± 0.02
Dissolved Oxygen (mg/liter)	8.3 ± 0.9	-	-	8.6 ± 0.5	8.8 ± 0.2
Hardness (mg/liter as $CaCO_3$)	69.3 ± 2.0	71.2 ± 4.1	70.9 ± 6.2	64.6 ± 3.6	62.9 ± 2.9
Alkalinity (mg/liter as $CaCO_3$)	40.2 ± 0.7	39.6 ± 4.0	42.8 ± 3.3	43.9 ± 3.0	42.7 ± 0.9
NH_3-N (mg/liter)	0.207 ± 0.029	0.166 ± 0.014	0.163 ± 0.021	0.142 ± 0.011	0.102 ± 0.013

[1]Detection limits were 4.8-10.7 x 10^4 fibers/liter for all samples.

LARVAL EXPOSURES

Newly hatched larvae were exposed to 0, 10^4, 10^6, 10^8, and 10^{10} fibers/liter for 13 weeks (91 days) in 4-liter aquaria in triplicate. Fifteen larvae were placed in each system. Water chemistry parameters (temperature, pH, dissolved oxy- alkalinity, hardness, and ammonia) were monitored twice monthly for the duration of the study by Standard Methods (n = 21 including all replicates, Table 21) (APHA et al. 1981).

Mortality was monitored twice daily, and dead fish were removed when observed. Data are expressed as percent mortality at each concentration relativized for control death. Growth (total length in mm) was evaluated on the seventh, fourteenth, twenty-eighth, fifty-sixth, and ninety-first days. Initial size was estimated using a cohort of 15 fish which had recently hatched and were not exposed. The first four measurements were made using a binocular microscope by removing fish from their tanks for observation. Fish were measured by vernier calipers (± 0.025 mm) on day 91.

TISSUE PATHOLOGY

Histology and cell ultrastructure were evaluated at the end of the fourth and thirteenth week of exposure. Fish were preserved in a 0.1M phosphate-buffered solution of 5% glutaraldehyde, 3% formalin, and 0.25% picric acid. The

heads of larger fish (13 weeks old) were removed to facilitate infiltration. Fish were post-fixed in 1.0% osmium tetraoxide, series alcohol dehydrated, infiltrated with propylene oxide and embedded in Polybed 812 resin. Thick sections were stained with toluidine blue and saphranin and viewed with a Leitz Dialux light microscope. Direct measurements of various regions of interest (e.g., kidney tubules, intestinal lining, liver, and epidermal tissues) were made, and photomicrographs were taken of representative tissue. Prior to staining, scans of thin-sections for chrysotile fibers were made using the TEM. Thin-sections were then stained with uranyl acetate and lead citrate and viewed by TEM. Micrographs were evaluated using planimetric (Sorenson and Bauer 1984) and grid counting techniques (Hughes 1972).

FIBER UPTAKE

Uptake of chrysotile asbestos by Medaka was evaluated at 0, 10^4, 10^6, 10^8, and 10^{10} fibers/liter. Since fish were too small to excise a sufficient amount of individual tissues, whole fish were prepared for analysis by the methods of Batterman and Cook (1981) and Patel-Mandlik (1981). The head and fins were cut from the body and the remaining tissue was placed in a porcelain dish that was previously sonicated in an acid bath. Each dish was tightly covered with aluminum

foil, and the tissue ashed at 500 C for 8 hours. The ash was suspended in 6M HCl, filtered and otherwise treated as a water sample in preparation for TEM analysis.

STATISTICAL ANALYSIS

In all cases, the mean ± 1 SE are given. Not all data were normally distributed, so for ease of comparison and consistency nonparametric techniques were used. For one-way layout data (egg hatchability, egg survival, larval growth, and tissue and cell structure) a one-way analysis of variance rank-analogue, the Kruskal-Wallis Test was used (Hollander and Wolfe 1973). If significant differences were found, a rank-like Least Signifcant Differences Procedure was used to compare means ($\alpha = 0.05$). In cases of paired data (liver tissue ultrastructure) Wilcoxon's Rank Sum Test was employed.

RESULTS

EGG HATCHABILITY AND SURVIVAL

Medaka eggs reared in petri dishes or aquaria and exposed to chrysotile took significantly longer to hatch (16.1-20.4 days in petri dishes and 13.9-14.8 days in aquaria) compared to controls (Table 22, $p < 0.005$). The pattern was inconsistent relative to nominal asbestos concentration, espe-

Table 22. Hatching and mortality of Japanese Medaka eggs (Oryzias latipes) exposed to chrysotile asbestos in petri dishes and 4-liter aquaria.

Asbestos Concentration	Individuals Observed (n)	Days to Hatch	Kruskal-Wallis (p-value)	Groups Tested (n)	Mortality (%) mean ± S.D.	Kruskal-Wallis (p-value)
Petri Dishes						
0	37	15.7 ± 3.2^{a}		4	7.5 ± 9.6	
10^{2}	20	20.5 ± 3.2^{d}		2	0 ± 0	
10^{4}	33	15.3 ± 2.2^{a}		4	17.5 ± 9.6	
10^{6}	37	17.2 ± 3.9^{bc}		4	7.5 ± 5.0	
10^{8}	40	16.5 ± 3.9^{b}		4	0 ± 0	
10^{10}	36	16.1 ± 3.5^{ab}	17.319 (0.005)	4	10.0 ± 8.2	9.731 (0.062)
Aquaria						
0	24	13.7 ± 2.7^{a}		3	20.0 ± 17.3	
10^{2}	25	13.9 ± 2.9^{a}		3	17.3 ± 7.1	
10^{4}	22	14.6 ± 3.7^{b}		3	26.6 ± 5.8	
10^{6}	21	14.1 ± 4.0^{ab}		3	30.0 ± 17.3	
10^{8}	24	14.8 ± 2.7^{c}		3	20.0 ± 22.0	
10^{10}	23	14.3 ± 1.9^{b}	22.874 (0.005)	3	23.3 ± 11.5	2.731 (0.500)

cially for fish exposed in petri dishes where the highest concentration (10^{10} fibers/liter) was not significantly different from controls and the lowest concentration (10^{2} fibers/liter) was significantly different from controls.

Medaka eggs did not suffer significant differential mortality at any asbestos concentration in either petri dishes or aquaria (Table 22). Egg mortalities ranged from 0% (10^{2} and 10^{8} fibers/liter in petri dishes) to 30.0% (10^{6} fibers/liter in aquaria). In general, eggs exposed in aquaria did not survive as well as eggs exposed in petri dishes.

LARVAL-JUVENILE SURVIVAL AND GROWTH

Medaka exposed to chrysotile asbestos at 10^{10} fibers/liter suffered nearly complete mortality (98%) by day 42 (Fig. 15). Fish exposed to 10^{6} and 10^{8} fibers/liter experienced gradual losses until day 63 whereafter further mortality was not observed. Fish exposed to 10^{4} fibers/liter did not suffer appreciably more mortality than controls. Actual mortalities (unadjusted for control death) were 17.7, 21.2, 28.3, 27.9, and 98.0% for Medaka exposed to 0, 10^{4}, 10^{6}, 10^{8}, and 10^{10} fibers/liter, respectively.

Growth in total length of Medaka exposed to asbestos was significantly reduced by the second week (Fig. 16). During the course of the study 10^{6}, 10^{8}, and 10^{10} fibers/liter were

Figure 15. Mortality of larval-juvenile Medaka (relativized for control death) exposed to 0, 10^4, 10^6, 10^8 and 10^{10} fibers/liter of chrysotile asbestos.

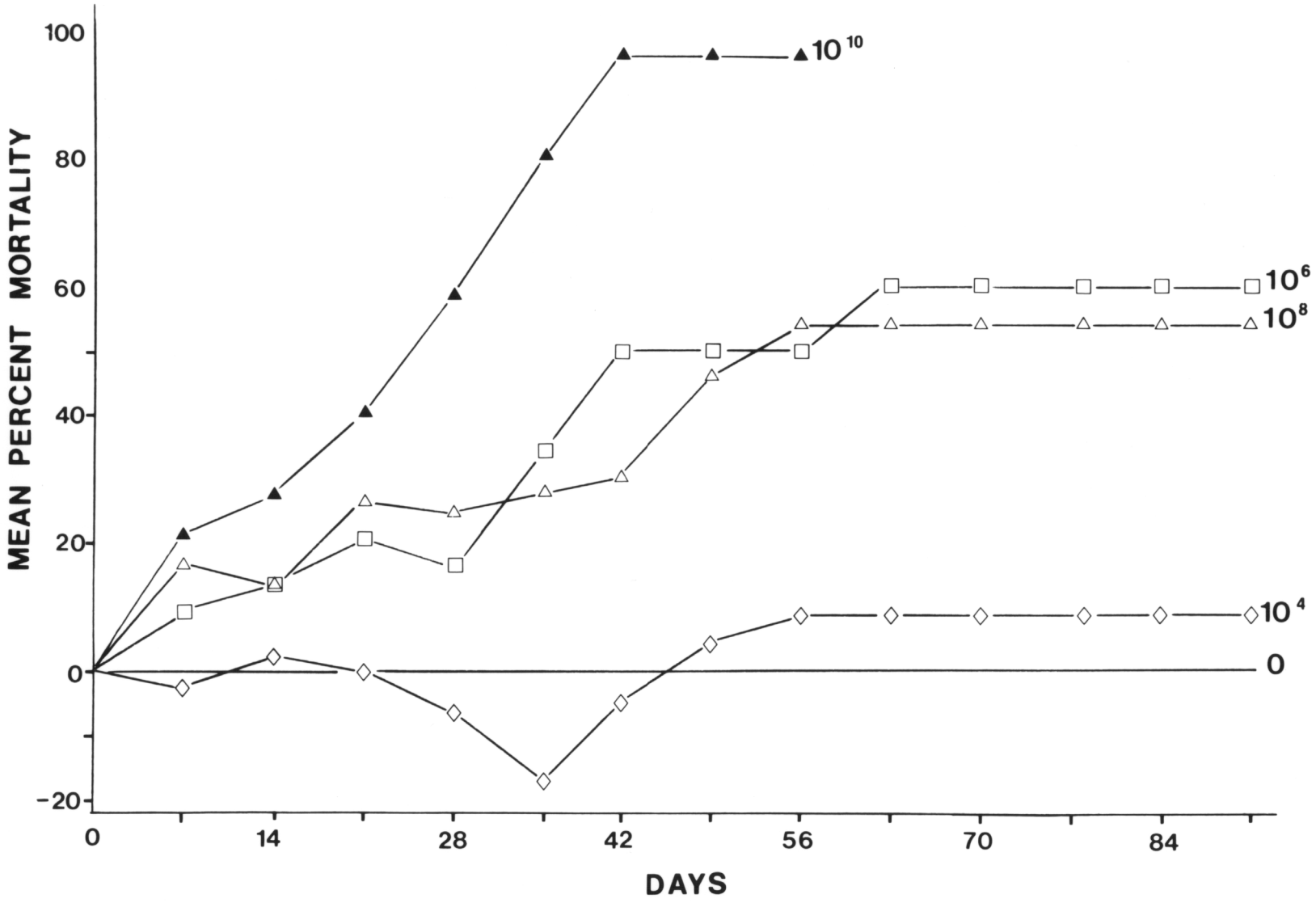
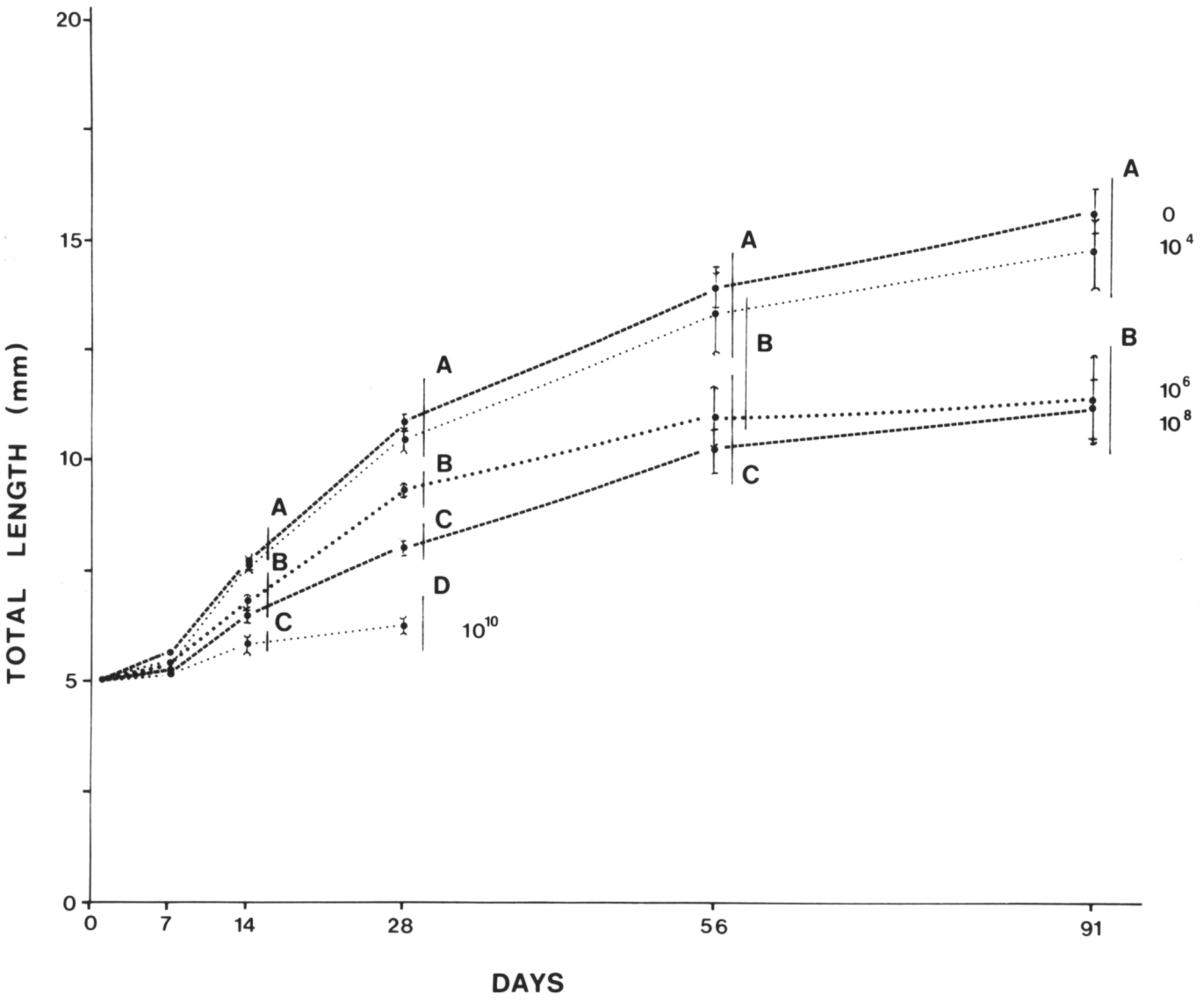

MEAN PERCENT MORTALITY
100
80
60
40
20
0
-20
0
14
28
42
56
70
84
DAYS
10^{10}
10^{6}
10^{8}
10^{4}
0

Figure 16. Growth of Medaka during asbestos exposure at 0 to 10^{10} fibers/liter. Vertical lines connect means which are not significantly different ($\alpha = 0.05$).


TOTAL LENGTH (mm)
20
15
10
5
0
0
7
14
28
56
91
DAYS
A
B
C
D
10^{10}
A
B
10^{6}
10^{8}
A
0
10^{4}

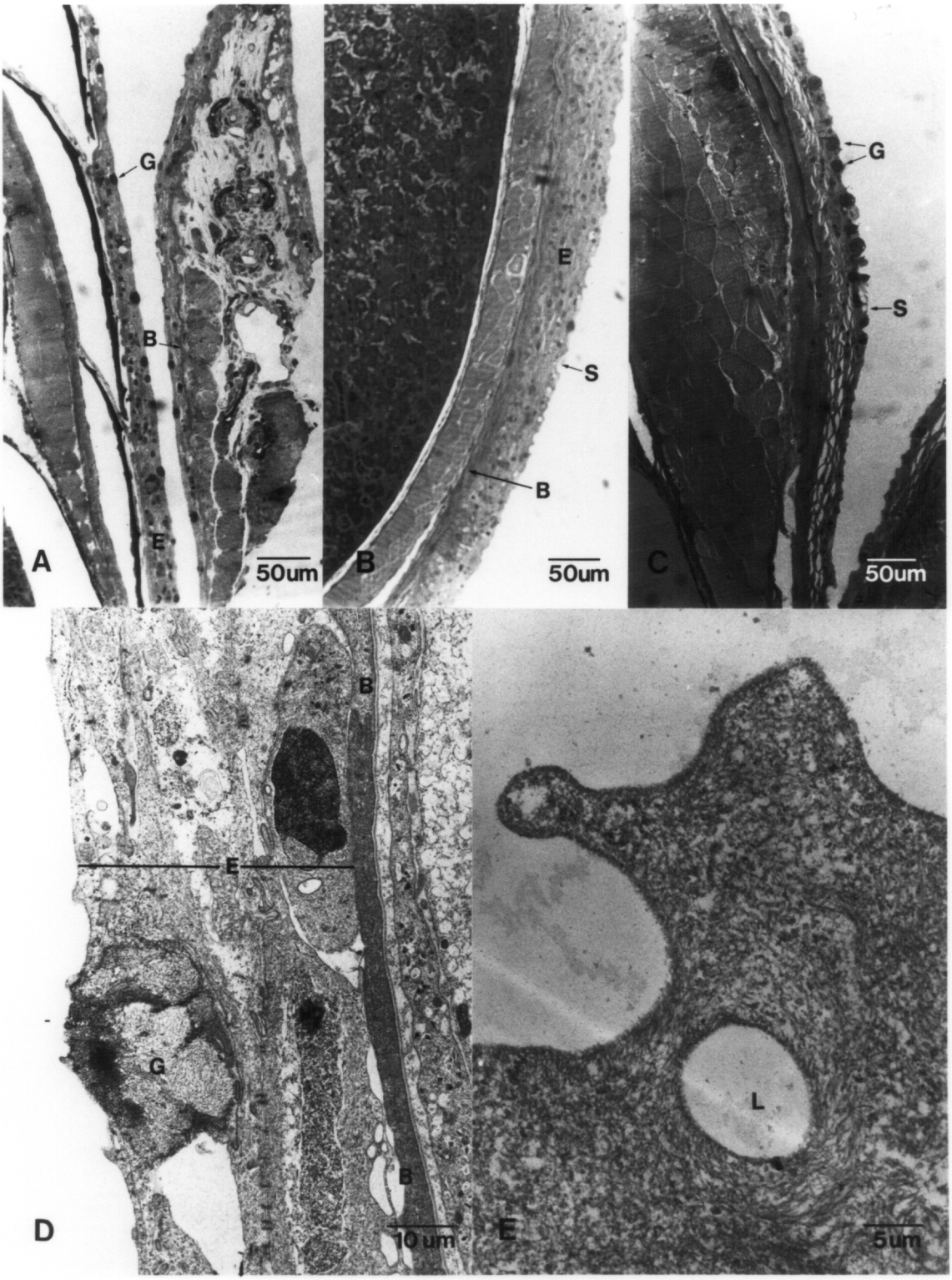
consistently significantly less than controls and 10^4 fibers/liter. By day 91 controls were approximately 30% larger than fish reared at 10^6 and 10^8 fibers/liter. Growth responses were similar to the observed levels of mortality.

TISSUE HISTOLOGY AND CELL ULTRASTRUCTURE

Several histological characteristics were noticeably altered at the light microscope level. Epidermal tissue was significantly thicker in asbestos-exposed fish. Control fish characteristically possessed 5-6 cells above the basal lamina (Fig. 17A) to an average thickness of 29.7 ± 3.0 µm (Table 23). However, fish exposed to 10^8 fibers/liter had up to 9 cells above the basal lamina (Fig. 17B) and the epidermal layer was 50.6 ± 1.1 µm. In addition, fish which expereinced asbestos exposure possessed a sculpted outer cell layer as opposed to the smooth appearance of control fish (Fig. 17C, 17D).

Fish exposed to chrysotile were found to have a greater number of goblet cells in the intestinal lining than exposed fish (Fig. 18). Controls, 10^4, 10^6, and 10^8 fibers/liter exposed fish had 0.0030 ± 0.0041, 0.0115 ± 0.0031, 0.2562 ± 0.0298, and 0.3601 ± 0.0580 goblet cells per µm of intestinal lining in cross-section (n = 2 fish for each group). The lack of significant differences between groups can be attributed to a small sample size.

Figure 17. A. Epidermal tissue of a control juvenile Medaka. The epidermal layer (E) was comprised of 3-5 cells tightly grouped above the basal lamina (B). Goblet cells (G) were common.
B. Epidermal tissue of a juvenile Medaka exposed to 10^8 fibers/liter for 91 days. The epidermal layer was thickened up to 9 cells above the basal lamina. The exterior cell surfaces were often sculpted (S) in response to asbestos irritation.
C. Epidermal tissue of a juvenile Medaka exposed to 10^8 fibers/liter for 91 days showing sculpturing of the outer cell layer.
D. Electron micrograph of the outer cell surface of the control epidermis. Lacunae (L) were common.
E. Electron micrograph of the outer cell surface from a fish exposed to 10^8 fibers/liter.


G
B
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50um
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G
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10 um
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5um

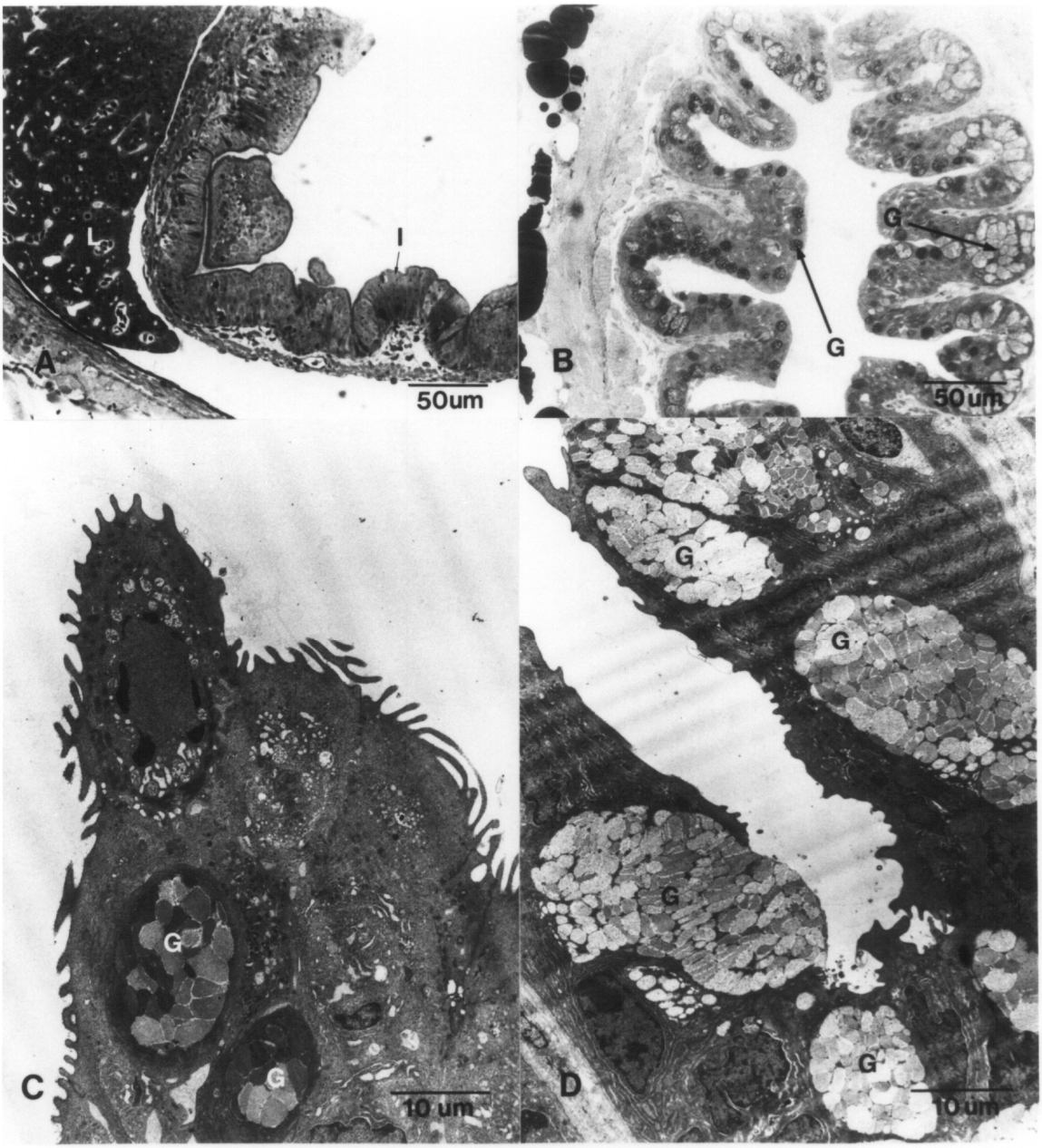
Table 23. Effect of chrysotile asbestos on epidermal and kidney tissue of Japanese Medaka exposed for 91 days. Groups which are significantly different are indicated by different letters ($\alpha = 0.05$).

Asbestos Concentration (fibers/liter)	Epidermis			Kidney		
	Number of Cell layers (range)	Thickness[1] (um)	Kruskal-Wallis (p-value)	Kidney tubule[2] diameter (um)	Kruskal-Wallis (p-value)
0	5-6	29.7 ± 3.0^a		31.4 ± 2.2^a	
10^4	4-7	31.4 ± 0.9^a		24.1 ± 0.5^b	
10^6	5-7	42.8 ± 0.9^b		13.9 ± 0.8^c	
10^8	6-9	50.6 ± 1.0^b	24.217 ($p < 0.005$)	16.7 ± 1.1^c	17.253 ($p < 0.005$)

[1] sample sizes were 12 for each group.

[2] sample sizes were 23, 24, 17, and 13 for 0, 10^4, 10^6, and 10^8 fibers/liter treatments, respectively.

Figure 18. A. Intestinal lining (I) of a control Medaka after 91 days of development. Few goblet cells were found.
B. Intestinal lining of a Medaka exposed to 10^8 fibers/liter. Goblet cells were profuse and were a dominant feature of the tissue.
C. Electron micrograph of the intestinal lining of a control fish showing goblet cells (G).
D. Electron micrograph of a fish exposed to 10^8 fibers/liter. Goblet cells were numerous and at least seven are visible in this field.


A
L
I
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B
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C
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G
10 um
D
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G
10 um

The cross-sectional diameters of fish kidney tubules were found to be significantly different relative to asbestos exposure (data were pooled for proximal and distal tubules) (Fig. 19, Table 23). Control tubules were 31.4 ± 2.2 μm and were double the size of tubules from fish exposed to 10^8 fibers/liter (16.7 ± 1.1 μm).

Two fish exposed to 10^{10} fibers/liter for 28-56 days developed epidermal outgrowths on the ventrum. The outgrowths were consistent with tumor (in the archaic sense of the word, meaning a swelling) and neoplastic characteristics by possessing an unorganized region of tissue surrounding a strongly staining region (Fig. 20). In addition, selective necrosis and vacuolated cells were present at the edge of the swelling. Observations of the region by TEM indicated a large amount of disorganized and necrotic tissue (Fig. 21). Some regions of the epidermis appeared fibrous and were pocketed with small holes (Fig. 21B). Unstained thin sections were found to contain chrysotile fibers in long and apparent cross-sections (Fig. 22).

Liver tissue was not noticeably altered at any asbestos concentration as observed by light microscopy, but under TEM some regions of the liver were found to contain dense aggregates of coiled endoplasmic reticulum (Fig. 23). Fish exposed to 10^8 fibers/liter had significantly greater amounts of lipid and endoplasmic reticulum relative to controls (Table 24). Control fish liver had a cell density of approxi-

Figure 19. A. Kidney tissue from a control Medaka. Proximal and distal tubules were approximately 30µm in diamter on the average. Hematopoietic tissue (H) and muscle surrounded the kidney.

B. Kidney tissue from a Medaka exposed to 10^8 fibers/liter for 91 days. Tubules were approximately 17µm in diameter on the average and some were highly constricted (T).

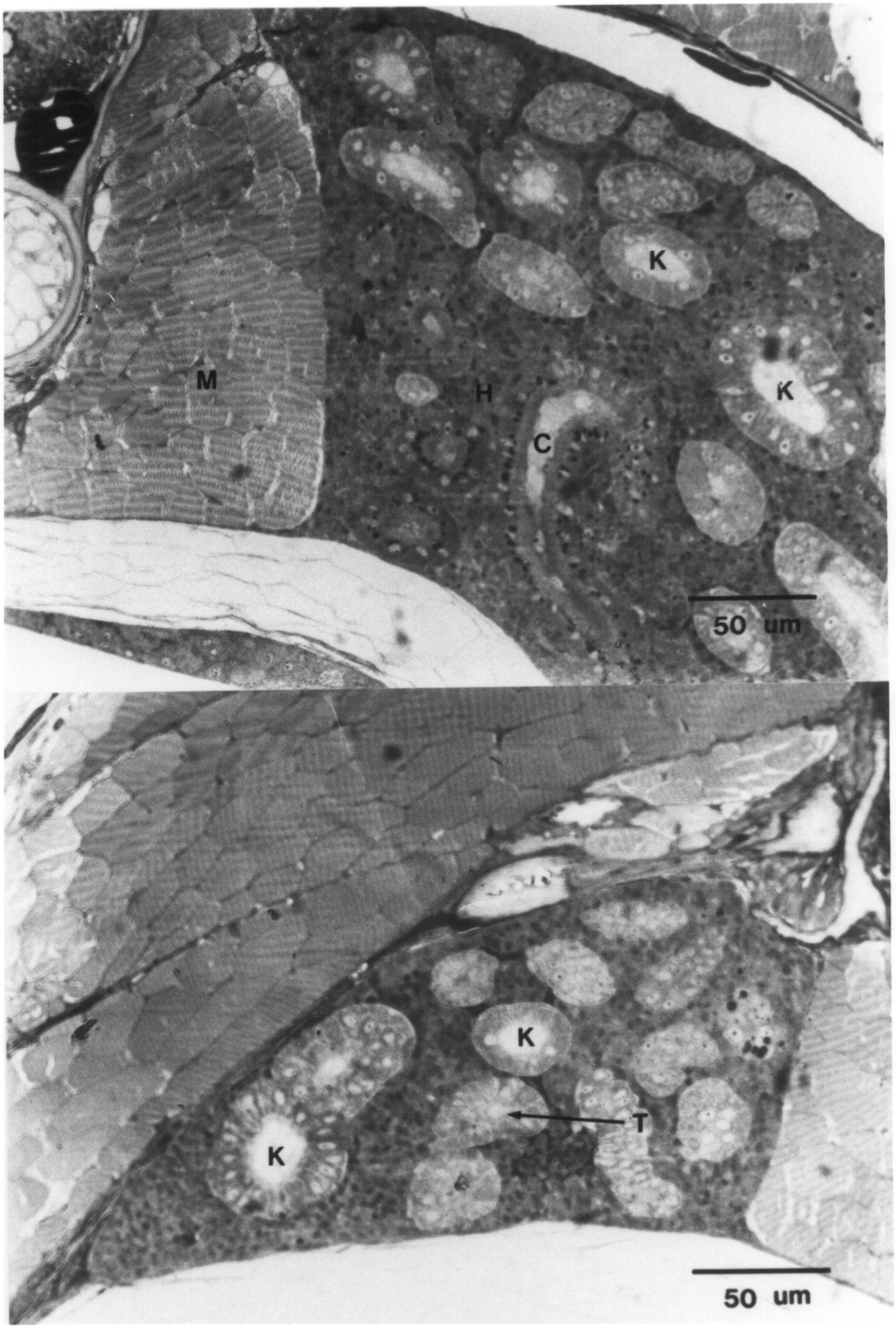
K
M
H
K
C
50 um
K
T
K
50 um

Figure 20. A. Histological section from an epidermal tumor of a Medaka exposed to 10^{10} fibers/liter for 28 days. The tumor was at the apex of the small remaining yolk sac (Y).
B. Histological section of the tumor showing a dark staining region (B) surrounded by enlarged (E) and vacuolated (V) cells.

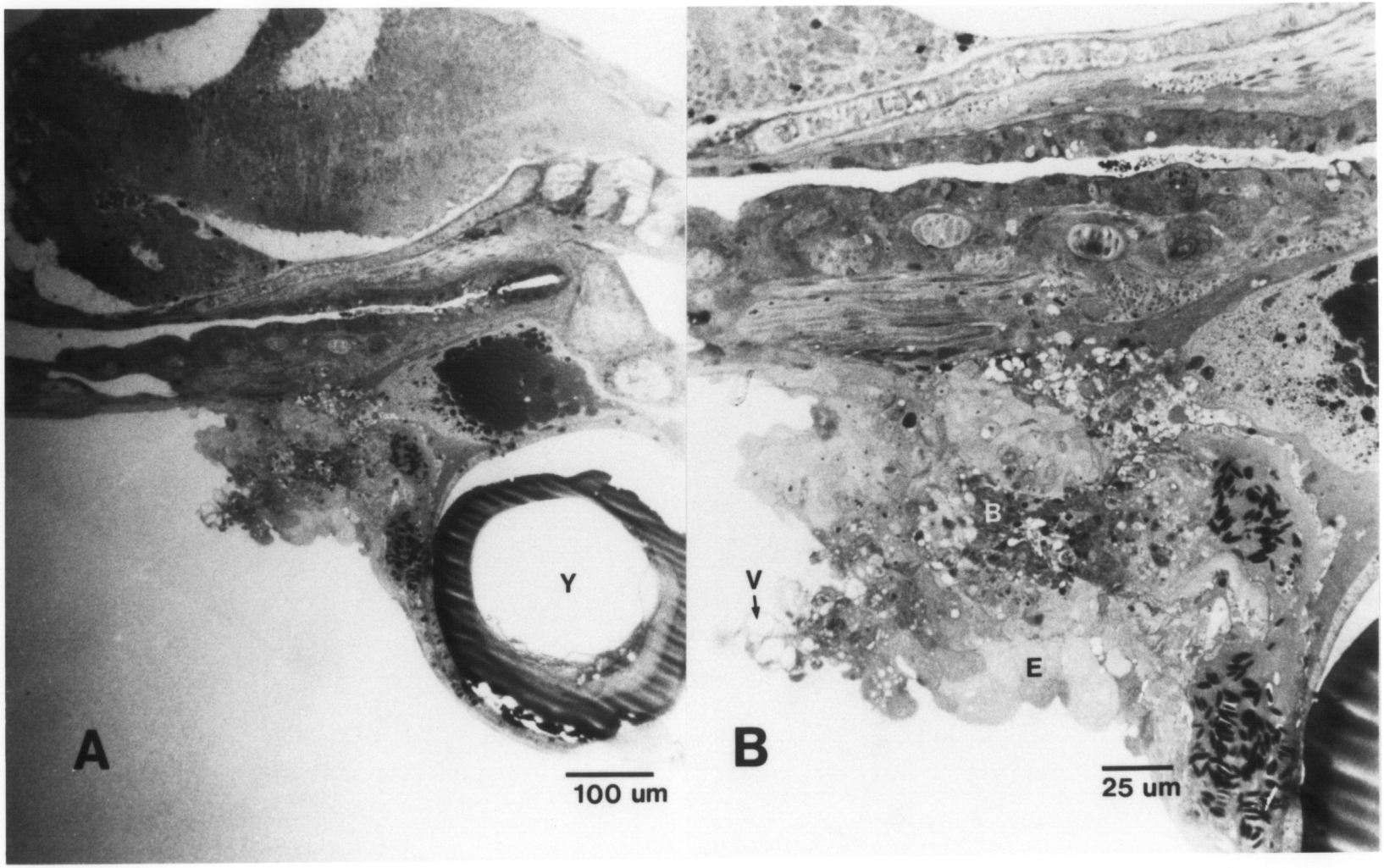
A
Y
100 um
B
V
B
E
25 um

Figure 21. A. Necrotic tissue (D) at the edge of the yolk sac (Y) was found in the tumor depicted in Figure 6. Remnants of cell membranes (M and arrows) and nuclei (N) were numerous.
B. Electron micrographs of cells at the periphery of the epidermis. Epidermal cells (E) in this region had lacunae (L) and copious amounts of collagen (C).
C. Several lacunae contained fragments of chrysotile asbestos (A).
D. Large amounts of collagen fibers (C) were found in these cells, probably in response to irritation by asbestos.

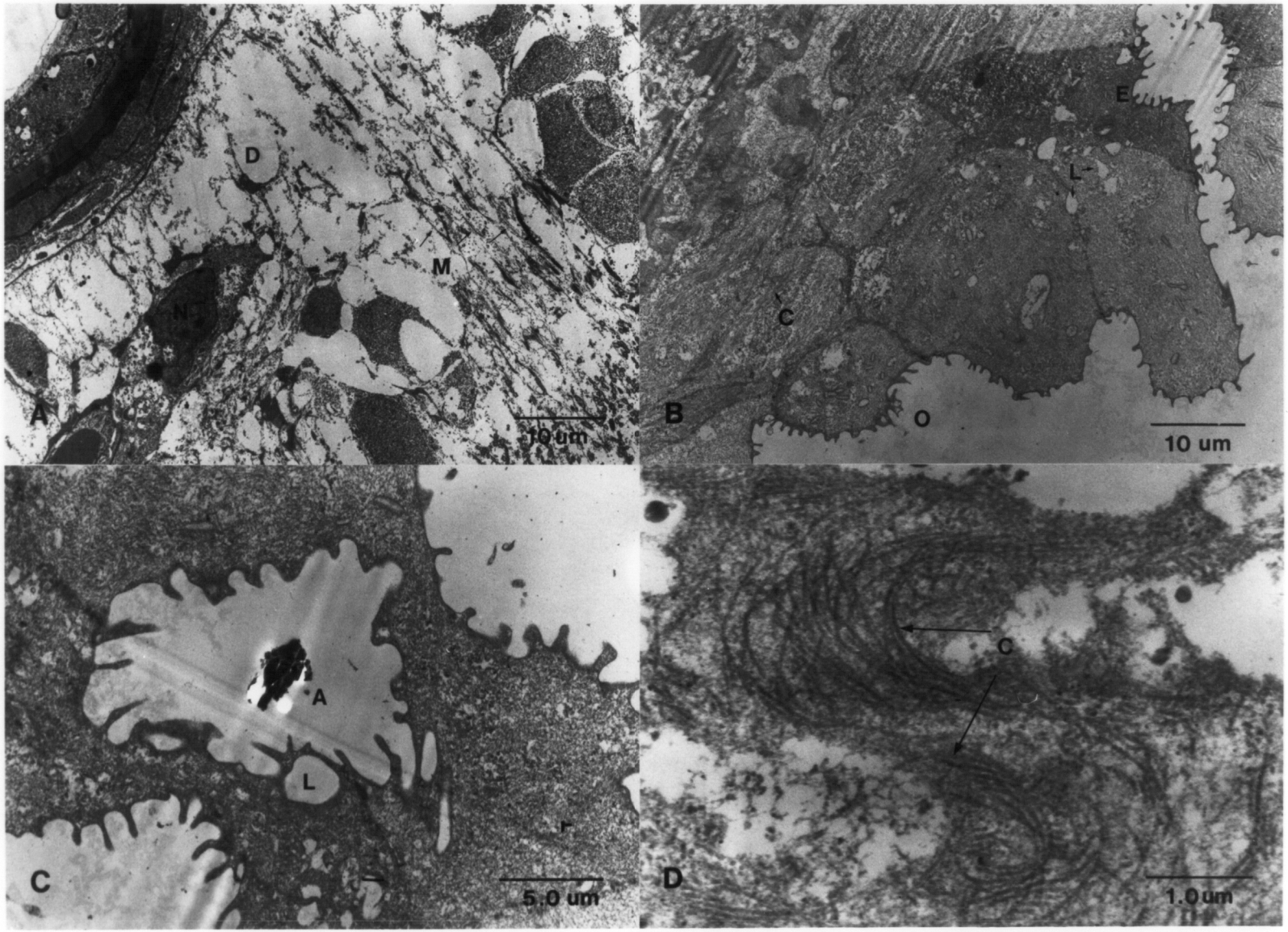
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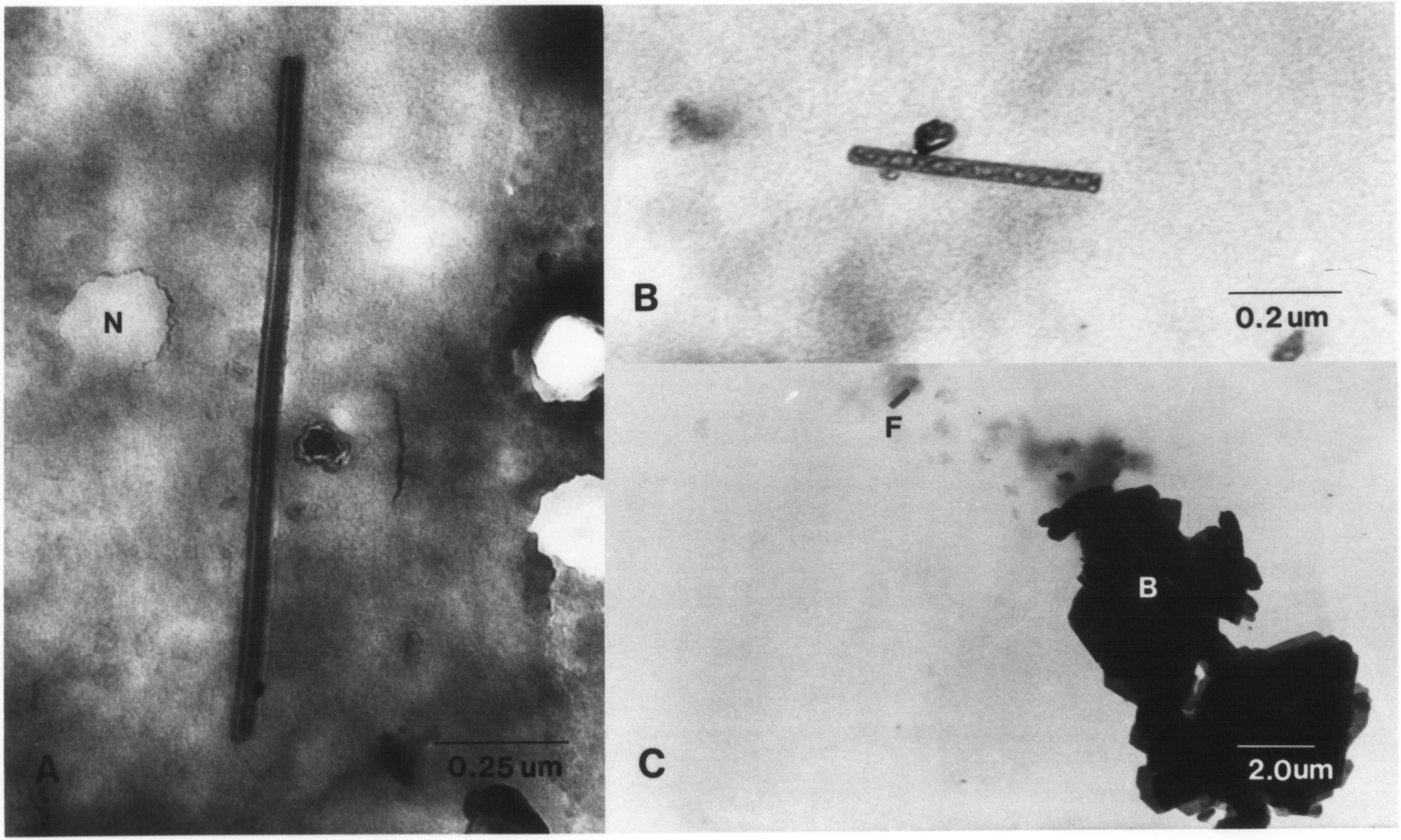
Figure 22. A. Chrysotile fiber (approximately 0.06μm in width) isolated from a Medaka exposed to 10^8 fibers/liter for 91 days.
B. Unstained thin section of a Medaka exposed to 10^{10} fibers/liter for 3 days showing an asbestos fiber in long section approximately 0.03μm wide).
C. Unstained thin section of a Medaka exposed to 10^{10} fibers/liter for 28 days. Large asbestos fragments (B) and small fibers (F) were observed.


N
A
0.25 um
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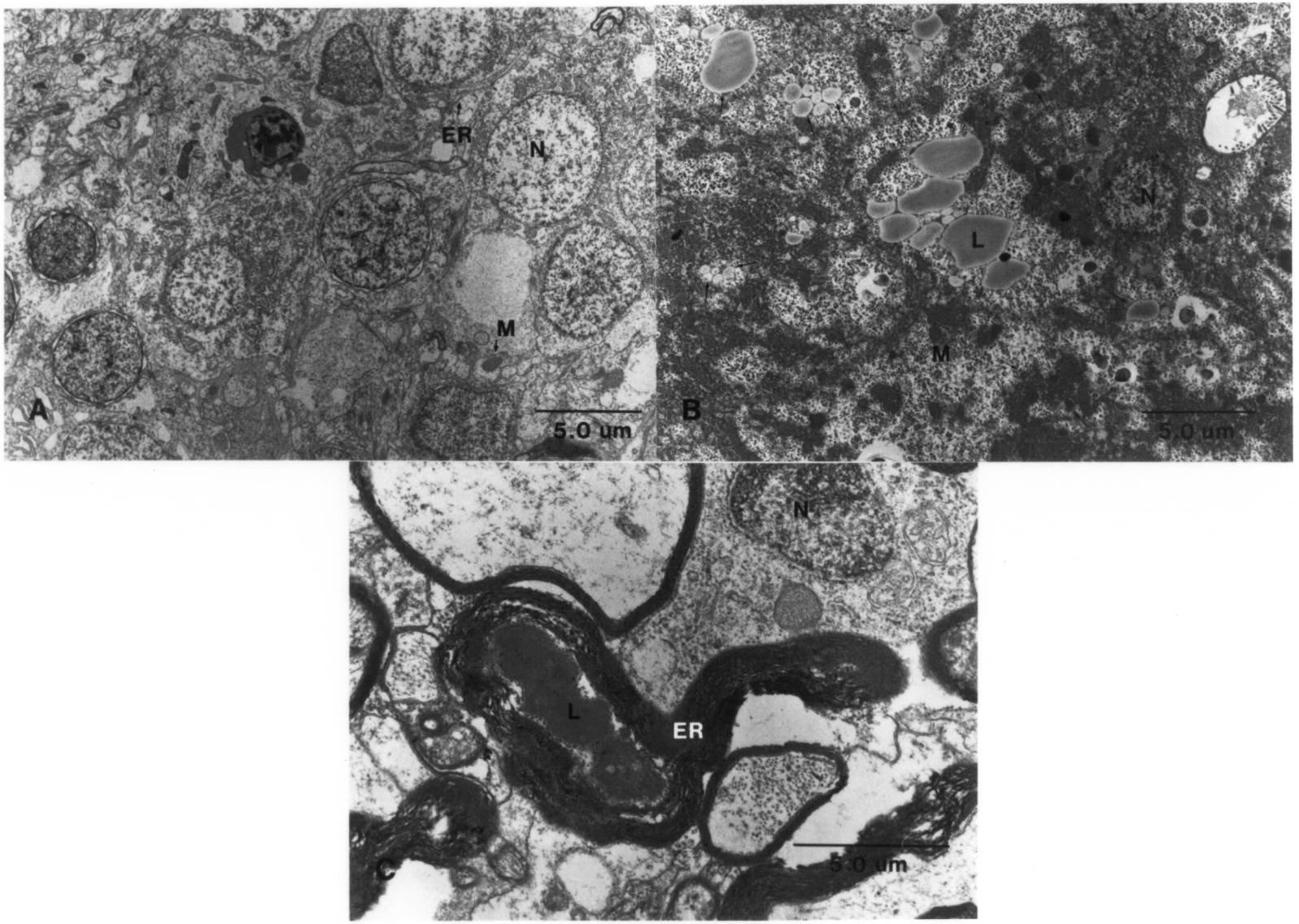
0.2 um
F
B
C
2.0um

Figure 23. A. Liver tissue of a 91-day old control Medaka. Round nuclei (N), moderate amounts of endoplasmic reticulum (ER), and mitochondria (M) were characteristic of this tissue.
B. Liver cells from a fish exposed to 10^8 fibers/liter for 91 days. Large numbers of irregularly shaped lipid droplets (L and arrows) were evident.
C. Necrotic liver tissue from the same fish as Figure 9B. Dense aggregates of endoplasmic reticulum around lipid and cytoplasm were abundant.


ER
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ER
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Table 24. Evaluation of cellular components (mean ± SE) and cell density of Medaka liver tissue after 91 days exposure to chrysotile asbestos (n = 6 for all parameters).

Parameter	Asbestos Concentration (fibers/liter) Control	10^8	Wilcoxon Rank Sum (p-value)
Cell density (cells/um^2)	52.3 ± 7.9	77.5 ± 21.4	49 (p =0.07)
Subcellular Components			
Nucleus (%)	25.6 ± 7.9	11.7 ± 6.2	56 (p =0.002)
Mitochondria (%)	10.7 ± 1.1	7.3 ± 3.1	43 (p =0.294)
Lipid (%)	3.7 ± 0.3	11.6 ± 2.4	58 (p =0.001)
Cytoplasm (%)	51.3 ± 3.2	53.7 ± 3.9	41 (p =0.409)
Endoplasmic reticulum (%)	4.6 ± 0.9	10.1 ± 6.5	49 (p =0.07)
Vacuoles (%)	2.1 ± 1.0	4.7 ± 0.9	40 (p =0.469)
Other (%)	2.0 ± 0.5	0.9 ± 1.1	44 (p =0.242)

mately 52.3 ± 7.9 cells/μm^2, whereas fish exposed to 10^8 fibers/liter for 91 days were approximately 77.5 ± 21.4 cells/μm^2.

FIBER ACCUMULATIONS

Fish did not accumulate chrysotile after only one month of exposure at any concentration except 10^{10} fibers/liter (375.7 fibers/mg, Table 25). By three months of exposure fish at 10^8 fibers/liter had nearly 500 fibers/mg. Detection limits were high and ranged from 149.8-291.3 fibers/mg in the group analyzed at 28 days and 126.8-258.6 fibers/mg in the group analyzed at 91 days.

DISCUSSION

EGG HATCHABILITY AND SURVIVAL

The effects of chrysotile asbestos on egg hatchability did not necessarily increase with dose and was inconsistent especially for eggs reared in petri dishes. Eggs in petri dishes were possibly dosed in a less effective manner (concentrations were not validated for this aspect of the study) due to smaller test volumes which would allow for greater room for error. Also, eggs exposed in aquaria took 2-6 days less time to hatch than groups exposed in petri dishes, but

Table 25. Total body burdens of chrysotile asbestos in Japanese Medaka (mean ± SE) exposed for 28 and 91 days.

Asbestos Exposure Concentration (fibers/liter)	Length of Exposure (days)	n	Asbestos Body Burden (fibers/mg dry weight)	Detection Limit (Range in fibers /mg dry weight)
0	28	3	0	171.3-200.2
10^4	28	3	0	149.8-183.1
10^6	28	3	0	189.9-193.8
10^8	28	3	0	170.2-216.4
10^{10}	28	1	275.7	291.3
0	91	3	0	130.2-141.2
10^4	91	2	0	157.4-258.6
10^6	91	3	0	158.6-168.0
10^8	91	3	486.4±47.9	126.8-169.6
10^{10}	91	-	-	-

suffered 10-20% more mortality. Some indications for dose-dependent hatchability response in aquaria is presented, but is probably biologically insignificant as hatching was de-only 0.6-1.0 days longer in asbestos exposures compared to controls.

Mortality was not significantly affected using either exposure method. In many hatchability studies the length of time to hatch was a variable indicator of stress because not all toxicants can penetrate the protective egg chorion (Eaton et al. 1978; Stevens and Chapman 1984; Woltering 1984). In Woltering's (1984) comprehensive review of organic and metal toxicants and chemical conditions to fish showed egg hatchability was intermediate in sensitivity to fry survival and growth (most sensitive) and adult survival and growth (least sensitive).

LARVAL SURVIVAL AND GROWTH

Medaka larvae were sensitive to chrysotile asbestos exposure and experienced substantial mortality at 10^6-10^{10} fibers/liter. Fish exposed to 10^4 fibers/liter (a concentration which was below detection, Table 22) did not suffer mortality much greater than controls. In general, growth and mortality data were in good agreement and controls and 10^4 fibers/liter exposed fish were never signifcantly different throughout the study. Larvae exposed to 10^6-10^8 fibers/liter

had lower densities in each chamber relative to lower asbestos concentrations throughout the study and still grew at a lower rate (i.e., controls did not suffer from density depensation (Woltering 1984)). Many studies dealing with effects of toxicants on fry growth and survival have found that the density of fish surviving a toxic episode will radically alter the observed growth responses as competition for food leads to greater variation in the growth of individuals (Ivan and Cella 1981; Goodman 1982). In this study, fish exposed at high asbestos concentrations (e.g. 10^{8}-10^{10} fibers/liter) appeared emaciated even though food was plentiful.

TISSUE HISTOLOGY AND CELL ULTRASTRUCTURE

Several histological and ultrastructural characteristics were found to change in fish exposed to chrysotile asbestos for 3 months. Intestinal goblet cells were more abundant and were most prevalent in asbestos stressed fish. Excessive mucous secretion activity in response to irritation by asbestos in the gut may diminish absorption of food items; however, this relationship has not been experimentally estbalished. Warheit al. (1984) and Kagan (1983) found cellular secretory activity to increase in response to asbestos irritation of lung tissue. The uptake of other particulates by gut epithelia has been demonstrated in fish by Woodhead

et al. (1981) and irritation effects were presumed present. The restriction in kidney tubule diameters found in this study is consistent with observations by Woodhead et al. (1983) who exposed Poecilia formosa to 0.1 and 10 mg/liter of chrysotile for 6 months. They found necrosis of kidney hematopoietic tissue, fibrous mats of endothelium, and dilation of kidney tubules. These pathologies were not as dramatic in Medaka which were exposed to a much lower (an environmentally realistic) level of chrysotile for a shorter time period. It is possible that smaller kidney diameters impaired osmoregulatory function and lowered fish activity. Belanger et al. (1985a) found asbestos-stressed coho salmon had reduced activity and swimming ability after 40-80 days exposure to chrysotile asbestos at 10^6 fibers/liter.

Epidermal tissues of asbestos-exposed Medaka were significantly thickened in this study. The primary response appeared to be mild hyperplasia, which is an increase in cell numbers and not size. Coho salmon exposed to asbestos responded similarly to asbestos exposure and also developed hypertrophic epidermal tissue (Belanger et al. 1985a). Medaka exhibited some sculpturing of the outer cell surface, especially at higher levels of asbestos exposure. Belanger et al. (1985a) described extensive epithelial vacuolation in the outer layers of coho salmon epidermis after asbestos exposure, reminiscent of the description of cellular hemolysis by Harington et al. (1975). In contrast, Amazon mollies did

not express any histopathology of the epidermis up to levels of 10 mg/liter (Woodhead et al. 1983). Apparently, a range of potential expressions of epidermal irritation to asbestos exists in a variety of fish species.

Histological and ultrastructural alterations of liver cells in Medaka exposed to chrysotile was unexpected since Batterman and Cook (1981) and Belanger (unpublished data) found relatively few fibers in arctic char and fathead minnow livers. Klaunig et al. (1984) and Aoki and Matsudira (1977) found Medaka to be disposed to a variety of tumor pathologies including trabecular hepatoma, cholangioma, and multiple liver foci after brief (1-10 days) exposures of larvae to acetate derivatives and DENA (N-methyl-N'-nitro-N-nitrosoguanadine) followed by 90-180 days further development. The liver of Medaka during normal development occupies the majority of the body cavity (Ishakawa et al. 1975) and is more physiologically dynamic (i.e, has greater fluxes of glycogen, lipid, and cellular components) than other model species used in cancer research such as small rodents and rainbow trout (James E. Klaunig, Department of Pathology, Medical College of Ohio, Toledo, Ohio, personal communication and Lipsky et al. (1978)). In this study several regions of liver from fish exposed to 10^8 fibers/liter for 91 days contained dense aggregations of endoplasmic reticulum and lipid droplets. These sites were found to be necrotic by electron microscopy. Chrysotile depressed fish growth by day 14 of

exposure. Early in the study, when fish fed on small protozoans, asbestos fibers may have interfered with feeding activity. Fish exposed to 10^{10} fibers/liter were emaciated prior to death and were found to have sunken eyes indicating starvation. Liver conditions often reflect an inadequate diet (Post 1983) and probably contributed to the observed liver condition in this study.

Epidermal outgrowths which were an assemblage of vacuolated cells, necrotic but intact cells, and mixed strong and weakly stained tissue can be tentatively diagnosed as an epidermal neoplasm. The growths had an exterior appearance to similar to those described by Belanger et al. (1985a) in coho salmon exposed to asbestos. In neither study was the growth invasive (metastatic) and was confined to epidermis and conncective tissue. Fish which developed these growths were near death and would have survived only a few days. It is important to note that mesothelioma has yet to be found in laboratory exposed fish (Black et al. 1982; Herman 1985).

FIBER UPTAKE

Uptake of chrysotile asbestos was not observed below 10^{8} fibers/liter. Accumulations were greatest in fish exposed to 10^{8} fibers/liter for three months. The body burdens included fibers present in the gastrointestinal tract. Individual organs were not assessed so as to lower the effective

detection limit for the entire organism. Batterman and Cook (1981) found fibers concentrated to the greatest extent in kidney tissue (up to 230.5 fibers/mg in arctic char). Belanger et al. (1985a) found fibers in epidermal tissue of coho salmon after asbestos. Belanger (unpublished data) determined that juvenile fathead minnows had up to 400 fibers/mg in kidney tissue after 30-day exposures to 10^8 fibers/liter. Other aquatic life bioaccumulate chrysotile including primary producers (Pfister 1980; Lauth and Schurr 1983; 1984), zooplankton (Stewart and Schurr 1980), and mollusks (Halsband 1974; Belanger et al. 1985b)

RELAVANCE TO ASBESTOS IN AQUATIC SYSTEMS

Microorganisms, invertebrates, and fish have been found to accumulate chrysotile asbestos in laboratory environments. The USEPA (1979) reported that bioaccumulation and the subsequent influence of asbestos to aquatic life was of a low probability. We are now moderately confident that asbestos can accumulate at all trophic levels of aquatic systems, although the transfer between levels has yet to be proven.

Several studies (Lauth and Schurr 1983, 1984; Stewart and Schurr 1980; Woodhead et al. 1983; Belanger et al. 1985a, 1985b) document toxic effects of asbestos in laboratory environments, and in some cases, structural degradation of a variety of tissues. The relavance to field exposures has

been inadequately addressed and is a logical next step for research. Few studies exist on this topic, but those that have been performed indicate ecological and toxicological effects can occur. Schreier and colleagues (H. Schreier, Department of Soil Science, Science and Wastewater Center, University of British Columbia, Vancouver, British Columbia, personal communication) found that microbial and plant densities were inhibited in the Sumas River, British Columbia during episodes of flooding which carried asbestos-rich sediments downriver. A direct relationship between asbestos fibers, associated tracer metals (Ni, Cr, Co, and Mn), and stream discharge existed. The influence of asbestos as a co-carcinogen and co-toxicant is well known (Selikoff and Lee 1978; Langer et al. 1979; Kandaswami and O'Brien 1980). The recent research evidence presented here and elsewhere suggests that the evaluation of asbestos discharge into aquatic receiving systems be comprehensively evaluated and assessed relative to probable impact on aquatic ecosystem and human health. The data relative to effects of chrysotile asbestos on Japanese Medaka indicate that the egg stage is not affected, but that the sensitive larval-juvenile stage is impaired at levels greater than or equal to 10^6 fibers/liter.

SUMMARY AND CONCLUSIONS

The effects of chrysotile asbestos at environmentally realistic levels (0-10^{10} fibers/liter) has been shown to impair sensitive life stages and species of fish and shellfish (Table 26). Larval Asiatic clams had greater mortality and were released at lower levels from adults at all levels of asbestos exposure (10^{2}-10^{8} fibers/liter). Juvenile and adult clams had depressed growth in long-term tests, probably as a result of reduced siphoning activity at 10^{4}-10^{8} fibers/liter. Clams accumulated chrysotile fibers at detectable levels only in the highest exposure concentration (10^{8} fibers/liter) which also affected gill structure Clams exposed to chrysotile asbestos under natural circumstances in the California Aqueduct System accumulated asbestos to levels greater than any values published in the literature. The fact that these excessively high fiber burdens were found indicates that asbestos is not acutely toxic and that Asiatic clams may make a suitable biomonitor for asbestos contamination.

Juvenile and adult fathead minnows were relatively resistant to chrysotile in 30-day tests up to 10^{8} fibers/liter. Juvenile minnows were impaired at 10^{6}-10^{8} fibers/liter and exhibited reduced weight at these levels. A negative correlation between asbestos concentration and ammonia in the test

Table 26. Summary and conclusions on the effects of chrysotile asbestos in acute to chronic exposures to aquatic life.

Chrysotile Asbestos Concentration (Fibers/Liter)	New River			California				Summary and Conclusions
	Larval Corbicula	Juvenile Corbicula	Adult Corbicula	Corbicula	Juvenile Fathead Minnows	Adult Fathead Minnows	Medaka larvae juveniles	
0	Marginal mortality in 2 days (~10%)	Positive tissue and shell growth, normal gill ultra-structure and siphoning activity during all seasons	Positive tissue and shell growth; normal siphoning activity; normal gill ultra-structure	——	Maintained good health in 30-day laboratory tests	Some decline in vigor after holding for 30-days in the laboratory	Growth was normal; tissues were of normal condition and indicated good health after 13 weeks in the laboratory	Laboratory populations had good vigor with the exception of adult minnows held in semi-static containers for 30 days.
10^2	Mortality was enhanced by 8% above control death; larvae released per adult was significantly lowered	Siphoning activity depressed in 30-day tests during all seasons; however, growth was not significantly reduced	Siphoning was depressed in 30-day tests; shell growth was positive but significantly lower and weight gain was not significantly reduced	——	——	——	——	Not harmful to most stages of Corbicula life history. Changes in siphoning reflect irritation by asbestos which can be overriden by a good diet. This concentration is 3 orders of magnitude below detection.
10^4	Mortality was enhanced by 14% above control deaths; larvae released per adult was significantly lowered	Siphoning was enhanced as were growth parameters in both seasons. Fibers were not accumulated to detectable levels	Siphoning and shell growth were significantly depressed. Weight gain was positive and not significantly reduced. Fibers were not accumualted to detectable levels	——	No mortality, slightly depressed ammonia content in water. Length and weight were comparable to control fish. No fiber accumulation indicated	No mortality or differences in length or weight compard to controls. Fish swimming performance was not affected. No fiber accumulation indicated. Ammonia content of water reduced.	Mortality and growth were comparable to controls. Fiber accumulation was not found. Tissues were in good condition.	Irritational effects of chrysotile reduce growth and siphoning to filter feeders in the laboratory fish were not impaired at this level which is 1 order of magnitude below detection. Acute toxicity is not suggested.

Table 26. Continued

Chrysotile Asbestos Concentration (Fibers/liter)	New River			California				Summary and Conclusions
	Larval Corbicula	Juvenile Corbicula	Adult Corbicula	Corbicula	Juvenile Fathead Minnows	Adult Fathead Minnows	Medaka larvae juveniles	
10^5	—	Siphoning was significantly depressed as were growth parameters in both seasons	Siphoning was shell growth were significantly depressed. Weight gain was positive and not significantly reduced	—	—	—	—	Irritational effects of chrysotile reduce growth and siphoning to filter feeders in the laboratory. This level is above detection. Field tests of asbestos induced effects are suggested as effects are chronic in nature.
10^6	Mortality was enhanced by 15% above controls; larvae released per adult was significantly reduced	Siphoning and shell growth was significantly reduced in both seasons. Weight gain was inhibited in the summer but not winter	Siphoning was significantly reduced. Shell degrowth occurred but weight gain was not significantly inhibited	—	No mortality, ammonia content depressed in 96-hr tests. Weight was significantly less than controls after 30-days. Length was not affected	No mortality, differences in length, weight or swimming performance. No fiber accumulation indicated. Ammonia content of water reduced	Mortality was greater than controls (~50%). Growth was permanently depressed by 14-days of exposure. Kidney tubules were constricted and epidermal tissue was thickened after 13 weeks. Fibers were not significantly accumulated	Filter-feeders and sensitive fish species are affected with pathological consequences. Toxicity is chronic and lower growth is observed. Field monitoring of sensitive species in environments at and above this level is suggested.

Table 26. Continued

Chrysotile Asbestos Concentration (Fibers/Liter)	New River			California	Juvenile Fathead Minnows	Adult Fathead Minnows	Medaka larvae juveniles	Summary and Conclusions
	Larval Corbicula	Juvenile Corbicula	Adult Corbicula	Corbicula				
10^8	Mortality was enhanced by 16% above controls; larvae released per adult was ½ of controls	Siphoning and shell growth was significantly less than controls. Shell growth was negative in the winter. Weight gain was reduced in summer, but not winter. Significant accumulations of fibers in gill and viscera with a decline in gill tissue condition	Siphoning was significantly inhibited shell and weight growth were negative. Fiber burdens were 1000 fibers/mg in viscera and 150 fibers/mg in gills. Gill tissue was impaired and clams had greater tissue water content	—	No excess mortality; ammonia content of water was reduced in 96-hr tests. Length not affected, but weight was significantly less than controls. Fiber burdens were 100 and 400 fibers/mg in liver and kidney tissue	Mortality, fish condition and swimming performance were not adversely affected. Fibers were found in liver and kidneys at levels below those documented for juveniles	Mortality was increased to 50% above control. Growth was permanently depressed by 14 days. Liver kidney epidermal and intestinal lining tissues were altered. Fibers were detected at 3 months of exposure in tissue (~500 fibers/mg)	Filter feeders and sensitive fish species are affected with pathological consequences. Death greatly reduced growth, and significant bioaccumulations of chrysotile asbestos. This level is harmful to sensitive species and life stages.
10^9	—	—	—	Visceral tissue contained up to 10^5 fibers/mg and gills up to 800 fibers/mg. Bioconcentration factors were moderate (50-100) for water and negligible for sediment. Suggesting water as the major route of uptake	—	—	—	Chrysotile asbestos is accumulated to levels in clams greater than any organism researched to date. Corbicula may make a suitable biomonitor for chrysotile contamination.

Table 26. Continued

Chrysotile Asbestos Concentration (Fibers/Liter)	New River			California	Juvenile Fathead Minnows	Adult Fathead Minnows	Medaka larvae juveniles	Summary and Conclusions
	Larval Corbicula	Juvenile Corbicula	Adult Corbicula	Corbicula				
10^{10}	—	—	—	—	Ammonia content of water was significantly reduced in 96-hr tests, no acute toxicity	Ammonia content of water was reduced in 96-hr tests. No acute toxicty	Nearly 100% mortality by 60 days; Growth was permanently reduced by 14 days. Evidence of epidermal neoplasms in fish exposed for less than two months	Pathology and toxicity to larval fish is significant. This level is tumorgenic to fish. Field investigations in regions at and above this level should be undertaken to determine the realized effects on fish and shellfish.
10^{12}	—	—	—	—	Ammonia content of water was reduced in 96 hr tests. No acute toxicants	Ammonia content of water reduced in 96-hr tests. No acute toxicity but 60 fibers/mg found in kidney tissue	—	This level is not acutely toxic, but levels of asbestos in kidneys and correlations with ammonia in the water indicate damage would occur to sensitive species.

water was determined and indicates osmoregulatory impairment could be found in longer, chronic tests. Fibers were detected in kidney and liver tissue in some fish exposed to 10^8 fibers/liter.

Japanese Medaka were sensitive to asbestos at concentrations of 10^6 fibers/liter and greater, displaying reduced growth, tissue and cellular pathology, and fiber accumulations at these levels. Tumors were recorded at 10^{10} fibers/liter after one month of exposure and 100% mortality by two months. Liver, kidney, and intestinal lining tissue were affected at 10^8 fibers/liter showing enhanced lipid and endoplasmic reticulum, constricted tubules, and greater densities of goblet cells, respectivley. Medaka eggs were not consistently affected at any asbestos concentration and responded erratically to exposure overall. The larval life stage was the most sensitive to chrysotile fiber exposure.

Senstive fish species at critical life stages and Asiatic clams were found to be impaired at 10^6 fibers/liter and greater. Clams, due to behavioral sensitivity, were found to be affected at all levels tested. Since asbestos is analytically detectable at 10^4 fibers/liter under optimal circumstances (low productivity and high clarity aquatic systems) these data suggest that asbestos discharge should be minimized to below detection limits when possible by water treatment technologies and that the release of asbestos into aquatic ecosystems be routinely monitored and evaluated.

APPENDIX A. CALCULATIONS OF BIOCONCENTRATION FACTORS

A. Conversion of sediment fiber concentration to mg chrysotile/mg sediment.

1. 1×10^{8} fibers = 0.07 ug chrysotile/liter, or 1.42×10^{8} fibers/ug of suspended chrysotile (McGuire et al. 1982).

2. 4.5×10^{-5} mg chrysotile/mg sediment occurs in Lake Silverwood (McGuire et al. 1982).

3. $$\frac{4.5 \times 10^{-5} \text{ mg chrysotile}}{\text{mg sediment}} \times \frac{1.42 \times 10^{11} \text{ fibers}}{\text{mg chrysotile}} = \frac{6.39 \times 10^{6} \text{ fibers}}{\text{mg sediment}}$$

B. Conversion of water-borne fiber concentrations to mg chrysotile/mg water in the field.

1. at 30m in Lake Silverwood 4.5×10^{9} fibers/liter is found (McGuire et al. 1982)

2. $$\frac{4.5 \times 10^{9} \text{ fibers}}{\text{liter}} \times \frac{\text{liter}}{10^{6} \text{ mg water at } 15^{\circ}\text{C}} = \frac{4500 \text{ fibers}}{\text{mg water}}$$

C. Conversion of water-borne fiber concentrations to mg chrysotile/mg water in the laboratory.

1. 4.789×10^{8} fibers/liter (mean measured concentration) used in laboratory exposures.

2. $$\frac{4.789 \times 10^{8} \text{ fibers}}{\text{liter}} \times \frac{\text{liter}}{10^{6} \text{ mg}} = \frac{479 \text{ fibers}}{\text{mg water}}$$

D. Example of a sediment BCF for a field exposed clam collected on 7 October 1983 for visceral tissue.

$$\frac{4.63 \times 10^{5} \text{ fibers/mg tissue}}{6.39 \times 10^{6} \text{ fibers/mg sediment}} = 0.72$$

E. Example of a water-borne BCF for a laboratory exposed clam for a whole clam homogenate.

$$\frac{916.3 \text{ fibers/mg tissue}}{479 \text{ fibers/mg water}} = 1.91$$

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Sigma Xi Grant-in-Aid of Research ($200)

Research Stipend Recipient (D.S. Cherry Principal Investigator) U.S. Department of the Interior, State Mining and Minerals Resources and Research Institute (MMRRI) ($6800). July 1983-June 1984. Effects and Uptake of Chrysotile Asbestos in Selected Aquatic Organisms with Potential Carcinogenic Consequences.

Researcher (D.S. Cherry, Principal Investigator). U.S. Department of the Interior, State Mining and Minerals Resources and Research Institute (MMRRI) ($7400). July 1984-June 1985. Pathological and Functional Responses of Asiatic Clams and Fathead Minnows to Chrysotile Asbestos.

Cherry, D.S. and S.E. Belanger. U.S. Department of the Interior, State Mining and Minerals Resources and Research Institute (MMRRI) ($7400) July 1985-June 1986. The Carcinogenic and Reproductive Effects of Chrysotile Asbestos to Fish Receiving Chronic Exposure.

PROFESSIONAL SOCIETIES:

American Fisheries Society, Sigma Xi, American Malacological Union, Society of Environmental Toxicology and Chemistry

JOURNAL AND GRANT REVIEWER FOR:

National Science Foundation
Maryland Power Plant Siting Program
Second International Corbicula Symposium
Canadian Journal of Fisheries and Aquatic Sciences
Transactions of the American Fisheries Society
Fisheries
Hydrobiologia
American Society for Testing and Materials
Bulletin of Water Research
Animal Behavior and Physiology

PROFESSIONAL ACTIVITIES:

Consulting Aquatic Ecologist, Design Management Associates, Orlando, Florida. Wetland management strategies for aquatic fowl, fish, and invertebrate species. 1982.

Consulting Aquatic Ecotoxicologist, Celanese Fibers Corporation, Narrows, Virginia. Control of biofouling of industrial installations by the Asiatic clam. 1983-1985.

Consulting Aquatic Ecotoxicologist, Consumers Power Company, Midland Michigan. Evalutation of potential synergisms between chemical and power company effluents. 1984.

Consulting Aquatic Ecotoxicologist, Union Camp Company, Virgina. Statistical analysis of fish preference-avoidance data. 1984.

Consulting Aquatic Ecotoxicologist, Borg Warner Chemical Company, Morgantown West Virginia. Development and

refinement of standard toxicity testing protocols and site-specific evaluations of potentially toxic effluents. 1984-1985.

Consulting Aquatic Ecotoxicologist, Proctor and Gamble Company, Cincinatti, Ohio. Collection and utilization of Asiatic clams in toxicity testing and hazard assessment. 1984-1985.

Consulting Aquatic Ecotoxicologist, Borg Warner Chemical Company, Parkersburg, West Virginia. Hazard evaluation and site assessment for a new plant location, production facility and associated effluent discharges. 1985.

Consulting Aquatic Ecotoxicologist, National Council for Air and Stream Improvement, New York, New York. Ten year evaluation of periphyton, invertebrate, and fish responses to pulp mill effluent and site visit/critique of testing facilities. 1985.

Invited Lecturer, Borg Warner Chemical Company, Parkersburg, West Virginia. Aquatic Ecotoxicology and Hazard Assessment. Jan. 11-13, 1985.

Consulting Aquatic Ecotoxicologist, Ecological Analysts, Shawsville, Virginia. Collection and use of the Asiatic clam in toxicity testing. 1985.

POSITIONS HELD:

Graduate Teaching Assistant, Bowling Green State University. 1980-1982. Courses instructed were as follows: General Biology, Man and the Environment, Ecology and Evolution, Phylogeny and Organ Systems. Instructional duties included preparation and grading of lecture and laboratory exams, assignment of course grades, supervision of lecture and laboratory classes, and organizing and conducting field trips.

Graduate Teaching Assistant, Virginia Polytechnic Institute and State University. 1982-1983. Instructional duties included teaching laboratory sections of General Biology and Principles of Biology, preparing, administering, and grading laboratory exams and exercises.

Graduate Research Assistant, Virginia Polytechnic Institute and State University. 1983-1984. Design and conduct short (96-hour) and long-term (30-day) tests to assess the effects of chrysotile asbestos on the Asiatic clam and fathead minnow. Objectives of these investigations were to evaluate fiber uptake with concurrent analyses of behavior, growth, and physiological functions. The assessment of the carcinogenic potential with these species was evaluate by cytological and ultrastructural studies of selected tissues. Additional duties included preparation of quarterly and final grant reports and assisting in writing of proposals.

Cunningham Dissertation Year Fellow, Virginia Polytechnic and State University. 1984-1985. Design field and laboratory tests for evaluating the toxicity of chrysotile asbestos to Japanese Medaka, fathead minnows, and Asiatic clams. These studies addressed early life history responses (growth, behavior, and pathology) associated with the accumulation of asbestos fibers.

RESEARCH INTERESTS:

Multivariate analyses of algal colonization of artificial and natural substrates as models for the interaction of biological with physico-chemical habitat variables. Periphytic algal communities offer a system to investigate the interplay of physical and chemical habitat on microbial colonization and succession by affecting recruitment rates and species composition of the resulting communities. The standardization of a variety of substrate types for optimizing the use of these techniques is being explored.

The use of novel techniques to estimate fish age. Aging techniques differ as to quality and ease of use for most species. Comparisons of boney structures has revealed scales are often the least reliable and most imprecise method available. Since age is an important factor in fish stock-recruit analysis it is imperative to use the best method available that is accurate and with narrow confidence limits. Otoliths and fin rays are most accurate but with respective drawbacks: otoliths require sacrificing the fish to be aged and fin clipping induces limited mortality once the fish is released. Future studies should resolve questions of age-structure differences and the parameters that influence them.

The toxicological effects of asbestos on aquatic life. Asbestos is a generic term for a group of intergrading minerals that have become widely distributed in aquatic systems as a result of anthropogenic activities. Research to date has been limited primarily to mammalian systems. Recent behavioral and histopathological studies have shown that coho salmon fry, juvenile green sunfish, and Asiatic clams are impaired at levels known from several aquatic environments. Further research is dictated to determine if uptake of asbestos is of a level high enough to warrant a reevaluation of asbestos discharge into aquatic environments as evaluated by federal, state, and industrial groups.

The use of the Asiatic clam, Corbicula sp., as an in situ biomonitor of the aquatic environment. Field and laboratory studies have shown that clams are efficient accumulators of heavy metals and mineral fibers. These studies have also

shown that ecological growth rates are retarded at low levels of metal and moderate levels of mineral fiber exposure. The bioaccumulatory potential of clams may be used in the hazard assessment of regions with no to low detectable toxicant concentrations to determine safe levels of exposure.

PUBLICATIONS:

Thesis:

Belanger, S.E. 1982. The effects of chrysotile asbestos on larval coho salmon (Oncorhynchus kisutch) and juvenile green sunfish (Lepomis cyanellus). M.S. Thesis. Bowling Green State University, Bowling Green, Ohio.

Books Reviewed:

Aquatic Toxicology and Hazard Assessment, Sixth Symposium. Bishop, W.T., R.D. Cardwell, and B.B. Heidolph (eds.). American Society for Testing and Materials, ASTM STP 802. 560p. Philadelphia, Pennsylvania. Fisheries 10(2): 43-44 and Transactions of the American Fisheries Society 114(2):313-314.

Journal Articles:

Belanger, S.E. and S.R. Hogler. 1982. A comparison of five aging methodologies applied to walleye (Stizostedion vitreum) in Burt Lake, Michigan. Journal of Great Lakes Research 8(4):666-671.

Belanger, S.E., J.L. Farris, D.S. Cherry, and J. Cairns, Jr. 1985. Sediment preference of the freshwater Asiatic clam, Corbicula fluminea. The Nautilus 99(2/3):66-73.

Belanger, S.E., R.L. Lowe, and B.H. Rosen. 1985. The effect of current and cell size on the epiphytic association of Synedra parasitica (W.Sm.) Hust. on Surirella robusta var. splendida V.H.. Transactions of the American Microscopical Society 104(4):378-386.

Belanger, S.E., K. Schurr, D.J. Allen and A.F. Gohara. 1985. Effects of chrysotile asbestos on coho salmon and green sunfish: evidence of behavioral and pathological stress. Environmental Research 34. In Press.

Belanger, S.E., D.S. Cherry, and J. Cairns, Jr. 1986. The uptake of chrysotile asbestos alters growth and reproduction of Asiatic clams. Canadian Journal of Fisheries and Aquatic Sciences 44. In Press.

In Review:

Farris, J.L., S.E. Bealnger, D.S. Cherry, A.E. Linkins, and J. Cairns, Jr. Functional responses of Corbicula fluminea to 30-day zinc exposures in field-laboratory artificial streams. Submitted to Environmental Toxicology and Chemistry.

Belanger, S.E., D.S. Cherry, and J. Cairns, Jr. Response of juvenile Asiatic clams (Corbicula fluminea) tested during winter and summer to chrysotile asbestos. Submitted to Water Research.

Belanger, S.E., J.L. Farris, D.S. Cherry, and J. Cairns, Jr. Growth of Asiatic clams, Corbicula sp., during and after long-term zinc exposure in laboratory and field artificial streams. Submitted to Archives of Environmental Contamination and Toxicology.

Trapp, K. and S.E. Belanger. Algal colonization of artificial and natural substrates in Big Stoney Creek, Virginia. Submitted to Transactions of the American Microscopical Society.

In Preparation:

Belanger, S.E., D.S. Cherry, J. Cairns, Jr., and M.A. McGuire. Validation of the bioaccumulation of chrysotile asbestos in Asiatic clams: a field and laboratory comparison. For submission to Journal of the Water Pollution Control Federation.

Belanger, S.E., D.S. Cherry, and J. Cairns, Jr. Growth and histopathological consequences of asbestos exposure to young fish: is water-borne asbestos a hazard? For submission to Science.

Farris, J.L., S.E. Belanger, D.S. Cherry, and J. Cairns, Jr. Interaction of aerial exposure and chlorination for control of the Asiatic clam, Corbicula sp.. For submission to The Nautilus.

Farris, J.L., D.S. Cherry, F.S. Colwell, R.B. Genter, S.E. Belanger, and J. Cairns, Jr. Community responses to low levels of zinc in site-specific artificial stream microcosms. For submission to Canadian Journal of Fisheries and Aquatic Sciences.

Abstracts and Papers Presented:

Belanger, S.E. and S.R. Hogler. 1982. A comparison of five ageing techniques for walleye in Burt Lake, Michigan. Poster Session, Twenty-Second Annual Ohio Fish and Wildlife Conference, Columbus, Ohio, February, 1982.

Belanger, S.E. and K. Schurr. 1982. The effects of chrysotile asbestos on larval coho salmon. Ohio J. Sci. 84(2):4. Conference Proceedings of the Ohio Academy of Science, Columbus, Ohio, April, 1982.

Hogler, S.R. and S.E. Belanger. 1982. On the use of various fish aging methodologies. Ohio J. Sci. 84(2):4. Conference Proceedings of the Ohio Academy of Science, Columbus, Ohio, April, 1982.

Belanger, S.E. and K. Schurr. 1982. The effect of chrysotile asbestos on coho salmon larvae (Oncorhynchus kisutch)and juvenile green sunfish (Lepomis cyanellus). Conference Proceedings of the International Association for Great Lakes Research, Sault St. Marie, Onario, May 1982.

Belanger, S.E., R.L. Lowe, and B.H. Rosen. 1983. Epiphytic association of Synedra parasitica (W.Sm.) Hust. on Surirella robusta var. splendida V.H.: observations of populations and associations in a Virginia Pond. Conference Proceedings of the Seventh North American Diatom Symposium, Columbus, Ohio, October, 1983.

Belanger, S.E. and K. Trapp. 1984. An evaluation of the use of artificial substrates in algal community studies. Conference Proceedings of the Annual North American Benthological Society Meeting, Raliegh, North Carolina, May, 1984.

Belanger, S.E., D.S. Cherry, and J. Cairns, Jr. 1984. Functional responses of Asiatic clams (Corbicula fluminea) exposed to chrysotile asbestos: growth, behavior, and fiber uptake. Poster Session at the Annual Meeting of the Society of Environmental Toxicology and Chemistry, Arlington, Virginia, November, 1984.

Belanger, S.E., D.S. Cherry, and J. Cairns, Jr. 1985. Responses of aquatic organisms to chrysotile asbestos fibers. To be presented at the Annual Meeting of the Society of Environmental Toxicology and Chemistry, St. Louis, Missouri, November, 1985.

Belanger, S.E., J.L. Farris, D.S. Cherry, and J. Cairns, Jr. 1985. Response of Corbicula fluminea to low levels of zinc in artificial stream systems. To be presented at the Annual Meeting of the Society of Environmental Toxicology and Chemistry, St. Louis, Missouri, November, 1985.

Farris, J.L., S.E. Belanger, D.S. Cherry, A.E. Linkins, and J. Cairns, Jr. 1985. Cellulase activity associated with chronic zinc exposure in laboratory and field artificial streams. To be presented at the Annual Meeting of the Society of Environmental Toxicology and Chemistry, St. Louis, Missouri, November, 1985.

Schurr, K., J. Lauth, S. Belanger, S. Shepka, and S. Stewart. 1985. Water-borne asbestos: injury to several species. Poster Session to be presented at the Annual Meeting of the Society of Environmental Toxicology and Chemistry, St. Louis, Missouri, November, 1985.