EPA/600/R-94/138
August 1994

FISH PHYSIOLOGY, TOXICOLOGY AND WATER QUALITY MANAGEMENT

Proceedings of 3rd Biennial International Symposium, Nanjing, PRC, November 3-5, 1992

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FOREWORD

Joint ecological research involving scientists from every country in the world is essential if global environmental problems are to be solved. Recognition of this international aspect of environmental pollution control is reflected in the joint activities undertaken under Annex 3, Item 4 of the USA-PRC Protocol for Environmental Protection. This component of the protocol provides for cooperative research on the environmental processes and effects of pollution on freshwater organisms, soils, and groundwater, and on the application of transport and transformation models.

Specific areas of cooperation in environmental research include inorganic chemical characterization and measurement; inorganic chemical transport and transformation process characterization; biological degradation process characterization; oxidation/reduction process characterization; field evaluation of selected transport, exposure and risk models; and application of models for environmental decision-making concerning organic pollution in semi-arid conditions, heavy metal pollution, and permissible loading of conventional and toxic pollutants in Chinese rivers. Activities include seminars, workshops, symposia, training programs, joint research, and publications exchange.

The third biennial symposium presented under the protocol was held on the campus of Nanjing University, Nanjing, Jiangsu Province, People's Republic of China, on November 3-5, 1992. Scientists from four countries presented papers at the symposium, which was sponsored by the U.S. Environmental Protection Agency, the PRC Environmental Protection Agency, the PRC National Science Foundation, the EPA of Jiangsu Province, the University of British Columbia, and the Nanjing University. The two earlier symposia were held in Guangzhou, PRC, on September 14-16, 1988, and Sacramento, Calif., USA, on September 18-19, 1990.

Symposia are an effective means of fostering cooperation among scientists from different countries as environmental organizations seek to gain the information necessary to predict the effects of pollutants on ecosystems and apply the results on a global scale. The symposia provide a forum through which distinguished scientists from laboratories and institutes from several countries can exchange scientific expertise on environmental problems of concern to EPA and the international environmental community.

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ABSTRACT

Scientists from four countries presented papers at the Third Biennial International Symposium on Fish Physiology, Toxicology and Water Quality Management, which was held on the campus of Nanjing University, Nanjing, Jiangsu Province, People's Republic of China. This proceedings includes 20 papers presented in sessions convened over 3 days. Papers address the regulation of growth hormones in fish, the reproductive effects of anthropogenic chemicals in fish, the effects of pollutants on physiological functions in fish, and the physiological responses of rainbow trout to copper and ammonia. Descriptions are provided concerning research on the effects of fenpropathrin on aquatic organisms, the bioaccumulation of organic chemicals in fish, the relationship between uptake of chemicals and oxygen consumption in fish, the toxic effects of meothrin on gill ultrastructure in grass carp, the bioaccumulation of hydrophobic organics in foodwebs, the monitoring of industrial effluents in Nanjing, the investigations of fish deaths in Lake Xuanwu, and the rehabilitation of streams degraded by mining wastes. Presentations also covered nonpoint sources and water quality, conversion of municipal wastewater into resources, modeling of toxic chemicals in the Yangtze River, regional water pollution control in rural China, adsorption of organic pollutants on soils and sediments, heavy metal speciation modeling on the Le An River, and uncertainty analysis using the QUAL2E model.

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ACKNOWLEDGMENTS

Organizing and presenting a symposium and preparing a proceedings is frequently a complex task, particularly when participants represent organizations in several countries. The cooperative work of those involved is gratefully acknowledged. Sponsoring organizations for this symposium include the U.S. Environmental Protection Agency, the Environmental Protection Agency of the People's Republic of China, the National Science Foundation of the People's Republic of China, the Environmental Protection Agency of Jiangsu Province, the University of British Columbia, and Nanjing University. The scientists, engineers, and environmental managers who participated in the symposium, of course, are deserving of primary recognition. In particular, recognition is accorded to the organizing committee composed of Dr. Hongjun Jin, Nanjing University; Dr. Haoren Lin, Zhongshan University; Dr. Yuhuan Lin, Academia Sinica; Dr. Rosemarie Russo, U.S. Environmental Protection Agency; Dr. David Randall, University of British Columbia, and Dr. Robert V. Thurston, Fisheries Bioassay Laboratory. As session chairpersons, they also assured the efficient functioning of the symposium.

THE REGULATION OF GROWTH HORMONE SECRETION IN CARP

H.R. Lin,[1] X.W. Lin,[1] Q. Zhang[1] and R.E. Peter[2]

INTRODUCTION

Recent progress has been made towards increasing our understanding of the processes involved in the neuroendocrine regulation of growth hormone (GH) secretion in goldfish (Chang et al., 1985; Marchant et al., 1989a,b,c). However, additional research is required before the role of the hypothalamus in the regulation of growth hormone secretion in teleosts, especially the cultured carps, is understood to the same extent that it is in homeothermic vertebrates. Such research is essential to provide a foundation for any subsequent studies on the stimulation of growth rates in these cultured fish.

In the present study, both in vitro and in vivo experimental approaches were used to examine the effects of hypothalamic peptides (gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH)), and several catecholaminergic drugs (apomorphine (APO), L-dopa, α-methyl-para-tyrosine (MPT), 6-hydroxydopamine (6-OHDA), epinephrine (E) and norepinephrine (NE)), on the regulation of growth hormone secretion in the Common carp (*Cyprinus carpio*) and Grass carp (*Ctenopharyngodon idellus*).

MATERIALS AND METHODS

The influence of hypothalamic peptides on GH release from the pituitary of mature common carp was examined in vitro using fragments of the pars distalis (adenohypophyses). Common carp (350-600 g body weight) were obtained from local suppliers (Guangzhou, P.R. China). Fish were maintained in recirculating, indoor, 250 L aquaria at room temperature (18-28 °C) and a natural photoperiod. Experiments were conducted using a column perfusion system, similar to that previously used for goldfish pituitary fragments (Mackenzie et al., 1984; Habibi et al., 1989), but with minor modifications. Pituitaries were dissected out from common carp anesthetized in tricaine methanesulfonate (TMS; Sigma, St. Louis, MO) and killed by decapitation. Pituitaries were immersed in iced Hank's balanced salt solution supplemented with 25 mM Hepes (Sigma) and 0.1% bovine serum albumin (HBSS; Sigma). The pituitaries were then washed twice with HBSS, and diced into fragments (smaller than 1 mm3) with surgical scissors. The fragments were pooled and again washed twice with HBSS. Groups of fragments, each equivalent to one-half of a complete pituitary, were placed between two layers of Cytodex microcarrier beads (Sigma) in a 0.3 ml perfusion chamber. The fragments were perfused overnight (8-10 hr) with Medium 199 containing Hank's salts, L-glutamine (Gibco Laboratories, Grand Island, N.Y.), 25 mM Hepes and 56 U/ml Nystatin (Sigma) at a flow rate of 5 ml/hr. Two hours prior to experimentation, the perfusion medium was switched to HBSS at 19±1 °C and the flow rate increased to 15 ml/hr. Fractions were collected at 5-minute intervals using an automated fraction collector and stored at -25 °C until analysis. Synthetic (D-Ala6, Pro9-NEt)-LHRH was provided by Ningbo Fish Hormone Factory, (Zhejing Province, China). (D-Trp6, Pro9-NEt)-LHRH, chicken GnRH-II, and sGnRH and its analogs (D-Arg6, Pro9-NEt)-sGnRH and (D-Ala6, Pro9-NEt)-sGnRH, were kindly provided by Drs. J.E. Rivier and W.W. Vale (The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla,

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CA). TRH was provided by the Laboratory of Reproductive Biology, Institute of Zoology, Academia Sinica (China). All peptides were diluted immediately before use with HBSS from a stock solution (10 μM for GnRH peptides, 100 μM for TRH).

The influence of (D-Ala6, Pro9-NEt)-LHRH and several catecholaminergic drugs on serum GH levels was examined in vivo in Grass carp fingerling. Grass carp fingerling (20-40 g body weight) were obtained from local suppliers (Guangzhou, P.R. China) and maintained in recirculating, indoor, 250 L aquaria at room temperature (19-24 °C) and a natural photoperiod. APO, L-dopa, α-MPT, 6-OHDA, epinephrine and norepinephrine (all purchased from Sigma, St. Louis, MO) were dissolved in 0.7% NaCl with 0.1% sodium metabisulphite. Drugs were injected into the caudal vasculature with a 25-gauge needle attached to a 1.0 ml disposable syringe, and then blood was serially sampled at 2, 6 and 24 hr post injection. Blood samples were allowed to clot on ice for several hours before the serum was separated by centrifugation and stored at -25 °C until hormone measurement.

GtH concentrations in the perfusates were determined by radioimmunoassay (RIA) (Peter et al., 1984) specific for Common carp GtH-II (Van Der Kraak et al., 1992). GH concentrations in the perfusates and serum samples of Grass carp were determined by RIA specific for Common carp GH (Marchant et al., 1989c). Serial dilutions of perfusates and serum samples from Grass carp resulted in displacement curves parallel to the Common carp GtH and GH standard curves in the respective RIAs. The hormone responses to each pulse of GnRH and TRH peptide were quantified according to Habibi et al. (1989), and expressed as a percentage of the average basal hormone levels (prepulse) in the three fractions collected immediately preceding each pulse (i.e. % of prepulse). Comparisons of hormone responses between different treatments were made by Student's t-test. Comparisons of hormone responses to different pulses of GnRH or TRH within an experiment were made using one-way analysis of variance followed by Duncan's multiple range test. The half-maximal effective dose (ED50) levels of GnRH-A and TRH in stimulating GH release were calculated according to Karber (1931).

RESULTS AND DISCUSSION

THE EFFECTS OF GnRH PEPTIDES ON GH RELEASE

All of the peptides tested (LHRH, (D-Ala6, Pro9-NEt)-LHRH, (D-Trp6, Pro9-NEt)-LHRH, chicken GnRH-II, sGnRH and its analogs (D-Arg6, Pro9-NEt)-sGnRH and (D-Ala6, Pro9-NEt)-sGnRH) (1, 10 and 100 nM), were found to stimulate dose-dependent increases in GtH and GH release from in vitro perfused pituitary fragments of Common carp (Fig. 1). Among the GnRH peptides tested, (D-Arg6, Pro9-NEt)-sGnRH (sGnRH-A) was the most effective stimulator of GH and GtH release. However, sGnRH-A stimulated GtH release more effectively than GH release. Chicken GnRH-II, sGnRH and (D-Ala6, Pro9-NEt)-sGnRH caused comparable stimulation of both GH and GtH release. Compared to sGnRH and its analogs, LHRH and its analogs were consistently less potent in stimulating GH and GtH secretion in this in vitro perfusion system (Table 1). Intraperitoneal injection of (D-Ala6, Pro9-NEt)-LHRH (LHRH-A) (0.01, 0.001 μg/g body weight) also significantly increased the circulating GH levels of Grass carp fingerling (Fig. 2).

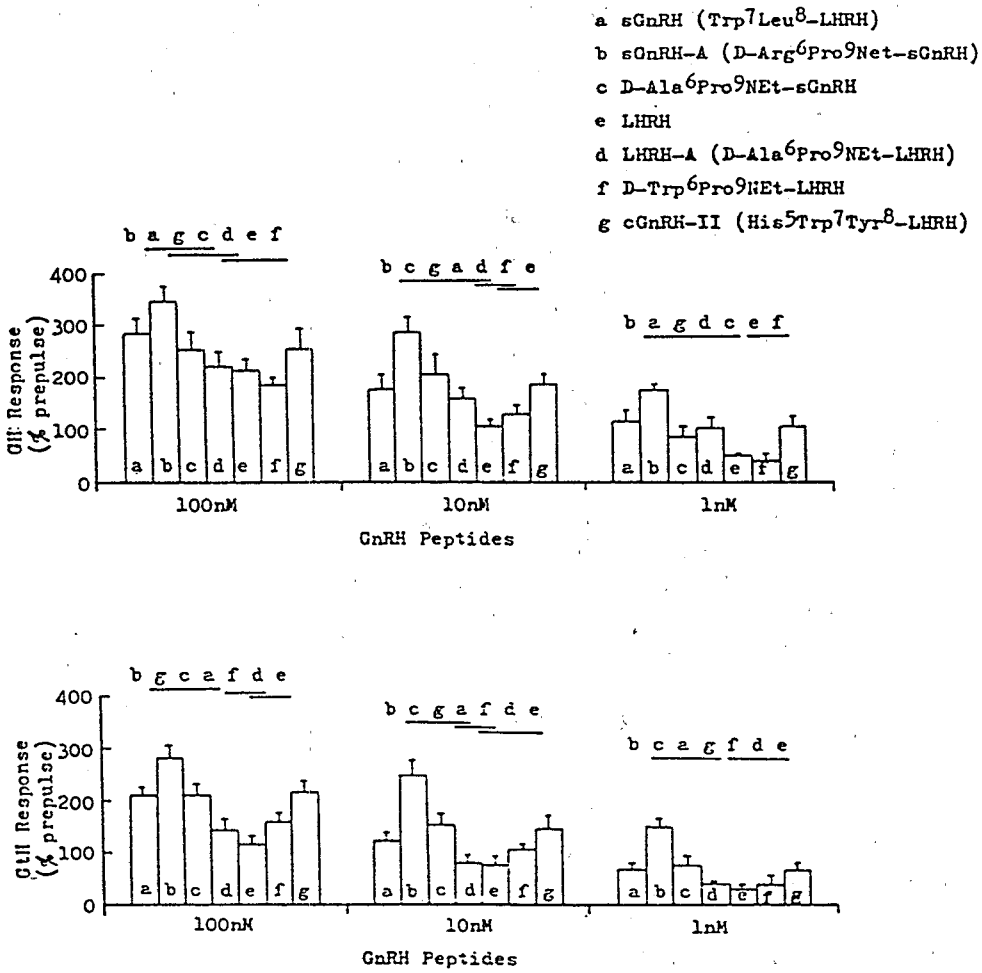

Figure 1. GH and GtH release from perfused pituitary fragments of common carp in response to different GnRH peptides administered in 2-min. pulse manner. Each value represents mean ± SE of 4 observations from 4 separate experiments. The average basal GH and GtH levels of the first prepulses were 191.4±88.0 ng/ml and 42.8±16.7 ng/ml, respectively. The letters in the column represent each treatment group in the range test, and groups with common underscoring are not significantly different (P>0.05, Duncan's multiple range test).

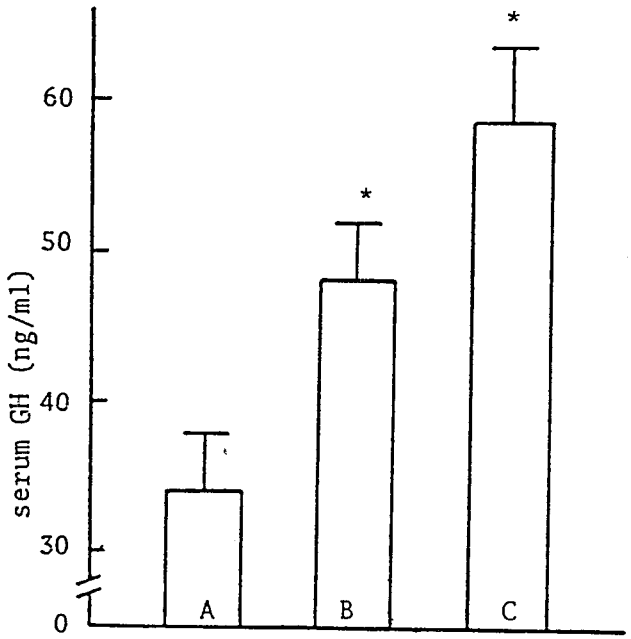

Figure 2. Effects of intraperitoneal injections of (D-Ala^6,Pro^9-NEt)-LHRH(LHRH-A) on serum GH levels of grass carp fingerling. A. Controls; B. LHRH-A 0.001 μg/g body weight/week for six weeks; C. LHRH-A 0.01 μg/g body weight/week for six weeks. *Signifcantly higher than controls ($P<0.05$).

Table 1. Comparison of the relative GH and GtH releasing potencies of GnRH peptides.

	Relative GH-releasing potency	Relative GtH-releasing potency
sGnRH	1.00	1.00
(D-Arg6, Pro9-NEt)-sGnRH	4.30	9.24
(D-Ala6, Pro9-NEt)-sGnRh	0.96	1.15
LHRH	0.31	0.28
(D-Ala6, Pro-NEt)-LHRH	0.60	0.35
(D-Trp6, Pro9-NEt)-LHRH	0.33	0.51
chicken GnRH-II	0.89	1.27

These results suggest that the structural modifications (i.e. GnRH analog A) that increased the potency of GnRH on GtH release also increased the potency of GnRH on GH release. The results agree with the findings of Marchant et al. (1989b, c), who found that GnRH peptides function as GH-releasing factors in goldfish. Such increases in circulating GH levels may be sufficient to accelerate body growth in Grass carp fingerling.

THE EFFECTS OF TRH ON GH RELEASE

Exposure of Common carp pituitary fragments to 5 minute pulses of increasing concentrations of TRH (0.1, 1.0, 10, 100 and 1000 nM) at 60 minutes intervals resulted in a rapid and dose-dependent stimulation of GH secretion so that GH levels were increased (upper panel, Fig. 3). The ED50 was estimated to be 9.7±2.3 nM by using 10 doses of TRH in order of increasing and decreasing concentrations. TRH did not effect GtH release (lower panel, Fig 3). These results indicated that the GH response to TRH was specific, and that TRH may act as a GH-releasing factor in carp.

THE EFFECTS OF CATECHOLAMINERGIC DRUGS ON GH RELEASE

Actions of Apomorphine (APO)

APO, a dopamine agonist, significantly increased basal GH release but decreased basal GtH secretion from perfused pituitary fragments of Common carp in a dose-dependent manner (10, 100 and 1000 nM) (Fig. 4). Intraperitoneal injection of APO in Grass carp fingerling significantly increased serum GH levels after 2 and 6 hours, while the GtH level remained unchanged (Fig. 5).
APO acts similarly to dopamine, which inhibits basal and GnRH-induced GtH release in goldfish and carp. The results of this study therefore, provide further evidence that the release-inhibitory factors regulating GH and GtH secretion in goldfish and carp appear to be separate and distinct.

Actions of 6-hydroxydopamine (6-OHDA)

Intraperitoneal injection of 6-OHDA (20 μg/g body weight), a neurotoxin for catecholaminergic neurons (Zambrano et al., 1975), significantly lowered serum GH concentrations of Grass carp fingerling 6 and 24 hours after injection, compared with control fish (Fig. 6).

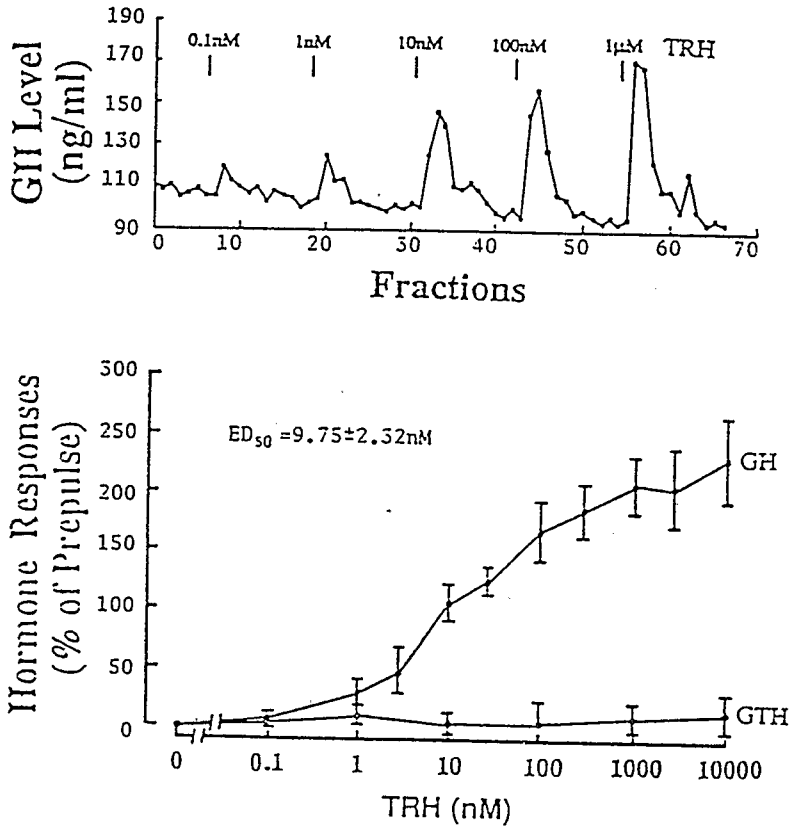

Figure 3. Representative profile of GH release from perfused pituitary fragments of common carp in response to increasing doses ofTRH administered at 5 min. pulses (black bar) every 60 min. (upper panel), and the dose response curve of TRH in stimulation of GH and the effects of TRH on GtH secretion (lower panel). All values in the dose-response are mean ± SEM of 6 observations from 3 separate perfusion runs. The average basal GH and GtH levels in these perfusion columns were 126.2±34.7 ng/ml and 3.3±3.1 ng/ml, respectively.

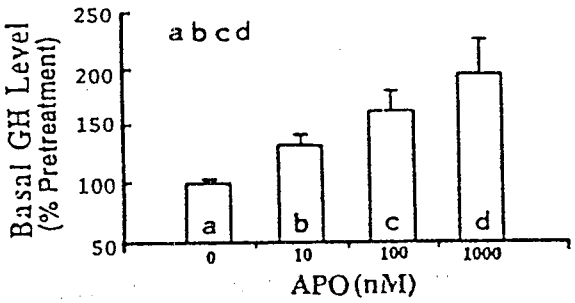

Figure 4. Influence of APO (10, 100, 1000 nM) on basal GH release from perfused pituitary fragments of common carp. The basal GH levels were expressed as the average hormone levels over the initial 15-min. period before exposure of the fragments to the peptides. Each value is mean $\pm$ SE of 4 observations from 4 individual perfusion runs. The average pretreatment GH level was 94.0$\pm$18.1 ng/ml. The letter in the column represents each experiment group in the range test, and groups without common underscoring are significantly different ($P<0.05$, Duncan's multiple range test).

Actions of L-ß-dihydroxyphenylalanine (L-dopa)

Serum GH levels of Grass carp fingerling were significantly higher 2 hours after intraperitoneal injection of L-dopa, the metabolic precursor of dopamine, (10 and 100 µg/g body weight) compared with control fish. However, at 6 hours after injection, serum GH secretion returned to the basal level (Fig. 7).

Actions of α-methy-para-tyrosine (α-MPT)

Injection of 50 µg/g body weight α-MPT, an inhibitor of L-dopa and catecholamine synthesis, significantly decreased serum GH levels in Grass carp fingerling 24 hours post injection (Fig. 8).

Actions of epinephrine (E)

Injection of 100 µg/g body weight epinephrine significantly decreased serum GH levels in Grass carp fingerling 2 and 6 hours post injection. Serum GH levels in Grass carp fingerling injected with a low dose of epinephrine (10 µg/g body weight) were also significantly depressed six hours after injection compared with control fish (Fig. 9).

Actions of norepinephrine (NE)

Serum GH levels in Grass carp fingerling injected with NE at either a low dose (10 µg/g body weight) or a high dose (100 µg/g body weight) were significantly decreased at 2 and 6 hours post injection compared with control fish (Fig. 10).

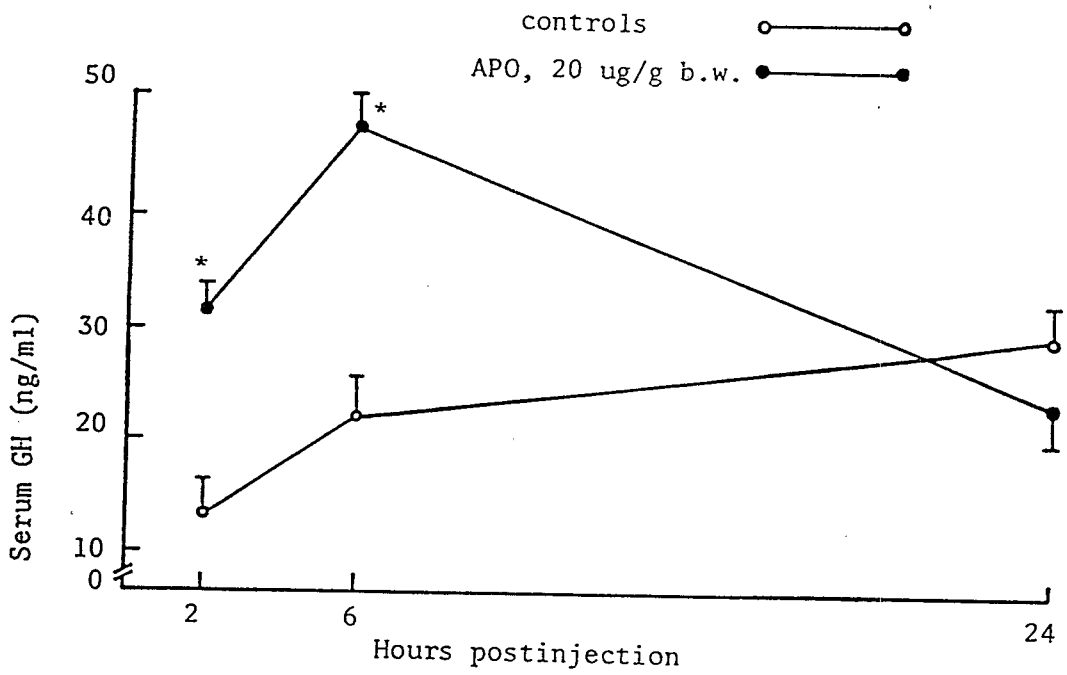

Figure 5. Effects of intraperitoneal injections of APO on serum GH levels of fingerling of grass carp. *Significant higher than controls (P<0.05).

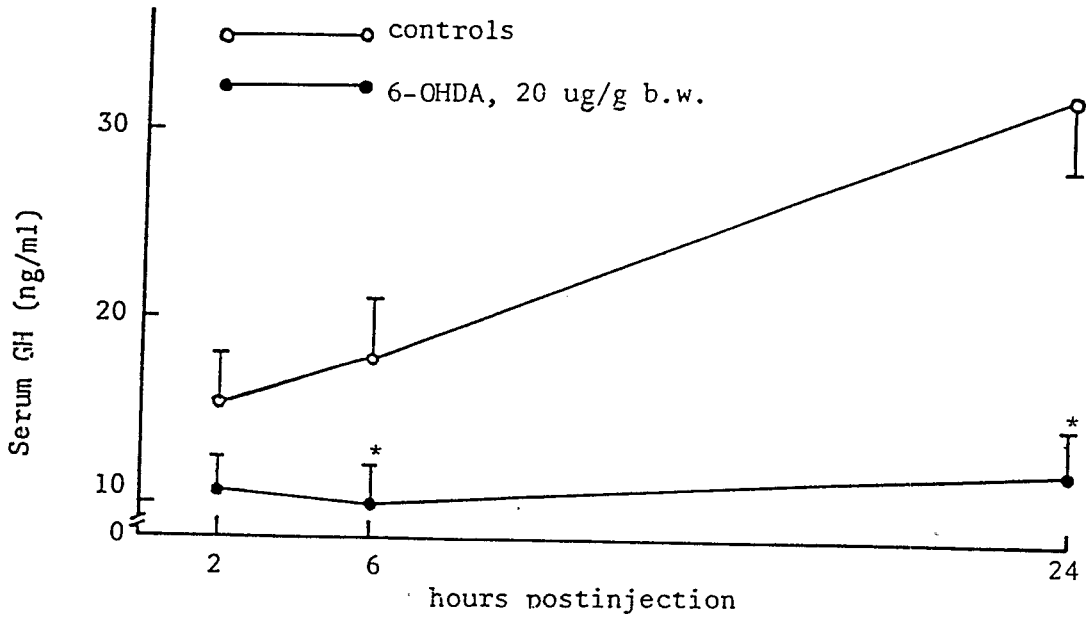

Figure 6. Effects of intraperitoneal injections of 6-hydroxydopamine (6-OHDA) on serum GH levels of fingering of grass carp. *Significantly lower than controls (P<0.05).

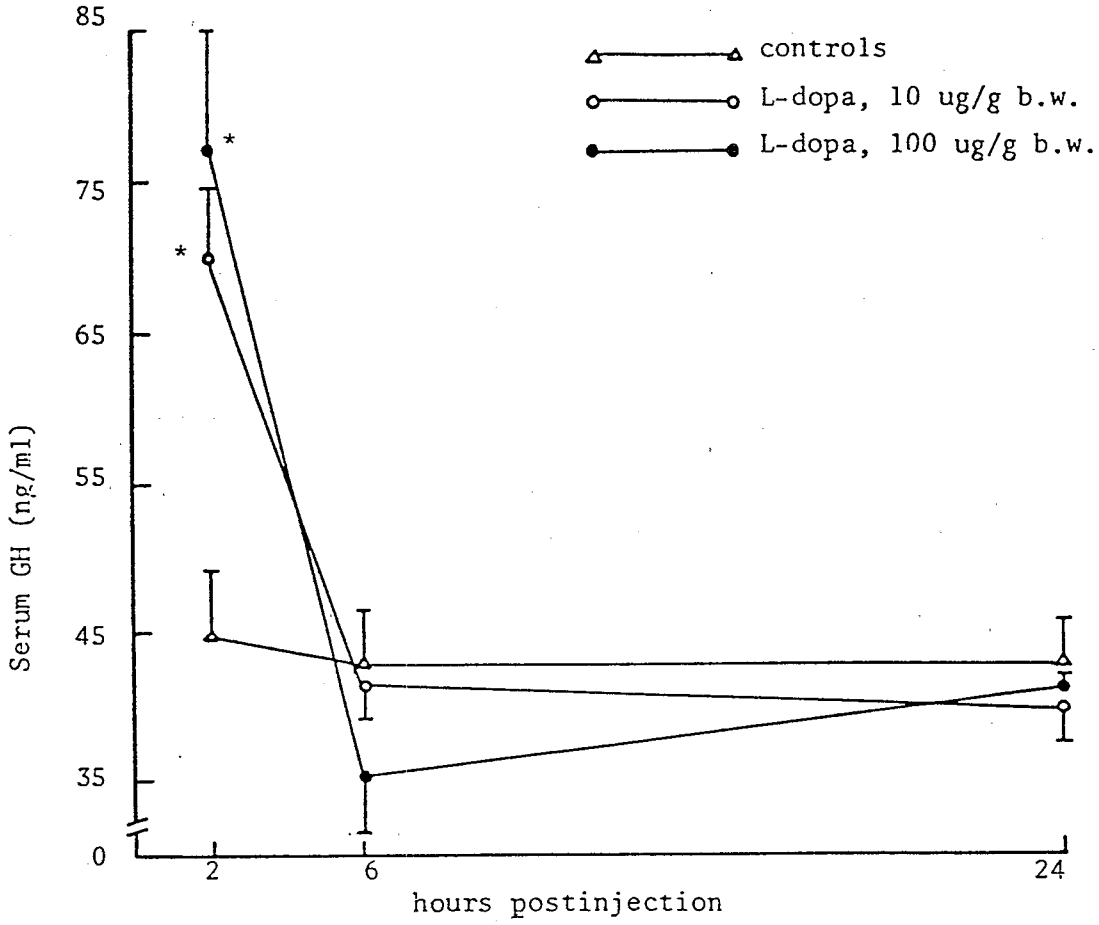

Figure 7. Effects of intraperitoneal injections of L-ß-dihydroxyphenylalanine (L-dopa) on serum GH levels of fingerling of grass carp. *Significantly higher than controls ($P<0.05$).

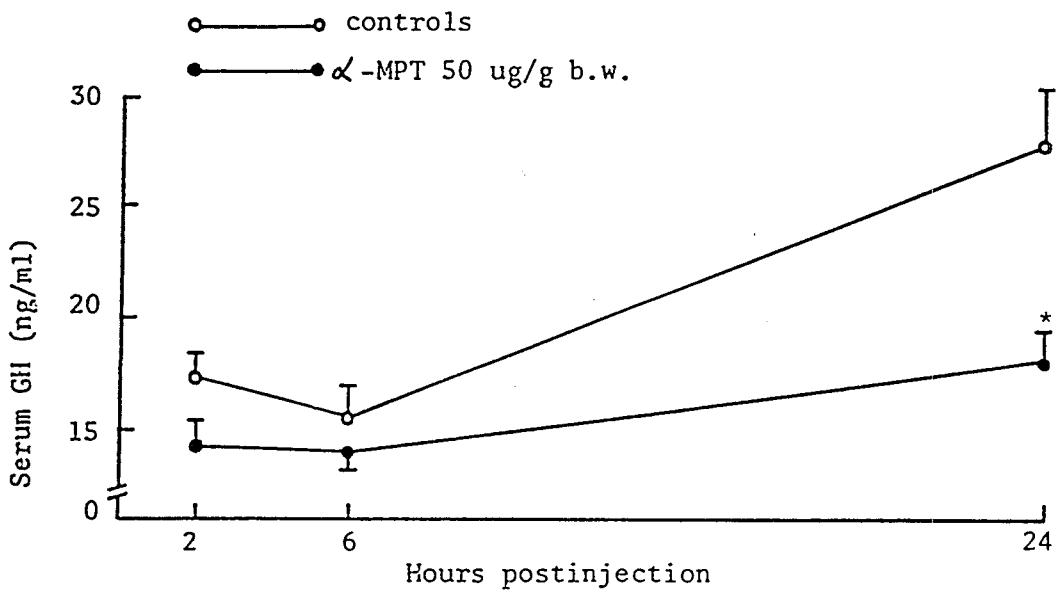

Figure 8. Effects of intraperitoneal injections of α-methyl-para-tyrosine (α-MPT) on serum GH levels of fingerling of grass carp. *Significantly lower than controls ($P<0.05$).

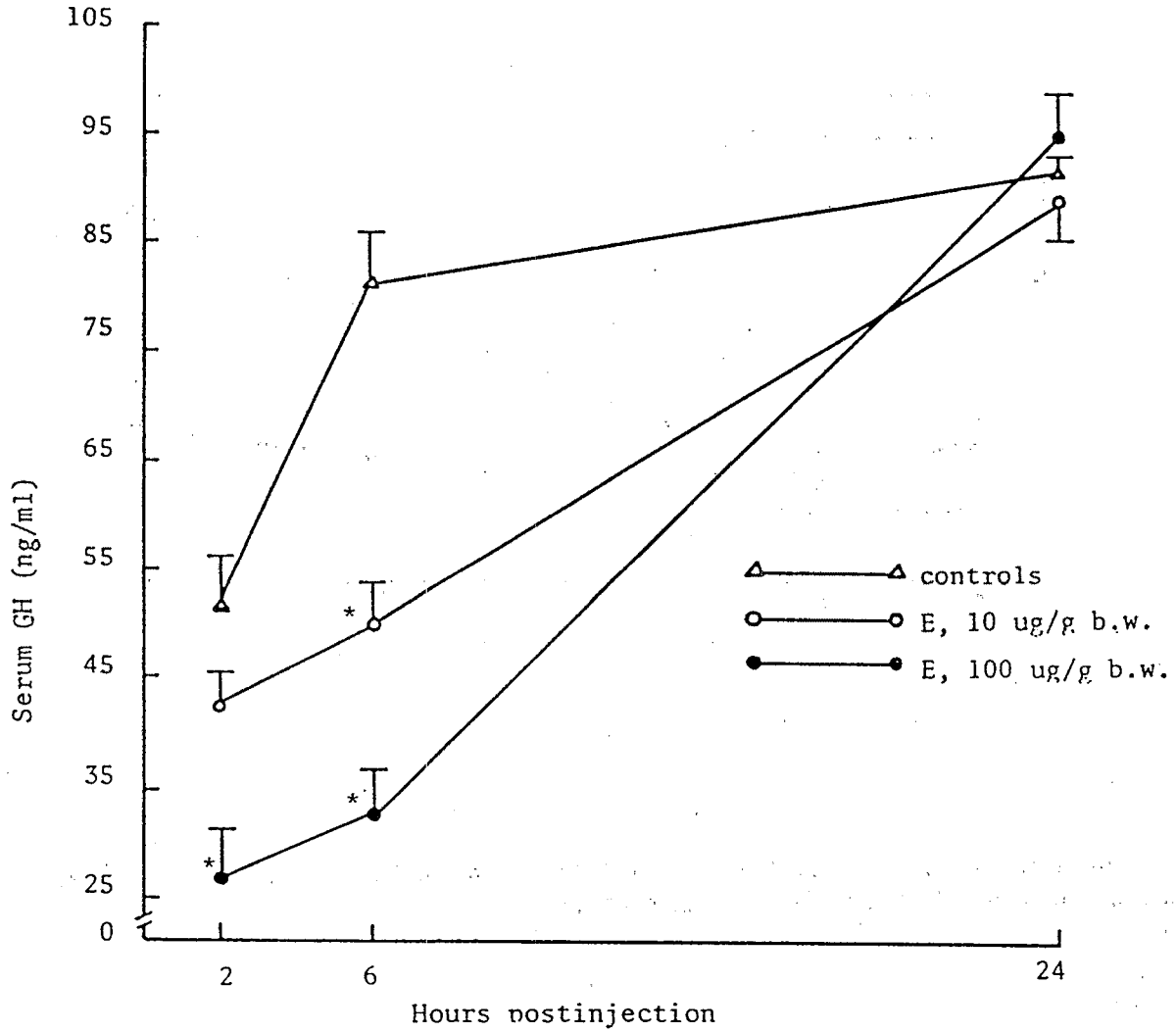

Figure 9. Effects of intraperitoneal injections of epinephrine (E) on serum GH levels of fingering of grass carp. *Significantly lower than controls (P<0.05).

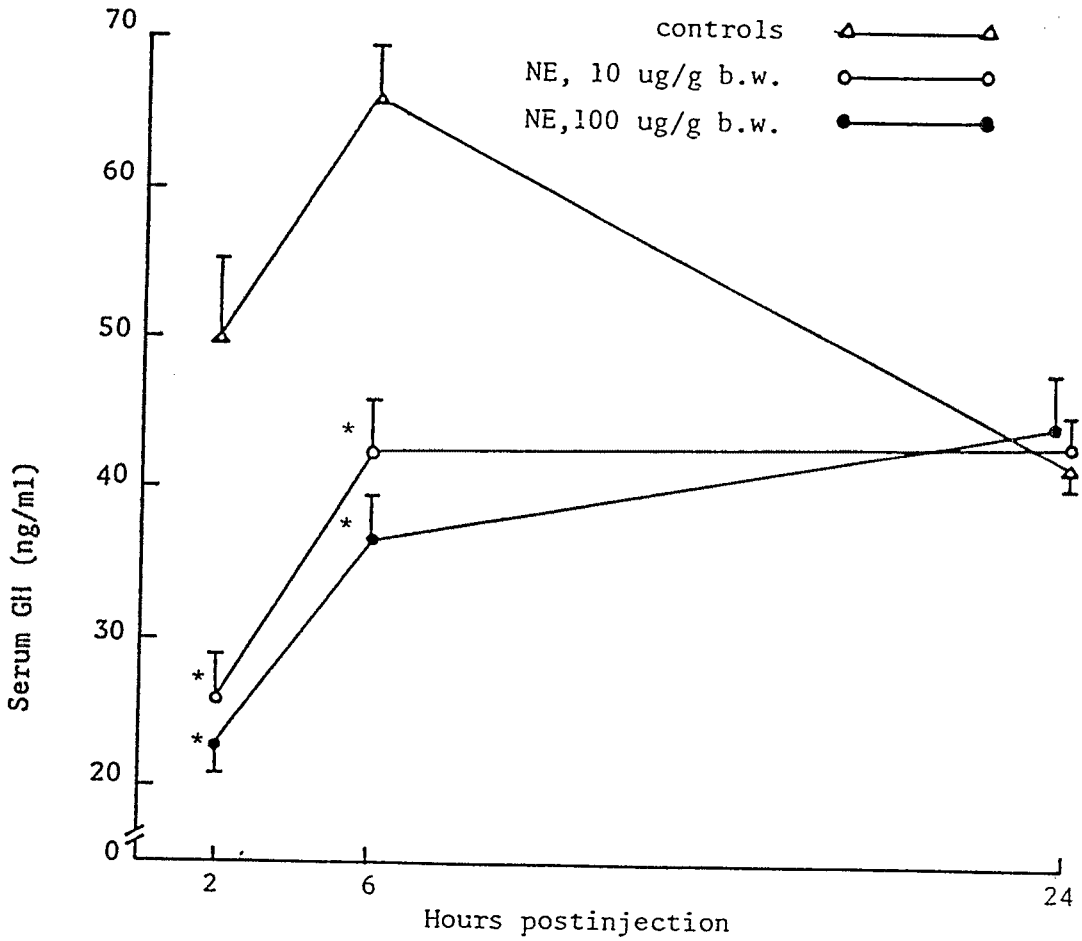

Figure 10. Effects of intraperitoneal injections of norepinephrine (NE) on serum GH levels of fingerling of grass carp. *Significantly lower than controls ($P<0.05$).

The results of the present study support the findings using goldfish (Chang et al., 1985), which indicated that catecholamines were involved in the neuroendocrine regulation of GH release in teleosts. Intraperitoneal injection of 6-OHDA, a catecholaminergic neurotoxin, or α-MPT, a catecholamine synthesis inhibitor, decreased GH levels. Intraperitoneal injection of L-dopa increased serum GtH concentrations, while intraperitoneal injection of APO, a dopamine agonist that crosses the blood-brain barrier, increased serum GH levels. Finally, serum GH concentrations were decreased by intraperitoneal injection of norepinephrine (NE) and epinephrine (E).

ACKNOWLEDGEMENTS

This work was supported by a grant 3-P-87-1028 from the International Development Research Centre of Canada to H.R. Lin and R.E. Peter.

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EVALUATION OF REPRODUCTIVE EFFECTS OF ANTHROPOGENIC CHEMICALS IN FISH

William H. Benson[1], Thomas Dillon[2] and Burton Suedel[3]

INTRODUCTION

Exposure of fish to toxic chemicals may yield effects varying from acute lethality to sublethal changes in reproduction, growth and development (Murty, 1986). A sublethal effect that warrants more detailed study is the influence of environmental exposure to anthropogenic agents on reproduction and offspring growth (Weis and Weis, 1989). Reproductive effects may arise in fish when some aspect of the environment alters the maternal contribution to yolk quality or quantity. With regard to anthropogenic agents, biologically important effects could arise through their direct incorporation into the yolk or through alteration in the egg yolking process. The manifestation of these subtle influences on reproductive biology could range from altered life histories of the affected generation to teratogenic effects.

There is evidence that maternal environment influences reproduction and offspring quality. Classic experiments documenting maternal effects have been conducted with cattle, rabbits and pigs (Pirchner, 1983) snails, fruit flies, rye grass, and tobacco (Mather and Jinks, 1982). Maternal effects are implicated in all reproductive modes, from live-bearing with extensive maternal-fetal exchange to egg or seed production. Maternal effects arise through contributions that the mother makes to her offspring's phenotype beyond her nuclear genes. Routes for these contributions are: cytoplasmic inheritance, pre-and post-natal nutrition, pre- and post-natal transmission of pathogens by feeding, imitative behavior, and interaction of sibs directly with each other or through the mother (Mather and Jinks, 1982). Maternal effects may arise from non-genetic or genetic sources. For example, non-genetic effects may arise from the environment in which the mother finds herself and a genetic source could be genetically based variation in maternal nourishment of offspring. Males can influence the maternal genetic effects of their grandchildren through their daughters, despite having no impact on their own offspring through this route. "Grandfather effects" such as these have been documented in species as diverse as horses, cattle (Pirchner, 1983), snails (Boycott and Diver, 1923; Sturtevant, 1923; Diver et al., 1925) and mosquitofish (Reznick, 1981).

Maternal environmental effects in fish on the size and number of eggs or offspring produced have been documented in several cases (e.g., Alm, 1949; Reznick, 1981; Cheong et al., 1984; Tanasichuk and Ware, 1987). Also, many cases are known where chemicals of environmental interest induce reproductive effects (Niimi, 1983; Heath, 1987; Weis and Weis, 1989). Such research has primarily focused on the reproductive effects of chemicals on fecundity and fertility though other, possibly subtle, effects of sublethal concentrations are likely.

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The purpose of this investigation was to evaluate the relationship between reproductive effects and polychlorinated biphenyl (PCB) tissue concentrations resulting from exposure to contaminated sediments from an inland waterway. Such "residues-effects" data are infrequently (ca., 6%) reported in the literature for fish or other aquatic organisms (Dillon, 1984). However, these types of data may be valuable when interpreting the biological significance of contaminant tissue concentrations in field-collected animals.

MATERIALS AND METHODS

SEDIMENTS

The PCB contaminated sediments were obtained from existing sediment samples previously collected from Sheboygan Harbor, Wisconsin and stored in 15-L glass jars at 4^0C. Sediments were mixed to produce treatment concentrations identified as low, medium and high which corresponded to PCB concentrations, as Aroclor 1254 equivalents, of 0.82, 14.0, and 27.0 µg/gm (dry weight), respectively.

EXPOSURES

Adult fathead minnows (*Pimephales promelas*) were obtained from Northeastern Biologists (Rhinebeck, NY). Females averaged 25 to 40 mm in length, and males averaged 40 to 50 mm in length. Following a 30 day acclimation to all experimental conditions except sediment, fish were placed in 40 L glass aquaria containing a 2 to 4 cm layer of PCB contaminated sediment. Aquaria containing only water served as controls. The aquaria were supplied with moderate aeration and a continuous flow of aged, charcoal filtered tap water having the following mean water quality characteristics: pH 7.8, hardness 59 mg $CaCO_3$/L and 8.1 mg/L dissolved oxygen. Flow rates (80 ml/min) were set to provide three volume additions every 24 h. Temperature and flow rates were monitored daily while total dissolved solids (TSS) was monitored on a weekly basis.

During the initial experimental phase, 7 male and 21 female fish per aquarium were exposed for 5 weeks with water temperature maintained at $20^0 \pm 1^0$C and photoperiod maintained at 12-h light:12-h dark. Fish were fed 3% freeze-dried brine shrimp in the morning and 3% commercial diet (Tetra-Min) in the evening, on an estimated body weight basis. Following the initial exposure phase, water temperature was increased 1^0C per day to $26^0 + 1^0$C. In addition, the photoperiod was lengthened 1 h every other day to 16-h light:8-h dark to induce gametogenesis, sexual dimorphism and reproductive activities in the test organisms. During this 10-d induction phase, spawning substrates were introduced into each of the aquarium. The use of frozen brine shrimp (3%) replaced freeze-dried brine shrimp to further induce spawning. Following induction, 1 male and 6 female fish were selected from each aquarium to evaluate treatment effects on reproduction. Fecundity and clutch size were monitored for an additional 9 weeks.

CHEMICAL ANALYSIS

Chemical analysis was conducted by the Tennessee Valley Authority (Chattanooga, TN). Briefly, fish were thawed, homogenized in petroleum ether and cleaned with concentrated sulfuric acid. Water was removed during repeated extractions with sodium sulfate and an aliquot of the homogenized fish tissue was removed for gravimetric determination of percent lipid prior to acid clean up. Extracts were run on a Hewlett-Packard gas chromatograph equipped with a 30 meter DB-5 fused capillary column and electron capture detector. All standards were purchased from Ultra Scientific (Hope, RI). The detection limit for Aroclor 1254 was 0.10 µg/g and for PCB congeners, 0.01 µg/g.

DATA ANALYSIS

Treatment effects on all parameters were analyzed by means of one-way analysis of variance. Differences were considered statistically significant at $p < 0.05$. Square root or log 10 transformations were used when data sets were not homogenous. Arc sine transformations were used for non-homogeneous data which were expressed as percentages. Mean separation for homogeneous data was achieved by means of Waller-Duncan k-ratio t test. When transformations were unsuccessful in achieving homogeneity a Proc Rank nonparametric procedure was used for mean separation (SAS Institute, 1985). To facilitate statistical analysis and presentation of the PCB analytical information, data reported to be less than the detection limit were considered to be equal to the detection limit.

RESULTS

Table 1 presents the major findings of the investigation. Survival was high in all PCB-contaminated sediment treatments and ranged from 80 to 100%. Regarding reproductive effects, there was a very clear separation between the control and low treatments and the medium and high treatments; with fish reproduction in the latter treatments being almost completely inhibited. This is substantiated by examination of fecundity and frequency of egg production. The mean number of eggs produced by fish in the control, low, medium and high treatments were 5,166, 3,346, 1,172 and 97, respectively, while mean number of clutches were 22.4, 14.4, 3.2 and 0.6.

Table 1. Survival, fecundity and frequency of egg production in fathead minnows exposed to PCB-contaminated sediment treatments

Treatment	Survival (%) male	Survival (%) female	Fecundity (total eggs)	Frequency (total clutches)
Control	100	100	5,166 ± 1,152 A	22.4 ± 3.3 A
Low	100	100	3,346 ± 417 A	14.4 ± 2.8 A
Medium	80	95	1,172 ± 1,114 B	3.2 ± 3.0 B
High	80	90	97 ± 9 B	0.6 ± 0.6 B

Values are reported as mean ± SE. Values within columns followed by the same letters are not significantly different ($p < 0.05$).

Tissue concentrations in fish from all experimental treatments were significantly different from one another after 7 and 16 week of exposure (Fig. 1). After 7 weeks of exposure, respective mean tissue concentrations in the control, low, medium and high treatments were 0.10, 5.25, 13.7 and 18.4 µg/g wet weight, expressed as Aroclor 1254 equivalents. PCB residues were also determined in fish at termination of the experiment. These residues, therefore, reflect 16 weeks of continuous exposure to the experimental treatments with the last 9 weeks under conditions to induce gametogenesis and spawning activities. Mean values after 16 weeks of exposure generally reflected patterns observed after 7 weeks except that the absolute concentrations were elevated over 2 fold. Significantly different PCB concentrations in the control, low, medium and high treatments were 0.10, 11.6, 36.0 and 47.2 µg/g wet weight, respectively.

Mean percent lipid values for all samples from this experiment are shown in Figure 2. At 16 weeks of exposure there is a significant increase in percent lipid in the medium and high treatments.

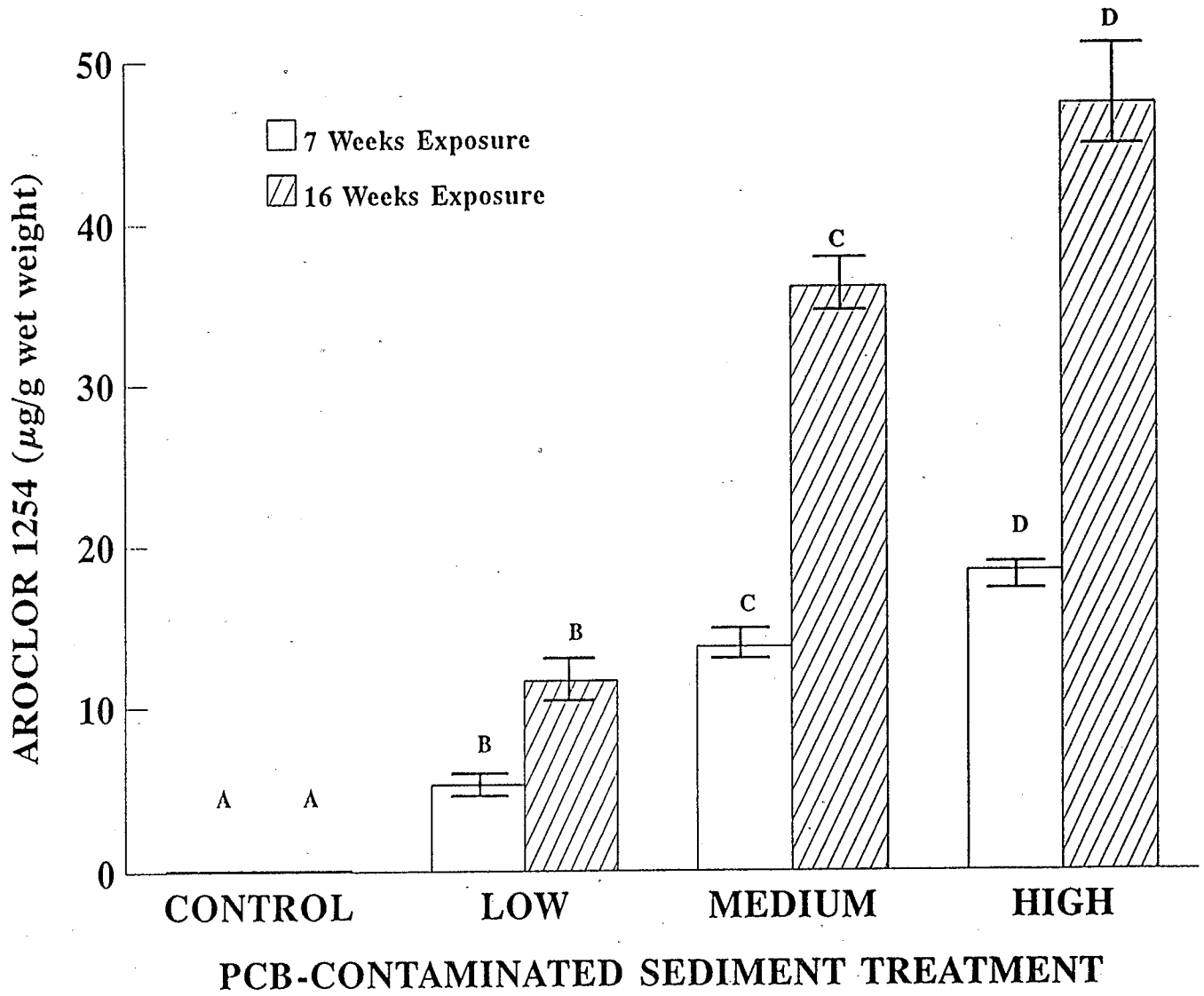

Figure 1. Tissue concentrations of PCB in fathead minnows exposed for 7 and 16 weeks to PCB-contaminated sediment treatments. Each bar represents the mean ± SE. Within each exposure period, means followed by the same letters are not significantly different ($p < 0.05$).

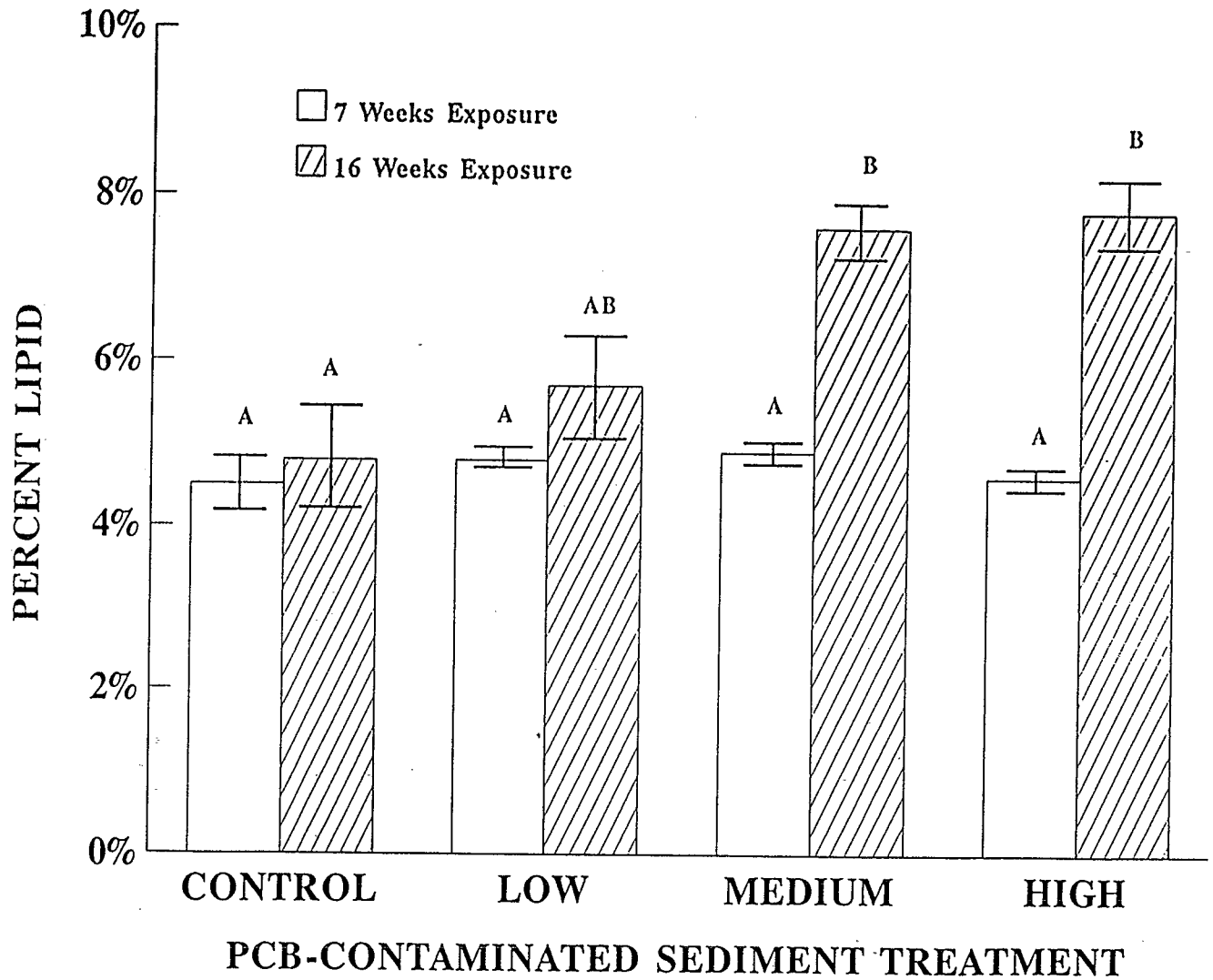

Figure 2. Percent lipid content of fathead minnows exposed for 7 and 16 weeks to PCB-contaminated sediment treatments. Each bar represents the mean ± SE. Within each exposure period, means followed by the same letters are not significantly different ($p < 0.05$).

DISCUSSION

One of the objectives of this investigation was to evaluate the biological consequences of bioaccumulation. To optimize this comparison one must have graded responses in both the consequences and the bioaccumulation components. In this investigation, both were observed.

The experimental treatments, represented by control, low, medium and high PCB-contaminated sediments, had a significant impact on reproduction fathead minnows. This is based on the observation that both fecundity and fertility were affected by these compounds in a dose-related fashion. Interestingly, fish exposed to PCBs at medium and high concentrations had a higher percent lipid content than those not exposed or exposed to low concentrations. This brings up the interesting prospect that the energy allocation pattern of exposed fishes may have been altered from reproduction to storage. PCBs are lipophilic, suggesting that lipid stores sequester concentrated levels of these compounds. Thus, even if environmental concentrations were to decrease, when and if these stores are metabolized, they could release high levels of PCBs into the organism. One characteristic of PCB activity is estrogenicity (Hansen, 1987). This property makes it likely that these compounds influence reproductive function. However, in addition to reproductive and hormonal effects, PCBs have a multiplicity of other toxicological effects including neurotoxicity, immunotoxicity, hepatotoxicity, as well as mutagenic and carcinogenic effects (Safe, 1984). Furthermore, commercial PCB products, such as Aroclor 1254, are mixtures of individual biphenyl molecules; or congeners. There are 209 theoretically possible congeners and recently, much attention has been given to their individual toxicological effects. For example, Dillon et al. (1989, 1990) examined the effects of individual PCB congeners on survival, growth and reproduction in the freshwater cladoceran, *Daphnia magna* (IUPAC numbers 52, 77, 101, 118, 138, 153, 180) and freshwater fish, *Pimephales promelas* (IUPAC numbers 52, 101, 138, 153, 180). These species accumulated substantial amounts of each congener examined. However, there were little to no detectable effects on survival, growth and reproduction. These investigators indicate that because the concept of PCB toxicity has been developed primarily through technical findings with mammals, caution should be exercised when applying those concepts to fish and other aquatic organisms.

ACKNOWLEDGEMENTS

Funding for this work was provided by the U.S. Army Corps of Engineers Long-Term Effects of Dredging Operations Program (LEDO).

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SUBLETHAL RESPONSES OF TWO LARVAL FISHES TO RICE FIELD PESTICIDES

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ABSTRACT

Newly hatched striped bass, *Morone saxatilis*, and Japanese medaka, *Oryzias latipes*, were exposed to "low" (environmental) and "high" (½ LC_{50}) sublethal levels of methyl parathion, carbofuran, molinate, and a combination of the three. Dry weight, RNA:DNA ratio, swimming performance, spontaneous activity, and acetylcholinesterase activity were measured immediately after the 4-d exposures and, again, after 10 subsequent days in clean water. Whereas pesticides had little effect on growth variables, acetylcholinesterase and swimming performance were affected, especially by the high doses in striped bass. Growth and movement variables of striped bass were greatly affected by food availability. "High" sublethal pesticide concentrations and/or low food availability would contribute to a weakened larval striped bass in the Sacramento-San Joaquin Estuary.

INTRODUCTION

Populations of striped bass, *Morone saxatilis*, an introduced predatory fish in the Sacramento-San Joaquin Estuary of California, USA, have undergone significant reductions over the past 15 years (Setzler-Hamilton et al. 1988). A valuable sportsfishing industry for this species has been severely damaged by this decline. Two possible reasons for the decline include: water diversions (and consequent loss of drifting striped bass larvae, as well as larval food where water is diverted from the River and Estuary for irrigation; and pollutants (resulting in poor survival of sensitive larvae or their prey. Unfortunately, striped bass spawning in the Sacramento River occurs at the same time and place when various insecticides and herbicides are flushed from surrounding rice fields into agricultural drains (such as the Colusa Basin Drain which flow into the Sacramento River. In low-runoff years, drain water can comprise up to 40% of the River flow at this point. The striped bass larvae drift in the river for 4-8 days until the water is considerably diluted by flows from the San Joaquin River and by tidal influences of saltier water in the estuary. Our research objective was to assess the effects of two insecticides and an herbicide using both molecular and whole-animal techniques on the growth and motor function of 4-10 day-old striped bass larvae and to compare these effects with those shown by Japanese medaka, *Oryzias latipes*. This comparison tested possible use of medaka as a surrogate species for striped bass in future larval toxicity studies. Medaka are much smaller as adult fish and can be spawned in the laboratory at any time, rather than only in May and early June when striped bass spawn.

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METHODS

We used methyl parathion, an organophosphate insecticide; carbofuran, a carbamate insecticide; and molinate, a thiocarbonate herbicide; and a combination of all three chemicals at two sublethal dosages. All were checked by actual gas chomatographic measurements in the California Department of Fish and Game Water Pollution Control Laboratory. The high dose approximated one-half of the larval striped bass LC_{50} for each chemical, whereas these concentrations were divided by 3 where they were mixed together for the combined high dose. The low dose approximated concentrations in the Colusa Basin Drain water. There were up to several orders of magnitude differences within dosages (Table 1). Larvae were exposed 4 days to these concentrations in 2-l beakers with daily water changes with new chemical. Pesticide exposures were conducted at the California Department of Fish and Game Aquatic Toxicology Laboratory and simulated their 4 days in the river.

Larvae were carefully transported to the University of California Department of Wildlife and Fisheries Biology and reared in clean water for 10 days to simulate time in the estuary. Larvae were held in polypropylene beakers with Nitex mesh panel sides, which were randomly assorted in a temperature and solute-constant water bath. Larvae inflate their swimbladders and commence feeding at day 6-8 post-hatch, so we fed 10 ml of a concentrated *Artemia* nauplii suspension twice daily in a randomized order. Measurements related to growth and movement were made at two periods: immediately after exposure to the chemicals, and after 10 days in clean water.

Growth was measured by comparisons of dry weights and by whole larvae RNA:DNA ratios. Larvae were fixed in 5% buffered formalin in individual wells of tissue plates, rinsed with distilled water, dried to a constant weight at 50°C, and weighed on a Cahn microbalance. RNA and DNA concentrations were measured using a modification of the spectrofluorometric method of Bentle *et al.* (1981).

Motor function was assessed by measurements of spontaneous activity, swimming performance, and acetylcholinesterase concentrations. Spontaneous activity was also measured in clean water, using a pyrex pan over a paper grid. Twenty seconds after being placed in the center of the pan, the number of grid lines crossed/minute was counted. In all of these measurements, a random number table was used to determine order of use, because the slowest swimming individuals might be captured first from the holding beakers with our plastic pipets. Also, the person conducting a given test was consistent across treatment groups, and a code system was used such that the experimenter did not know the fish's treatment group. Swimming performance was measured in clean water using "racetrack," consisting of a plastic petri dish with an inverted smaller dish serving as a centerpiece, forming a circular aquatic "track.". An individual larva was randomly selected and after 2 minutes adjustment to the "track", was gently chased with a glass rod as it might be chased

TABLE 1

Mean (± SD) pesticide concentrations (ppb) and water quality data ranges during 4-d exposures of fish larvae[1]

Pesticide	Concentrations				Water Quality	
	Low Nom	Low Meas.	High Nom.	High Meas.	Temp.	pH
STRIPED BASS						
Parathion	0.66	1.04±0.59	1800	1650±150	17.0-17.7	8.2-8.7
Carbofuran	1.3	1.1±0.2	110	79±7.9	16.6-17.6	8.0-8.9
Molinate	70.0	59.0±2.9	4050	3125±330	17.0-17.6	8.1-8.9
Combined:						
Parathion	0.66	0.55±0.08	600	470±14		
Carbofuran	1.3	1.08±0.18	37	29±4.2	16.7-21.7	7.9-8.4
Molinate	70.0	43.0	1350	930±57		
MEDAKA						
Parathion	0.66	3.17±0.15	1800	1567±58	21.9-23.3	8.3-8.6
Carbofuran	1.3	1.5±0.10	110	88±14	23.1-23.7	8.0-8.6
Molinate	70.0	54.0±2.1	4050	3001±183	22.2±22.7	8.2-8.5
Combined:						
Parathion	0.66	0.41±0.01	600	515±10		
Carbofuran	1.3	1.1±0.12	37	31±5.6	23.1-24.5	7.8-8.5
Molinate	70.0	41.0±2.1	1350	920±48		

[1] As shown in Heath et al. (1993a, b).

by a predator, and the number of radial lines it crossed in 1 minute was recorded. Larvae were frozen for acetylcholinesterase activity, which was assayed in Dr. Joseph Zinkl's laboratory at the University of California, Davis, using a modified Ellman *et al* (1961) procedure.

Analyses of variance, or Mann-Whitney U-tests, were conducted on the pooled triplicate beakers for each treatment giving an "n" of 8-16 larvae/treatment for the acetylcholinesterase measurements, 21 for the spontaneous activity measurements, and 15 for the other data. Further details on methods are in Heath et al. (1993).

RESULTS AND DISCUSSION

STRIPED BASS

There was very little mortality induced by the pesticide exposures. However, we did observe that immediately after the 4-day exposure to methyl parathion, the majority of the striped bass larvae were swimming on their sides. This behavior persisted through the subsequent 10 days in clean water.

There was virtually no increase in dry weight over the 10 days in clean water in the methyl parathion and molinate experiments (Table 2). The fish were observed to be feeding and had food in their guts, but the 300 um Nitex panels were not effectively holding the nauplii in sufficiently high concentrations in the beakers. We switched to 200 um mesh panels for subsequent experiments and larvae grew faster (dry weight tripled). The less-dense food suspension was adequate for maintenance needs (preventing starvation, but allowing no growth), and may have more accurately simulated the estuary under food-limited conditions. Larvae from the low parathion dose had reduced weight immediately after exposure. This may have been a statistical fluke, because they were still operating on endogenous yolk - they had not yet begun to feed. But the RNA:DNA ratio was also depressed, suggesting a depressed protein synthesis rate. Weight of the high-dose molinate-exposed striped bass, after 10 days in clean water was significantly greater than controls. The same phenomenon was measured in medaka (Table 3). This could represent a type of hormesis where an organism metabolically over-compensates to an exogenous stressor (Stebbing 1987).

The RNA:DNA ratio was also elevated in this group (high molinate). The low ratios in all three treatments (including controls) in the 10-day combined pesticide group might reflect an error in preparation of the nucleic acid standard, because the same standard was used for both dosages and the controls. Overall, from both weight and RNA:DNA ratio data, these concentrations of these three pesticides show little evidence of growth suppression.

Striped bass exposed to methyl parathion at both doses showed a slight but significant depression in swimming performance immediately after exposure (Table 2). After the 10 days in clean water, only the high-dose fish showed a significant depression compared with controls. As stated above, most of this swimming was on their sides. The high dose of molinate also reduced swimming performance, which persisted after the 10 days in clean water. The larvae with reduced food abundance (methyl parathion and molinate-exposed, including controls) all showed depressed swimming, which suggests that this measurement might be useful as a biomarker for low food consumption.

TABLE 2

Mean (± SE) effects of 4-d exposure of striped bass to 3 pesticides and combined pesticides (low and high doses: see Table 1). Numbers of fish were generally 10-15 (see text). Asterisks indicate significant statistical difference ($p<0.05$) from controls, and "na" indicates not available.

Pesticide/ Dose	Swim Perf. (lines/min)	Spont. Act. (lines/min)	AchE (ug/mg prot.)	Dry Wt. (ug)	RNA/DNA
METHYL PARATHION					
		Immediately After Exposure			
Control	20±1	53±4	487±33	171±12	2.34±0.11
Low Dose	*16±2	47±4	*389±20	*125±10	*1.87±0.05
High Dose	*16±2	53±4	*356±29	199±17	2.44±0.08
		After a Subsequent 10 d in Non-Contaminated Water			
Control	9±1	12±2	585±71	176±39	na
Low Dose	10±1	12±2	*333±23	130±9	na
High Dose	*4±2	*6±4	*402±11	132±22	na
MOLINATE					
		Immediately After Exposure			
Control	22±1	36±3	409±19	120±8	2.07±0.22
Low Dose	22±2	42±4	498±38	118±11	1.74±0.08
High Dose	*17±1	*28±3	465±21	*95±7	2.11±0.13
		After a Subsequent 10 d in Non-Contaminated Water			
Control	10±1	10±1	686±49	120±6	1.46±0.10
Low Dose	*5±1	12±2	613±23	125±6	1.36±0.15
High Dose	*4±1	11±1	*487±24	*153±9	*1.92±0.10

Table 2 (continued)

CARBOFURAN					
			Immediately After Exposure		
Control	23±2	46±6	286±37	113±5	1.91±0.06
Low Dose	20±2	42±6	253±25	113±5	*2.21±0.09
High Dose	*17±2	18±5	202±16	113±6	1.850.06
			After a Subsequent 10 d in Non-Contaminated Water		
Control	24±2	35±4	386±22	306±27	2.32±0.06
Low Dose	27±3	31±4	*671±52	386±51	2.20±0.09
High Dose	21±2	41±4	*574±46	312±26	*1.97±0.10
COMBINED PESTICIDES					
			Immediately After Exposure		
Control	22±2	36±5	289±19	138±8	2.95±0.25
Low Dose	24±2	49±4	245±8	130±11	2.71±0.11
High Dose	20±2	*53±4	*212±16	154±13	3.30±0.19
			After a Subsequent 10 d in Non-Contaminated Water		
Control	22±2	25±4	612±58	315±25	1.88±0.15
Low Dose	20±2	26±5	485±52	343±28	2.05±0.13
High Dose	23±3	24±4	*371±20	355±44	2.07±0.08

Spontaneous activity measurements were somewhat variable, including a significant elevation for combined pesticides (escape behavior?). Striped bass showed more significant reductions at the high dose, indicating impaired movement, but there were fewer reductions than in their swimming performance data, indicating that this measure of activity is somewhat less sensitive than swimming performance to pesticides. However, it appears especially sensitive to food availability (Table 2). This result plus the significantly-reduced swimming performance may point to a contributing cause of the striped bass decline in the Sacramento-San Joaquin Estuary. Significant reductions in larval food supplies may make these young striped bass much more vulnerable to predation (Webb 1986).

Acetylcholinesterase activity showed significant reductions, mostly at the high dose for all of the chemicals, including the combination (Table 2). Our measured reductions are small, but reflect whole body levels. Methyl parathion and carbofuran are both known acetylcholinesterase inhibitors (Zinkl et al. 1991). Acetylcholinesterase inhibition by a thiocarbamate herbicide is more surprising. The prolonged effect (at 10 days) may indicate central nervous system developmental inhibition in larval striped bass or acetylcholinesterase inhibition from a molinate metabolite, because Tjeerdema and Crosby (1987) measured molinate's half-life to be 1 day!

Summarizing the larval striped bass results: exposing these newly-hatched fish to individual pesticides for 4 days resulting in some sublethal effects, even at concentrations several orders of magnitude below the LC50. Some of these effects persisted in larvae kept for 10 days in clean water. Swimming performance and acetylcholinesterase inhibitions were the most sensitive variables examined, with the swimming being especially sensitive to food shortage. Methyl parathion appears to cause the greatest harm of the 3 pesticides tested. Combining the 3 produced a less than additive effect on most of the measured variables.

MEDAKA

Medaka showed virtually no mortality from pesticide exposures and the subsequent 10-day clean water period. They were eager eaters and they gained weight rapidly. Medaka reached sizes 2-4 times that of the striped bass larvae (Table 3). The significant increases in day weight after the high molinate dose was the potential hormesis also observed in striped bass larvae.

A bad batch of DNAase enzymes from the supplier spoiled some of our RNA:DNA ratio samples, but like the dry weight data these pesticides at these concentrations seem to have little effect on growth (Table 3).

Medaka swimming performance showed some variability (a large SE for methyl parathion low dose), but the only consistent effect might have been a significant reduction after exposure to the high dose of carbofuran.

Spontaneous activity showed significant increases immediately after exposure to molinate and to combined pesticides (Table 3). Interestingly, striped bass showed the same response to combined pesticides (Table 2), but molinate-exposed striped bass shoed significant depressed spontaneous activity - the opposite response of medaka. Medaka acetylcholinesterase showed more effects than the swimming or spontaneous activity showed.

TABLE 3

Mean (± SD) effects of 4-d exposure of medaka to 3 pesticides and combined pesticides (low and high doses: see Table 1). Numbers of fish ranged from 7-15 for acetylcholinesterase activity measurements to 21 for spontaneous activity measurements (see text). Asterisks indicate significant statistical difference ($p<0.05$) from controls, and "na" indicates not available.

Pesticide/ Dose	Swim Perf. (lines/min)	Spont. Act. (lines/min)	AchE (ug/mg prot.)	Dry Wt. (ug)	RNA/DNA
METHYL PARATHION					
Immediately After Exposure					
Control	32±2	na	1651±167	86.2±4.4	2.29±0.32
Low Dose	26±2	na	*664±82	71.8±4.	1.79±0.75
High Dose	24±3	na	*604±72	73.2±4.9	1.70±0.22
After a Subsequent 10 d in Non-Contaminated Water					
Control	76±5	na	2488±227	145±20	5.04±0.41
Low Dose	97±14	na	*1933±129	172±36	5.68±0.71
High Dose	76±7	na	2022±195	172±23	4.69±0.82
MOLINATE					
Immediately After Exposure					
Control	18±1	8±1	1464±164	98±6	na
Low Dose	18±1	10±1	1336±239	91±3	na
High Dose	16±1	*13±1	*942±50	88±5	na
After a Subsequent 10 d in Non-Contaminated Water					
Control	44±4	12±3	1960±134	545±46	na
Low Dose	39±5	12±2	*1533±69	616±61	na
High Dose	44±4	12±3	*1430±124	*719±39	na

Table 3 (continued)

Pesticide/ Dose	Swim Perf. (line/min)	Spont. Act. (lines/min)	AchE (ug/mg prot.)	Dry Wt. (ug)	RNA/DNA
CARBOFURAN					
Immediately After Exposure					
Control	16±1	5±1	na	76±4	na
Low Dose	13±1	6±1	na	68±3	na
High Dose	*11±1	3±1	na	66±3	na
After a Subsequent 10 d in Non-Contaminated Water					
Control	54±4	7±2	3218±247	108±8	3.19±0.26
Low Dose	54±3	9±2	3773±195	111±12	3.35±0.33
High Dose	*42±3	8±3	3044±170	90±8	*2.40±0.20
COMBINED PESTICIDES					
Immediately After Exposure					
Control	14±1	6±1	na	67±3	6.02±0.46
Low Dose	16±2	5±1	na	68±2	4.99±0.34
High Dose	12±1	*10±1	na	66±5	5.98±0.31
After a Subsequent 10 d in Non-Contaminated Water					
Control	36±5	4±2	na	90±9	9.17±1.07
Low Dose	45±3	5±2	na	104±11	8.39±0.45
High Dose	46±3	4±2	na	88±8	8.00±0.90

In conclusion, we have found that there are some sublethal effects on newly hatched striped bass larve from exposure to pesticide conscentrations several orders of magnitude below the LC50. Of the variables measured in the larvae, swimming performance and inhibition of acetylcholinesterase were the most sensitive to the pesticides. Medaka larvae are clearly less sensitive to these pesticides (as measured here) than are striped bass larve so their use as a surrogate species is not supported.

ACKNOWLEDGEMENTS

We thank Drs. Rose Russo, Dave Randall, Vance Thurston, Lin Haoren, Lin Yu-huan, and Hong-jun Jin for inviting JJC to speak at Nanjing University, and we thank the USEPA for travel-related support. We appreciate the logistical support of Dr. Paul Lutes and Mr. Bill Bentley of the University of California Aquaculture and Fisheries Program and facilities of the Califorina Department of Fish and Game Aquatic Toxicology Laboratory and the University of California Institute of Ecology. We acknowledge the USEPA and the CDF&G for supporting our research. JJC was partially supported by the USEPA (R819658) Center for Ecological Health Research at UC Davis. We also acknowledge several University of California undergraduate students who assisted with the data collection: Mike Steele, Kevin Reimer, Laurie Martin, Jon Hamm and Scott Cech.

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THE EFFECTS OF WATER POLLUTANTS ON SOME PHYSIOLOGICAL FUNCTIONS OF FISH

Yiming Huang[1] and Weihong Lin[1]

China, a nation abundant with fish resources, is developing its own aquaculture industry. However, fisheries production and indeed the survival of many fish species is threatened today by pollution from industrial wastes, mine runoff, petroleum, pesticides, etc., and the number of natural bodies of uncontaminated water are becoming scarce. The effects of water pollutants on the physiology of fish is receiving wide-spread attention, and this article is a brief survey of the most recent studies on this topic.

THE EFFECTS OF WATER POLLUTANTS ON FISH RESPIRATORY FUNCTION

Respiration is a major function of the fish gill, and recording opercular movement (respiratory frequency) is a basic measure of the effect of pollutants on respiration. It has long been known that pollutants, and in particular industrial wastes such as from synthetic fabric mills, decrease breathing frequency to a lethal level. Ammonia inhibits respiratory frequency, but is also taken up by the gills and affects metabolism by its alkalinizing effect on the blood. Severe NH_3 poisoning can result in respiratory failure and fish mortality (28). Phenol is a common industrial pollutant, and concentrations of 5 - 50 ppm can increase the respiratory frequency of catfish in a manner directly correlated with concentration and exposure time. High concentrations of phenols can lead to whole body muscle convulsions (18). Water containing 0.005 mg/L phenol can strongly inhibit the respiratory center of Common carp. Fresh water carp such as Common carp, Silver carp, and Grass carp are very sensitive to heavy metal ions (Hg^{2+}, Cu^{2+}, Ag^{+}) in water (6, 7, 8), and respiration is perturbed at very low concentrations (0.05-0.1 mg/L). Increasing concentrations lead to coughing and a decrease in opercular movement. This may be caused by the heavy metal ions flowing into the gills with respiratory movement and becoming attached to the gill surface. The ions may combine with the mucus excreted by the gill epidermis to form a unsoluble metal-protein compound. The higher the concentration of heavy metal ions, the more compound formed, which reflexively increases cough frequency in an attempt to eliminate the accumulated pollutants and restore gas exchange. Coughing is apparently a protective mechanism, occurring almost immediately when fish are exposed to heavy metal ions, and lasting a prolonged period of time. High concentrations of these ions severely inhibit respiratory function and lead to death. The effects of heavy metal ions on fish respiratory function differ depending upon both the ion and the fish species involved. Exposure to several metal ions simultaneously increases cough frequency to a greater degree than exposure to a single ion, implying an additive or synergistic effect. Research on the effects of heavy metals on fish respiratory function is of both theoretical value and of practical significance in the establishment of water quality criteria to define safe and allowable limits of pollutants and encourage biological monitoring of water contamination.

THE EFFECTS OF WATER POLLUTANTS ON FISH BLOOD AND CARDIOVASCULAR SYSTEM

There has been considerable research lately on the effects of organic materials (petroleum and its by-products, pesticides, benzene and its salts) and heavy metals (chromium, zinc, cadmium) on the cardiovascular system of fish. Fish exposed for some time to water with a concentration of 200-400

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mg/L of the toxin aniline or its salts (24) exhibited abnormal behaviour and hyperventilation. Death occurred after 2-3 days exposure. When chronically exposed to a low concentration of aniline (0.05-50 mg/L), examination of the blood showed altered erythrocyte size and shape, a decreased erythrocyte count and hemoglobin content, and an increased number of leucocytes. These toxins also reduced total blood protein and plasma albumin levels, although the content of gamma-albumin increased. The results indicated that aniline and its salts affected not only blood tissue structure and function but also protein synthesis. Heavy metal ions have similar effects. Exposure to 15 ppm PbNH3 for 90 hr decreased the blood cell count 53%, decreased hematocrit 43% and reduced hemoglobin content by 37% (16). Blood neutrophils were altered and increased in number when fish were exposed to 50 ppm cadmium for 8 hr (3). Treatment with chromium changed blood ion concentrations and decreased plasma osmotic pressure in a dose-dependent fashion (15). Exposure to 0.1 ppm mercury for 10 days resulted in anemia and a decreased hemoglobin content (4).

We determined biophysical characteristics of blood using hemorheology and blood from Silver and Variegated carp exposed to industrially polluted water. The characteristics of blood from exposed fish were significantly different from those of normal fish ($P<0.05$). The most significant changes were a decrease in blood viscosity, plasma osmotic pressure, hematocrit, and erythrocyte sedimentation rate, and an increase of red cell electrophoresis rate. Phenol and rogor had a similar effect on Common carp and catfish (19).

Many pollutants also affect cardiac frequency (10), and our results have shown that the heart rate of catfish increases dramatically with increased pollutant concentration. In other experiments, free-swimming Common carp were exposed to 4 ppm rogor, and blood pressure chronically monitored through a dorsal aortic cannula. Systolic and diastolic pressures, and arterial pulse rate gradually decreased after 1, 7, 12 and 24 hr exposure. It is clear that many pollutants have serious and extensive detrimental effects on the cardiovascular system of fish.

THE EFFECTS OF WATER POLLUTANTS ON FISH GASTROINTESTINAL FUNCTION

The effects of oral perfusion of different concentrations of DDT and petroleum on the gastrointestinal function of cod has been studied by chronically recording electromyogram activity (30). The results showed that even short-term perfusion (2-5 min) by 10^{-8} M DDT destroyed basic electrical rhythm, resulting in an irregular frequency and amplitude. Short-term perfusion of 50 mg/L petroleum can strongly inhibit electrical activity. However, the long-term effects of DDT and petroleum (7 days) are different from the short-term effects. Gastric muscle electrical activity began to decrease in both frequency and amplitude on the third day of exposure. Amplitude then remained stable, while the frequency of activity continued to decline and finally vanished by the seventh day. Considering the physiological response to short-term exposure to these toxins, the data suggests that gastrointestinal activity could recover if the fish were returned to clean water. Injection of atropine (0.5-1.0 mg/L) to block M-cholinergic receptors could also eliminate the effects of DDT and petroleum. The effects of these pollutants on gastrointestinal activity may be indirect through effects on the central nervous system.

THE EFFECTS OF WATER POLLUTANTS ON THE CENTRAL NERVOUS SYSTEM AND BEHAVIOUR OF FISH

Many pollutants exert effects directly on the central nervous system. An example is petroleum and its by-products, which have an anaesthetic effect. Salmon and perch exposed to 10% phenol lose their sense of balance and swim in a circular, spiral or erratic manner before appearing to enter an essentially anesthetized state. Fish exposed to acute pesticide poisoning display a similar behaviour.

In the 1970s, there was considerable electrophysiological research on the effects of pollutants on the fish nervous system. DDT has been shown to alter salmon lateral line nervous activity (2), paper mill effluent perturbs fish olfactory function (26), while lethal levels of phenol and pesticides damage synaptic transmission in the central and peripheral nervous system of perch (29). There have also been acute and chronic recordings of both spontaneous and evoked action potentials in the brain during exposure to toxic pollutants. Electrical activity of the cerebellar dorsal surface changed when goldfish were placed in 10^{-6} M DDT, and the changes in frequency and amplitude were correlated with the behaviour of the fish. In the first 25 hr of exposure, the fish kept their balance and there were no marked changes in electrical activity. Further exposure resulted in the fish losing their balance, with a corresponding decrease in the mean amplitude of potentials from 49 μv to 84 μv, and a decrease in frequency from 12.8 Hz to 10.5 Hz. These changes were reversed when the fish were placed in uncontaminated water, with balance recovering in 24-120 hr. Evoked potentials in the brain can be used not only as a meticulous method of studying structure-function relationships in the central nervous system, but also as both normal and pathological indicators of different parts of the brain. Evoked potentials in the shark procerebrum and dorsal surface of the midbrain caused by stimulating the optic nerve were increased in amplitude by exposure to petroleum (27). The increase in the amplitude was dose-dependent, and could be raised by up to 50% in the procerebrum and 25% in the surface of the mesencephalon. Removal of the pollutant led to gradual recovery of the potentials to control levels. Functional and structural analysis of the mesencephalic surface showed that the negative and positive waves of an evoked potential reflected the nerve cells original synchronous activity after the afferent impulses arrived along the optic nerve. The increase in evoked potential amplitude suggested that excitability was increased in the dorsal, mesencephalic surface of the visual nervous system.

Neuromorphological studies indicated that neurons in the medulla changed in a manner similar to that during malnutrition when fish were exposed to acute or sub-acute petroleum poisoning. The neurons became swollen, the shape of the nucleus was altered, and cell cytoplasm was absent. *Huso dauricus* exposed to 0.02 mg/L pesticide for 60 days showed swelling in the spinal cord and changes in the shapes of individual neurons and their microstructure. Behaviourally, the fish were quiescent and did not feed. Post-mortem examination disclosed that the pollutants accumulated primarily in the brain and liver (14). Fish exposed to 1×10^{-6} ^{14}C-DDT for 5 min had a concentration of 0.3×10^{-6} in the brain, while exposure for 1 hr increased the concentration in the brain to 1×10^{-6}. Protein metabolism, and both neurotransmitter and cholinesterase levels changed in a manner compatible with the functional state of the fish after exposure to pollutants (20, 21).

We recently recorded the electrical activity of the maxillary beard afferent nerve of catfish to study the effects of pollutants on fish taste response (9). There was no marked change in electrical discharge of the taste-sensitive afferent nerve after direct stimulation of the upper jaw beard with a solution of DDT (0.02 ml/L) and $CuSO_4$ (0.02 g/L). However, after 2 hr of perfusing the surface, the pollutants reduced the intensity of the taste response to such stimuli as extract of earthworm, L-arginine and ascorbic acid. Prolonged exposure to 0.01 mg/L phenol first increased, then decreased the taste response of fish to various stimuli. A weakened taste response can affect normal feeding and thus hinder development and survival.

The active mechanism of pollutants on the central nervous system is specific to the type of pollutant. Oil and its by-products have an anaesthetic effect, while organic pesticides act as an anti-cholinesterase. Phenol inhibits oxyphosphorylation, and organic chlorine pesticides can damage synaptic transmission.

Fish behaviour is determined by their environment, and is directly affected by pollution. Young salmon and Mirror carp exposed to pesticides were lethargic and maintained a relatively long distance from each other even when in the same shoal (17). Experiments investigating the formation and maintenance of conditional reflexes in fish have shown that high-level processing of nervous activity was damaged if fish were raised in polluted water. Both the growth rate and survival rate of young sturgeon exposed to 50-70 mg/L petroleum were significantly inhibited (23). Exposure to lower concentrations could increase the survival rate to 100%, but behaviour was still abnormal in that the fish had a decreased appetite and a lethargic response to environmental stimuli. If crude petroleum was added to the water (30 mg/L), fish feeding reflexes and defence reflexes were seriously damaged. Exposure for 1 day to water containing petroleum decreased the feeding reflex in sturgeon by 80%, and in Mirror carp by 100%. If fish were returned to clean water the second day, the defence response remained inhibited for that day but returned to control levels on the third day.

Pollutants also affect the ability of a fish to distinguish different illumination, temperature and salinity levels. The ability of salmon to distinguish light levels decreased linearly, and defence reflexes were inhibited after feeding on food containing 30-80% of the LD50 concentration of DDT (12). Salmon exposed to low concentrations of DDT (1×10^{-11}) chose a water temperature 2°C lower than the water temperature selected by control salmon, while exposure to high concentrations of DDT (5×10^{-11}) led to selection of a water temperature 4°C higher than the temperature selected by control fish (13). The ability to distinguish water salinity was also affected in mosquito fish by DDT (5).

There are sufficient facts to prove that pollutants in the aquatic environment damage normal fish physiological functions, and threaten fish survival. In order to protect fish production, yet rationally utilize fish and other aquatic resources, the active mechanism of pollutants on fish must be quickly discerned.

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THE PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT TO COPPER AND AMMONIA: MECHANISMS OF TOXICITY AND AMMONIA EXCRETION

by
E.W. Taylor[1] and R.W. Wilson[2]

INTRODUCTION

Estuaries are environments in which large variations in the physical and chemical nature of the water, such as temperature, salinity and dissolved oxygen, occur both seasonally and tidally. In addition to these natural variations, the estuaries of industrialised countries frequently suffer from the man-made problems of pollution via effluent discharges from refineries, chemical plants and power stations as well as sewage outfalls. Many anadromous or catadromous fish species such as the salmonids make their periodic migrations via these polluted estuaries. The present study considered the responses of a salmonid fish, the rainbow trout, *Oncorhynchus mykiss* to ammonia and copper, two pollutants discharged into the Tees estuary in N.E. England. The assessment of the toxicity of copper and ammonia to fish has been the subject of numerous studies and reviews (Alabaster & Lloyd, 1980; Campbell & Stokes, 1985; WHO, 1986). Such studies of toxicity and the modulating effects of various water qualities, such as temperature, pH and relative hardness, are useful in predicting the toxicity of pollutants in the natural environment but we require a better understanding of the mechanisms of toxic action, including the physiological changes produced by lethal and sublethal levels of toxicants.

AMMONIA PRODUCTION AND TOXICITY IN FISH

Ammonia is one of the commoner pollutants of rivers and estuaries, and can enter these waters from a number of sources including sewage, agricultural and industrial discharges (Alabaster & Lloyd, 1980). It is also the chief metabolic waste product of fish and can therefore become a problem in the crowded conditions typical of intensive fish culture.

Ammonia is highly toxic to all vertebrates if allowed to accumulate internally (WHO, 1986) and so must either be rapidly excreted or converted to a less toxic form of waste nitrogen. Aquatic animals have the advantage that water acts as an infinite sink for ammonia and so continuous excretion over permeable outer surfaces such as gills is usually sufficient to balance the rate of production and maintain low, sub-toxic, internal levels. This is the case for most teleosts studied, which excrete the majority of their nitrogenous waste (60-95%) simply as ammonia (e.g. Smith, 1929; Sayer & Davenport, 1987), and are therefore referred to as ammoniotelic. In fish an acute increase in the ambient ammonia concentration can cause a reduction in excretion and a net gain of ammonia from the environment; the result is an accumulation of ammonia in the body which may be deleterious to the fish (Randall & Wright, 1987).

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COPPER TOXICITY

Copper is one of the more abundant transition metals and a small amount (less than 0.1/μmol l^{-1}) is normally present in both freshwater and marine environments. Elevated concentrations of copper in natural waters occur mainly through mining and smelting processes, but more recently the acidification of freshwater lakes by acid precipitation has been associated with high levels of copper due to leaching from bedrock (Spry et al., 1981). Copper is also frequently found in certain industrial wastes, which has resulted in toxic concentrations occuring in heavily populated and industrialised estuaries and coastal areas (Abdullah et al., 1972; Tort et al., 1986).

The toxicity of pollutants such as metal ions, protons and surfactants can often be explained by their surface activity, particularly on the structurally and physiologically delicate gills (Stagg & Shuttleworth, 1987; McDonald et al., 1989). The fish gill is a complex and multifunctional organ, being the principal site of gas exchange, ion regulation, acid-base balance and excretion of nitrogenous waste. Any pollutant interfering with one or more of these functions will have a potentially toxic effect on the fish. LaurΘn & McDonald (1985; 1986) showed that during short term (1^{2}-24 hours) exposure of freshwater rainbow trout to copper, toxicity resulted from the disruption of gill ionoregulatory function. Plasma Na^+ and Cl^- levels declined due to an inhibition of active ion uptake at copper concentrations as low as 0.2 μmol L^{-1}. At higher copper concentations stimulation of passive ionic effluxes also occured. The effects of copper on marine fish are less well documented. However, high levels of ambient copper (2.67 μmol L^{-1}) caused elevations in plasma Na^+ and Cl^- in the seawater-adapted flounder, *Platichthys flesus* (Stagg & Shuttleworth, 1982a).

RESULTS AND DISCUSSION

A recent study in our laboratory considered the separate and combined effects of high levels of ammonia and copper on rainbow trout. The levels of pollutants were based on those measured in the Tees estuary (NE England) and the fish was chosen to represent the salmonids (trout and salmon); migrating species which have to negotiate estuaries, are sensitive to pollution and of great commercial value.

EXPOSURE TO HIGH EXTERNAL AMMONIA

Exposure to 1 μM total ammonia (T_{Amm}) at pH 7.9 was non-toxic to trout adapted to either fresh water ($[NH_3]$ = 21.6 μM) or sea water ($[NH_3]$ = 14.3 μM). The fish survived and were able to maintain plasma ammonia lower than ambient levels despite the high permeability of the gill epithelium for NH_3 and its continuous production as a waste product of the fish's metabolism (Fig 1,a). Maintenance of a negative gradient for ammonia was accompanied by a net accumulation of non-respiratory protons (but no net pH changes) in freshwater, but a reduction in non-respiratory protons (and a subsequent alkalosis) in seawater (Fig 1,b). We interpreted these data as indicating that the fish were able to actively excrete ammonia against a diffusion gradient, most likely as the ammonium ion NH_4^+, in exchange for sodium in seawater (leading to net efflux of protons) and in exchange for an influx of protons in freshwater (Wilson and Taylor, 1992). However, more recent data suggest that, in freshwater at least, trout may not possess an active NH_4^+ exchange mechanism (Wilson et al., 1993). Instead, an acidified boundary layer at the external gill surface may sufficiently reduce the local $[NH_3]$ to allow passive diffusion of NH_3 from blood to the water, even when the $[NH_3]$ in the bulk water is much higher than that in the blood. Currently, our understanding of the mechanisms for excretion of ammonia over the gills of fish is incomplete.

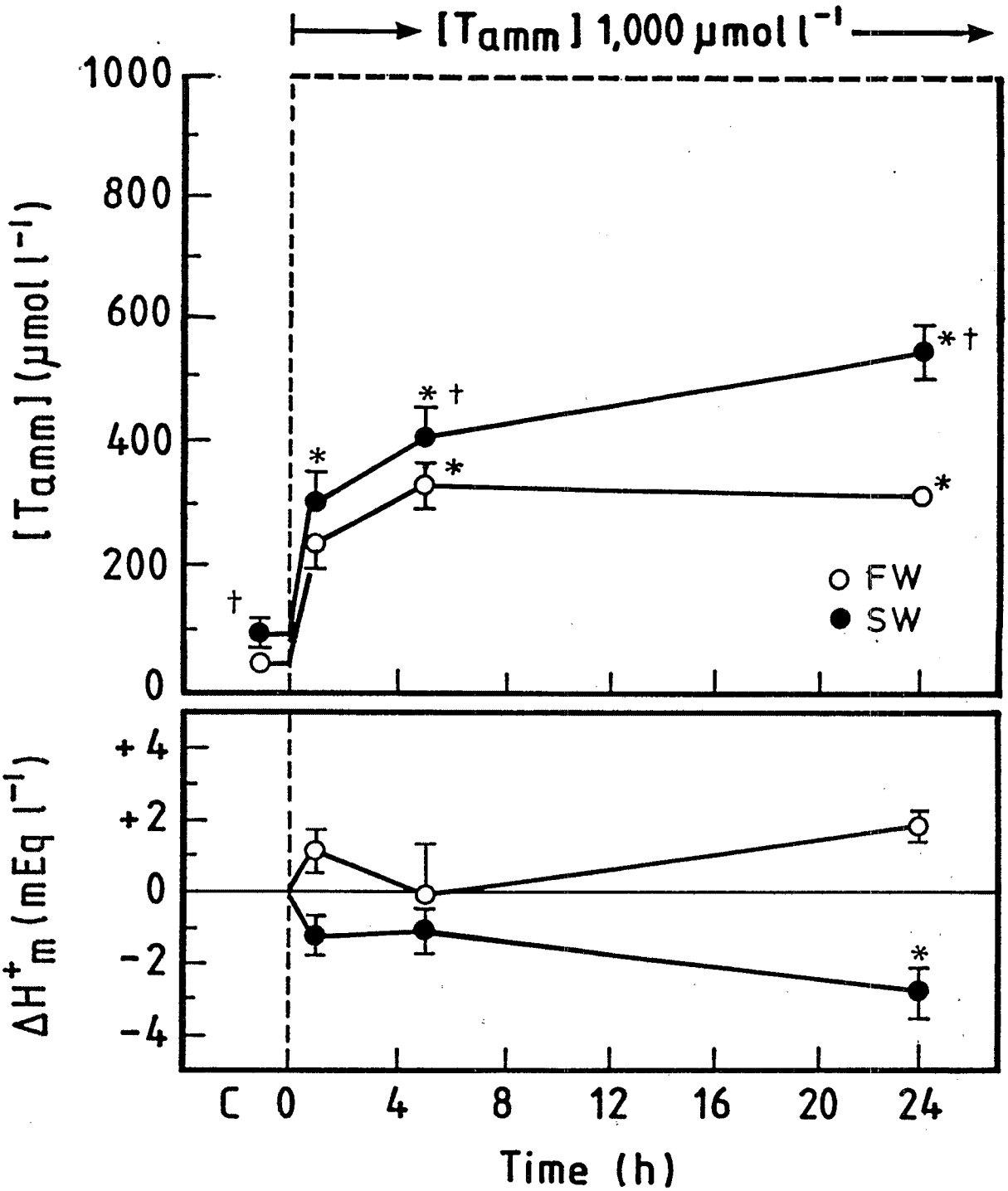

Figure 1a: Plasma total ammonia $[T_{Amm}]$ in freshwater and seawater acclimated trout prior to and during 24 hours of exposure to high external ammonia concentration (Tann 1,000 µmol l^{-1} pH 7.9, 15°C). External T_{Amm} during the control period (C) was less than 30 µmol l^{-1}. * denotes values significantly different from control values and + denotes values significantly different when compared with the corresponding value from freshwater trout ($P<0.05$; Student's unpaired t-test). Mean values are shown ±1 S.E.M., N=6 and 5 for freshwater and seawater trout, respectively.

Figure 1b: The non-respiratory acid load for freshwater and seawater trout during the control period (C) and during 24 hours of exposure to high external ammonia concentration (mean ±1 S.E.M.; for N values see Fig. 1,a) * denotes a value significantly different from the control mean within the group ($P<0.05$).

RESPONSES TO COPPER IN FRESHWATER TROUT

Exposure to copper was acutely lethal to freshwater trout. The fish died within 24 hours of exposure to 4.9 μM copper (the level measured in the Tees estuary). The physiological changes occurring during this period gave clues to the physiological effects of the toxic metal ion and these are illustrated in Figure 2 a-i. The fish's ability to ionoregulate broke down so that sodium and chloride levels in plasma progressively fell (Fig. 2a). Plasma calcium levels were regulated at first but rose precipitously at the point of death. In contrast potassium levels increased progressively (Fig. 2b), and we interpretated this as indicating potassium efflux from tissues1 secondary to plasma ionic dilution.

The disruptive effects of copper on branchial ionoregulation were likely to arise from: i) the inhibition of active ion uptake at the gills, and ii) an increase in the permeability of the gills, and consequently accelerated diffusive efflux of ions. For example, it has been demonstrated that copper inhibits branchial ATPases both in vitro (Shephard & Simkiss, 1978; Bechman & Zaugg, 1988), in isolated gill or opercular membrane preparations (Stagg & Shuttleworth, 1982b; Crespo & Karnaky, 1983), and in vivo in freshwater rainbow trout (Laurén & McDonald, 1987b). The inhibition of active NaCl uptake is most likely to be due to the high affinity of copper for sulphydryl groups (-SH) on transport enzymes such as Na^+, K^+-ATPase located in the gill epithelium (Shaw & Grushkin, 1964; Stagg & Shuttleworth, 1982b).

The increase in gill permeability mentioned above is probably related to the ability of copper to displace calcium from biological ligands (Nieboer & Richardson, 1980). Since calcium is known to be important in controlling the integrity and permeability of the branchial epithelium in fishes (Potts & Fleming, 1971; Cuthbert & Maetz, 1972; McDonald & Rogano, 1986), Laurén & McDonald (1985) proposed that the stimulation of ionic effluxes by copper may be a result of increased ionic permeability of the gill, secondary to the displacement of Ca^{++} ions from paracellular tight junctions. The toxicity of copper is therefore dependant on the ambient calcium levels during exposure (e.g. Alabaster & Lloyd, 1980; Reid & McDonald, 1988). At low enough concentrations of copper, ionoregulatory disturbances can occur without apparent physical damage to the gills (Laurén and McDonald, 1985). However, at higher levels of copper the inhibition of active ion pumps and displacement of intercellular and membrane-bound calcium may lead to a breakdown in branchial cell volume control and epithelial organisation. Indeed, in our freshwater trout exposed to 4.9 μM copper we observed severe gill histopathologies which included cell adhesion failure, swelling, and pillar cell detachment leading to numerous haematomas (red cells pooling in regions of lamellae).

The structural disruption of the gill epithelium in freshwater trout exposed to copper had drastic effects on respiratory gas exchange. The fish became progressively hypoxic and as a consequence haemoglobin oxygenation was reduced so that oxygen supply to tissues was reduced (Fig. 2,c). Carbon dioxide accumulated (Fig. 2,d) and the fish switched to anaerobic metabolism so that lactic acid levels in the blood rose (Fig. 2,e). The blood became progressively acidotic (Fig. 2,f) due to a combined respiratory (CO_2) and metabolic (lactic acid) acidosis; this in turn caused a rightward Bohr shift on the fish's haemoglobin and oxygen uptake and transport were further curtailed. Physical damage to the gills (and reduced diffusion of NH_3) may also be responsible for the progressive accumulation of plasma ammonia (Fig. 2,g). However, inhibition of active ammonium ion transport NH_4^+/Na^+ or H^+ exchange) may also contribute to this effect. As part of its attempt to compensate for these changes by increasing oxygen transport the fish exhibited an increase in haematocrit to values as high as 50% (Fig. 2,h). This was partially due to a reduced plasma volume, partly due to increased red cell numbers (probably released from the spleen) and partly due to red cell swelling. These changes are likely to have resulted in an increase in apparent blood viscosity. Simultaneously the fish

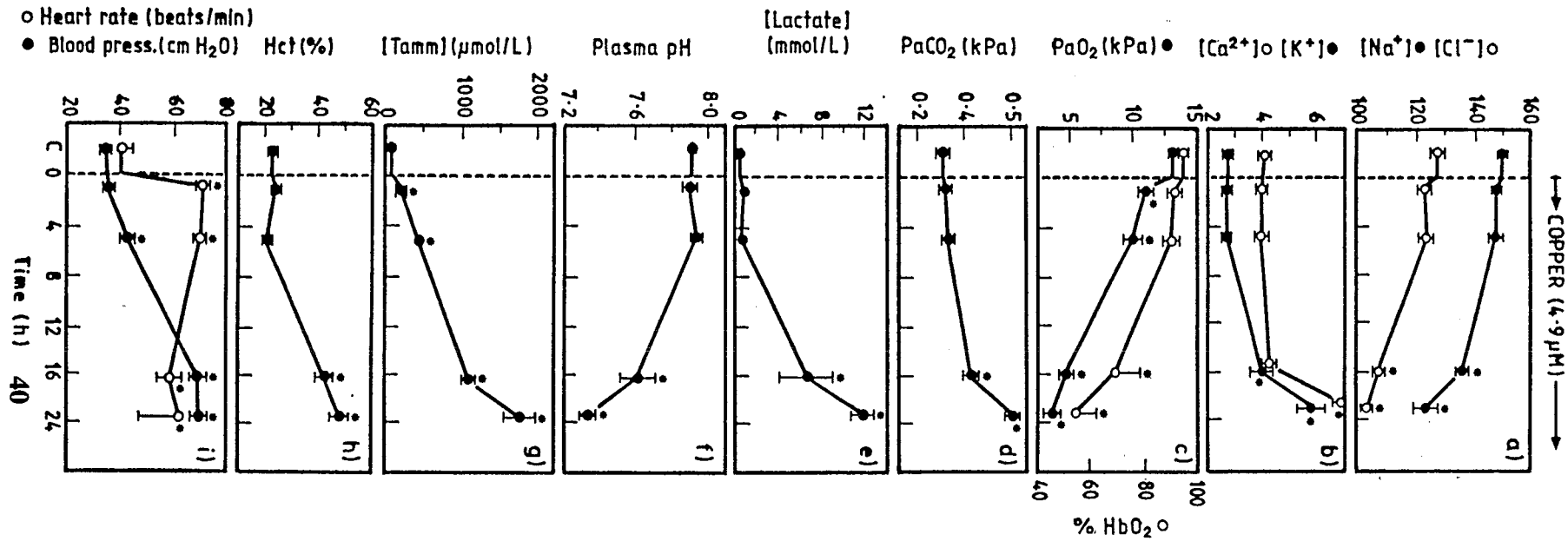

Figure 2: Changes in measured plasma ion, blood gas and metabolite levels and cardiovascular variables in freshwater rainbow trout exposed to copper (4.9 µmol l^{-1}) at 15°C. Values are means ±S.E.M. (N varies from 4 to 13) * denotes values significantly different from the control mean (C) for each variable. For a description see text.

showed increased heart rate and blood pressure (Fig. 2,i), and together with the changes in haematocrit these indicate that circulating catecholamines were elevated. The combined effect of these responses was probably cardiac failure leading to death. Similar trauma have been described from fish exposed to very acid waters (the product of acid-rain) (Milligan & Wood, 1982). The fish dies because its integrated physiological responses to the toxic environment culminate in its destruction. The breakdown appears to start with disruption of ionoregulation which in freshwater is vital to survival because the ionic gradients between the fish and its environment are very large.

RESPONSES OF SEAWATER TROUT TO COPPER

We have seen that acute exposure of freshwater trout to 4.9 µmol copper l^{-1} at pH 7.9 caused 100% mortality within 24 hours, as a result of severe ionoregulatory and respiratory disturbances. In contrast, trout acclimated to full-strength sea water (external [NaCl] three- to fourfold higher than blood [NaCl]) and exposed to 6.3 µmol copper l^{-1} at the same pH for 24h, exhibited no significant change in plasma ion concentrations, no respiratory problems and survived with apparently undamaged gill lamellae. It is clear that copper toxicity, and the physiological responses during acute exposure, are greatly reduced at high salinities. These results were unexpected; (i.e. our prediction was that an ionoregulatory inhibitor such as copper would cause a net increase in plasma ions in fish maintained in a hypersaline environment).

Thus, seawater-adapted teleosts are less sensitive to copper than freshwater species as previously described by Eisler & Gardner (1973); Voyer (1975); Taylor et al. (1985). This may be a function of physicochemical factors such as higher calcium levels, resulting in greater competition with copper for membrane binding sites (Laurén & McDonald, 1985; Reid & McDonald, 1988), or high carbonate alkalinity and the presence of chelating agents which can reduce the toxic forms of copper by complexation (Chakoumakos et al., 1979; Miller & Mackay, 1980). Alternatively, the lower toxicity could be related to physiological differences in the mechanisms of branchial salt transport and the location of the key transepithelial ion transporting enzymes in seawater acclimated fish.

Environmental Ca^{2+} and Mg^{2+} are thought to play an important role in controlling the branchial permeability to Na^+ in both freshwater and seawater teleosts (Potts and Fleming 1971; Bornancin et al. 1972; Isaia and Masoni 1976). Copper is thought to displace surface Ca^{2+} in freshwater trout causing increased gill permeability Laurén and McDonald (1985). However, the ambient [Ca^{2+}] in seawater is more than 26 fold higher than in fresh water which would obviously provide more competition for external binding sites with copper. This may explain the differential responses found in seawater trout in the present study. If it is assumed that the basolateral Na^+K^+ -ATPase enzyme is unaffected in 24h (due to the slow intracellular uptake of copper), then any ionoregulatory disturbances can only be attributed to changes in the passive diffusion of Na^+ and Cl^- which is Ca^{2-} (and Mg^{2+}) dependent. In seawater the high external [Ca^{2+}] may simple out-compete the external copper, resulting in no ionoregulatory disturbances despite the large Na^+ and Cl^- gradients across the gills. In contrast, in freshwater trout osmotic and ionic gradients are large and calcium levels relatively low. Addition of copper may displace much of the calcium leading to spatial disruption of the gill epithelium and affecting specific ATPases (see Fig. 3). However, to fully test this hypothesis would require the use of isotopic unidirectional ion flux measurements and synthetic seawater media with controlled Ca^{2+} and Mg^{2+} levels to determine whether differences in [Ca^{2+}] and [Mg^{2+}] are in fact the determining factors in the differential responses to copper exposure seen in trout acclimated to freshwater and seawater. Longer term exposure of fish to copper results in uptake over the gills or via the gut and subsequent damage to internal organs such as the liver (Baker 1969). Consequently, basolateral ion regulation may be affected by long term exposure.

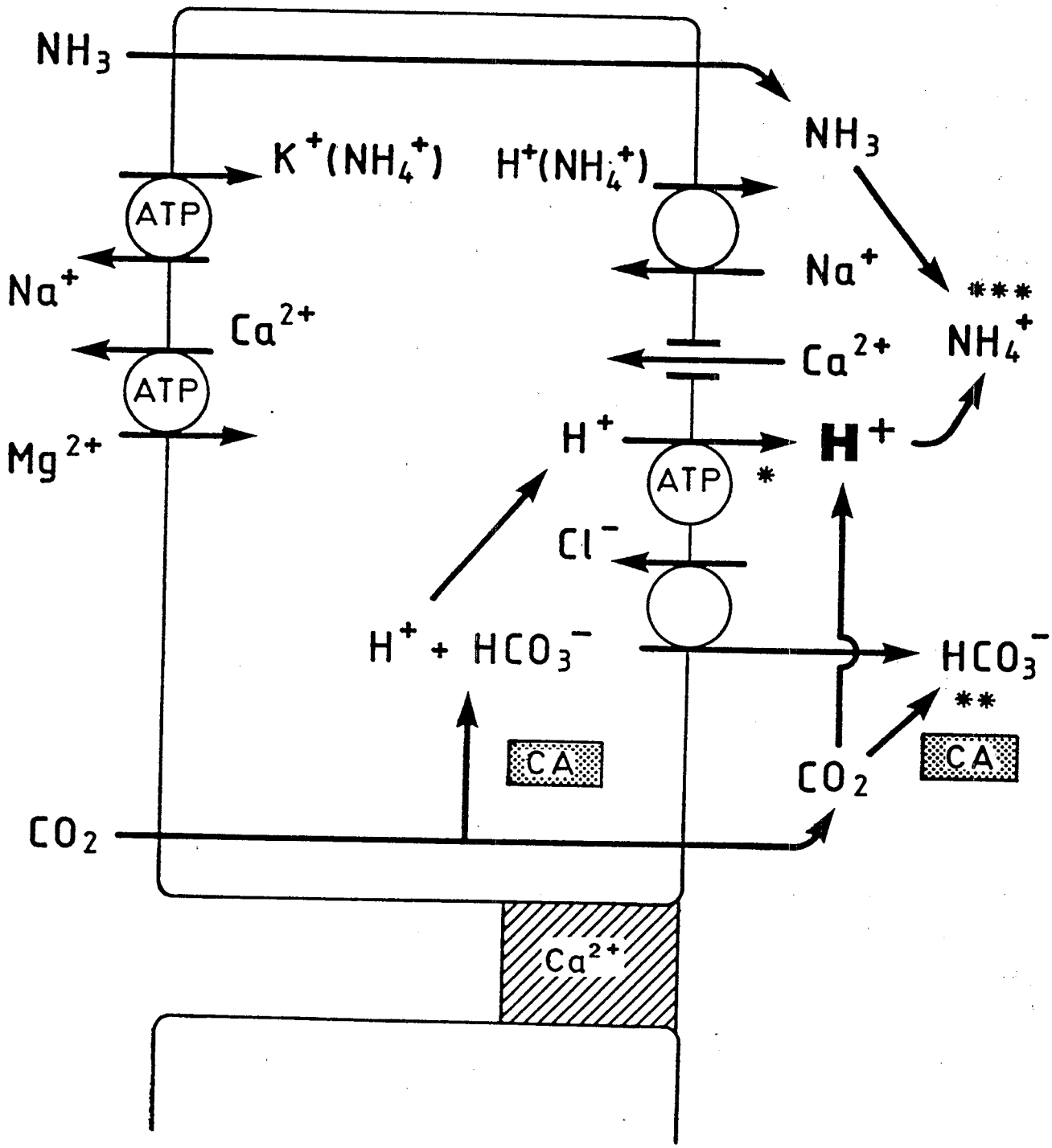

Figure 3: Plasma total ammonia concentration in the arterial blood of the seawater acclimated rainbow trout exposed to 6.3 μmol^{-1} copper l^{-1} at 12°C. Points represent mean values ± 1 S.E.M.) during the pre-exposure period and following 1, 5 and 24 hours of exposure at pH 7.9 * denotes mean values significantly different from the pre-exposure mean (C) ($P<0.05$; Student's paired t-test).

Exposure of freshwater trout to copper caused a large and progressive increase in plasma ammonia levels (Fig. 2,g). Seawater trout similarly experienced elevated ammonia levels when exposed to copper but they reached a plateau after 4 hours at levels half that reached by 4 hours and one tenth of the value after 19 hours in freshwater trout (Fig. 4). Copper exposure caused no increase in O2 consumption in the seawater trout. If the normal relationship between $\dot{M}O_2$ and $\dot{M}NH_3$ (Brett and Zala 1975; Heisler 1984) remained constant throughout exposure to copper, then the elevation of plasma $[T_{Amm}]$ cannot be explained by any change in the rate of endogenous ammonia production. Under normal pre-exposure conditions branchial ammonia excretion was probably the result of a combination of NH_3 diffusion and apical Na^+/NH_4^+ exchange (Wright and Wood 1985). It seems unlikely that any changes in the gill NH_3 diffusion capacity occurred since no major changes in the other blood gas tensions P_aO_2 or P_aCO_2 were observed. This argues for an inhibition of apical Na^+/NH_4^+ exchange by copper as suggested by LaurΘn and McDonald (1985) in freshwater rainbow trout. The stabilisation of plasma $[T_{Amm}]$ after 5 hours in seawater trout could then be explained if it is assumed that the rate of branchial NH_3 clearance by diffusion will again match the endogenous rate of NH_3 production once a new, larger PNH_3 gradient over the relatively undamaged gill lamellae is established. In freshwater the gills are structurally damaged and ionoregulation breaks down after copper exposure (Fig. 2), so that apical Na^+/NH_4^+ exchange and NH_3 diffusion are likely to be impaired. Consequently, ammonia levels continue to rise rather than reaching a plateau.

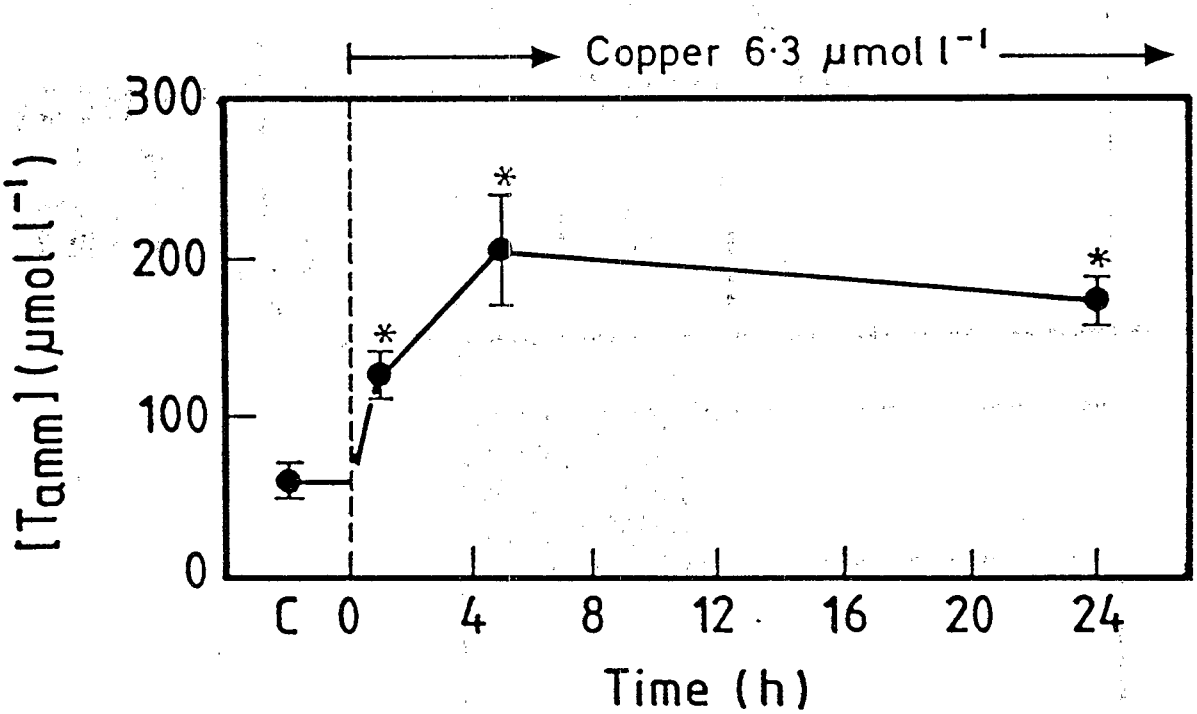

Figure 4: Schematic diagram illustrating the possible routes for NH_3 and CO_2 excretion, ionoregulation and acid-base regulation of a generalised gill epithelial cell from a freshwater trout. As well as $Na^+/K^+(NH_4^+)$ and Ca^{2+}/Mg^{2+} exchanges on the basolateral membrane and $Na^+/H^+(NH_4^+)$ and Cl^-/HCO_3^- exchangers on the apical membrane the diagram includes the proton pump (*), additional acidification of the boundary layer by hydration of CO_2 catalysed by carbonic anhydrase (CA) ** and ammonia trapping in the acid boundary layer, *** (for additional description see text).

CONCLUSION

Exposure of trout to elevated levels of ammonia or copper in freshwater or seawater has provided useful indicators of the mechanisms of ammonia exchange over the fish gill. Trout are able to maintain ammonia excretion against high external levels either by active excretion of NH_4^+ (in exchange for H^+ or Na^+) or by excretion of NH_3 into an acid boundary layer (sustained by an electrogenic H^+ pump (Lin & Randall, 1991) and/or by local hydration of CO_2, catalysed by carbonic anhydrase (Randall & Wright, 1987)). These processes are summarised on Fig. 4. Copper, because it inhibits branchial ATPases and competes with calcium for ion channels and ion binding sites, disrupts ionoregulation and in freshwater can cause breakdown of cell homeostasis and cell adhesion in gill epithelia (cf Fig. 4). In seawater calcium levels are relatively high so that elevated levels of copper are less likely to cause physical damage to the gill lamellae and ionoregulation is apparently unaffected. However, ammonia accumulates in the plasma, indicating that some apical transport processes involved in ammonia excretion (e.g. Na^+/NH_4^+ exchange) are affected by copper; whereas, the principle basolateral mechanisms involved in sodium and chloride regulation are apparently unaffected . In support of this, we observed a significant increase in plasma [HCO_3^-)] in the seawater trout exposed to copper, indicating inhibition of apical Cl^-/HCO_3^- exchange in addition to apical Na^+/NH_4^+ exchange.

The breakdown of the gill epithelia in freshwater trout, in the presence of copper, compromised ionoregulation and respiratory gas exchange. The fish became markedly hypoxic and hypercapnic and accumulated lactic acid; resulting in a combined respiratory and metabolic acidosis. As part of a physiological response to these accumulating problems, probably mediated by elevation of circulating catecholamines, the fish showed greatly increased haematocrit, heart rate and blood pressure, resulting in death from heart failure.

ACKNOWLEDGMENTS

Ted Taylor's attendance at the Symposium was funded by the US Environmental Protection Agency and he is grateful both to the EPA, the staff and students and in particular Professor Hongjun Jin of Nanjing University, PRC for organising this successful international meeting.

Rod Wilson was supported by a studentship from the Science and Engineering Research Council and the research was conducted in cooperation with Zeneca Environmental Research laboratories, Brixham, Devon, UK. The authors are grateful to Sheila Craggs for her patient processing of the manuscript.

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ECOTOXICOLOGICAL STUDY OF FENPROPATHRIN ON SOME COMMON CHINESE AQUATIC ORGANISMS AND EVALUATION OF ITS EFFECT ON THE AQUATIC ECOSYSTEM

Yiwei Yin[1], Zhaohui Wang[1], Yongyuan Zhany[2], Ying Xu[2], Lihong Xu[2], Wengzhong Wu[2] and Guoshong Chen[2]

ABSTRACT

Fenpropathrin is acutely toxic to some common Chinese aquatic organisms. The toxic mechanisms of this pesticide in fish involve inhibition of Na^+,K^+-ATPase, modification of the Na^+ channel in the nervous system, perturbation of ion metabolism, and ultrastructural injury of the fish gill. However, this pesticide does not accumulate persistently in the body of fish. Most of it is rapidly absorbed by sediment after entering a body of water, and the residue in the aquatic phase degrades within a few days. The pesticide contained in the sediment also completely degrades within several days. While the toxic effect of fenpropathrin is not persistent, use of this pesticide within any area containing fish must be carefully monitored to avoid acute poisoning.

INTRODUCTION

Fenpropathrin (a-cyano-3-phenoxy-henzyl-2,2,3,3-tetramethyl-cyclopropane-propane-carboxylic ether) is a new synthetic analogue of pyrethroids that was synthesized in China in 1991 and manufactured under the commercial name of Meothrin. Fenpropathrin is not very toxic to mammals (including humans), but are highly toxic to a wide variety of micro-organisms at a relatively low dose. The toxicity of fenpropathrin to aquatic organisms, especially fish, is essentially unknown. Coats and O'Donnell-Jeffery (1979) have reported that the octanol/water partition coefficient of fenpropathrin is 1070 and the 24 hour LC50 is 76.7 ppb in Rainbow trout, which is considered to be of high toxicity. The half-life of fenpropathrin in soil under aerobic and anaerobic conditions at 25 °C is 10 and 25 weeks, respectively (Su and Fan, 1989). It is not known whether the degradation pattern would be similar in the benthic environment of water bodies. Since fenpropathrin is a highly toxic agent, utilization of this pesticide could be a significant problem to fisheries in China. Therefore, this study examined the ecotoxicological effects of fenpropathrin on fish and other aquatic organisms, and its degradation in both the aquatic and benthic phase.

METHODS, RESULTS AND DISCUSSION

ACUTE TOXICITY OF FENPROPATHRIN TO AQUATIC ORGANISMS

While most pyrethroids are considered to be highly toxic to aquatic organisms, some are not, making it necessary to precisely determine the toxic level of fenpropathrin to some common Chinese organisms. Fenpropathrin was dissolved in acetone to form a stock solution, and appropriate volumes of stock solution were added to dechlorinated tap water to make up the required concentrations. Since

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preliminary studies showed that the lethal concentration of fenpropathrin was lower than its soluble limit (340 ppb), no emulsifiers or solvents were used in these tests. Acute toxicity tests were carried out on the following representative Chinese aquatic organisms; Silver carp (*Hypophthalmichthys molitrix*), Grass fish (*Ctenopharyndon idell*), Mosquito fish (*Gambusia affinis*), *Daphnia magna* H.B., *Daphnia carinata* and *Chronomus* sp.. The results of these tests are given in Table 1. It is clear that fenpropathrin is highly toxic to aquatic organisms, and extreme caution in its use around fisheries areas is highly recommended.

Table 1. Acute toxicity of fenpropathrin on some common Chinese aquatic organisms.

Species	LC50 or EC50 (ppb)	Time (hr)	Temperature (°C)
Mosquito fish (*Gambusia affinis*)	1.8	96	23-25
Silver carp (*Hypophthalmichthys molitrix*)	1.8	96	25-27
Grass fish (*Ctenopharyndon idell*)	2.5	96	16.5-17.5
Daphnia magna HB	1.8	48	20±5
Daphnia carinata	0.7	48	20±5
Chronomus sp.	5.5	48	22±5

TOXICOLOGICAL STUDY OF FENPROPATHRIN IN FISH

While no aquatic toxicological studies of fenpropathrin have been conducted, pyrethroids in general appear to effect fish Na^+ channel activity (Khan, 1983; Cole et al., 1984; Laurence and Caside, 1983), ATPase activity (Clark and Matsumura, 1982), ion metabolism (Bradbury, 1989) and gill structure. The toxicological studies outlined below investigate these four perturbations.

A. Effect of Fenpropathrin on ATPase Activity in the Gill and Kidney of Silver Carp

Silver carp were exposed to fenpropathrin in vivo and the gills and kidneys removed for assay. Gills and kidneys were also removed from unexposed fish and then immersed in fenpropathrin in vitro. Isolation of gill and kidney tissues, preparation of tissue homogenates, and measurement of ATPase activity were done according to the method of Matsumura et al. (1982) and Xu et al. (1987). Results showed that Na^+,K^+-ATPase and total ATPase activities were higher in gill homogenates than in kidneys (results not shown). The inhibition of ATPase in gill and kidney tissue of Silver carp after exposure to 10-3 M fenpropathrin is shown in Table 2. At a fenpropathrin concentration of 10-3 M, inhibition of Na^+,K^+-ATPase in gill and kidney homogenates was 43.9% and 29.2%, respectively, but inhibition of total ATPase in the two homogenates was only 23.2% and 16.8%, respectively. The inhibition of ATPase in the two homogenates was dose-dependent, and inhibition was significant above a concentration of 10-6 M.

Table 2. Inhibition of ATPase activity in gill and kidney tissue of Silver carp after exposure to 10-3 M fenpropathrin

	Gill (% inhibition)	Kidney (% inhibition)
Total ATPase	23.0 (P<0.01)	16.8 (P<0.05)
Na^+,K^+-ATPase	43.9 (P<0.10)	29.2 (P<0.01)

The inhibition of Na^+,K^+-ATPase activity in gill homogenates of Silver carp exposed to a fenpropathrin concentration near the LC50 for 24 hr is shown in Table 3. Table 4 shows inhibition of total ATPase and Na^+,K^+-ATPase in gill homogenates of Silver carp after exposure to fenpropathrin at a concentration well above the LC50 (5 ppb) for 5 hr. The results indicate that similar inhibition of Na^+,K^+-ATPase activity (approximately 40%) occurred when fish were exposed to either 1 ppb for 24 hr or 5 ppb for 5 hr. Since fish mortality occurred while Na^+,K^+-ATPase activity was significantly inhibited, this suggests that inhibition Na^+,K^+-ATPase activity could be one of the causes of death.

Table 3. Gill Na^+,K^+-ATPase activity of Silver carp in vivo when exposed to different concentrations of fenpropathrin for different periods of time

Exposure		Control	0.5 ppb	0.75 ppb	1.0 ppb
24 hr	Activity (µM Pi/µg/hr)	4.03±0.26	4.01±0.33	4.04±0.25	2.52±0.11
	% inhibition				37.47**
	% mortality		0	0	37.5
48 hr	Activity (µM Pi/µg/hr)	4.07±0.26	4.02±0.30	3.62±0.24	2.45±0.19
	% inhibition			11.07	39.80**
	% mortality		0	12.3	41.8
72 hr	Activity (µM Pi/µg/hr)	4.15±0.12	3.86±0.14	3.61±0.32	2.50±0.24
	% inhibition			13.01*	39.76**
	% mortality		6.89	15.6	46.3
96 hr	Activity (µM Pi/µg/hr)	4.02±0.21	3.66±0.17	3.22±0.26	2.32±0.20
	% inhibition		8.35	19.90**	42.29**
	% mortality		8.7	29.5	47.5

n=4; t-test; results expressed as average ± standard deviation

Table 4. ATPase activity in gill tissue of Silver carp after exposure to 5 ppb fenpropathrin for 5 hr.

		Control	Exposed
Total ATPase	activity*	9.18±0.39	7.15±0.46
	% inhibition		22.11**
Na^+,K^+-ATPase	activity*	4.10±0.20	2.29±0.29
	% inhibition		44.15**
Mortality (%)			55.5

* μM Pi/μg/hr, mean±SD; ** t-test, significantly different

B. Effect of Fenpropathrin on the Na^+ Concentration in the Blood of Grass Carp

Urinary Na^+ and K^+ concentration and excretion rates are significantly elevated in trout after exposure to fenvalerate (Bradbury et al., 1987), which may be associated with the effect of fenvalerate on ion metabolism and osmoregulation (Symonik, 1989). We therefore examined the effect of fenpropathrin on the Na^+ concentration in the blood of Grass carp.
Grass carp fingerlings (14 cm in length) were exposed to fenpropathrin at concentrations between 1-3 ppb. The test solution was changed every 2 hours. Blood was sampled from the gill aorta, and weighed and nitrated. The blood Na^+ concentration was measured with an ILC-I liquid chromatograph (Waters Company).

Blood Na^+ concentration decreased approximately 25% after several hours of exposure (Fig. 1) and decreased more rapidly in the higher concentrations of fenpropathrin (3 ppb). The fall in Na^+ concentration in the blood may be associated with disturbances in ion metabolism and osmoregulation, and could be involved in the lethal action of fenpropathrin on fish.

C. Effect of Fenpropathrin on Na^+ Channel Activity of Cardiac Muscle of Silver Carp

Pyrethroids have been reported to increase transmembrane sodium influx by delaying inactivation of the sodium channel (Narahashi et al., 1967). Decamethrin caused a positive inotropic effect on isolated guinea pig atrial muscle which was abolished with tetrodotoxin, implying that the effect of decamethrin is due to modification of the sodium channel (Berlin et al., 1984). The current study was undertaken to investigate the inotropic action of fenpropathrin on the isolated heart of Silver carp.

Cardiac muscle function was studied using the method of Berlin et al. (1984) and Li et al., 1989. The heart was removed from the fish and a cannula inserted into the liver vein to perfuse the heart. The perfusate was pumped out through the aorta. The heart was equilibrated in physiological buffer solution for 20-30 minutes and then 2 ml fenpropathrin (10-4 - 10-6 M) dissolved in 10% ethanol was injected into the cannula. The results are shown in Fig. 2. Fenpropathrin caused a dose-dependent (10-4 M to 10-6 M) increase in heart rate which peaked in 8-10 sec and then recovered to resting levels. When 1 ml 10-7 M tetrodotoxin was injected during the tachycardia induced by 2 ml 10-5 M fenpropathrin, the heart stopped beating for several seconds but then regained its rhythm. Therefore, the positive inotropic effect of fenpropathrin on cardiac muscle was blocked by tetrodotoxin. Since tetrodotoxin blocks the increase in Na^+ influx in nerve endings (Berlin et al.,

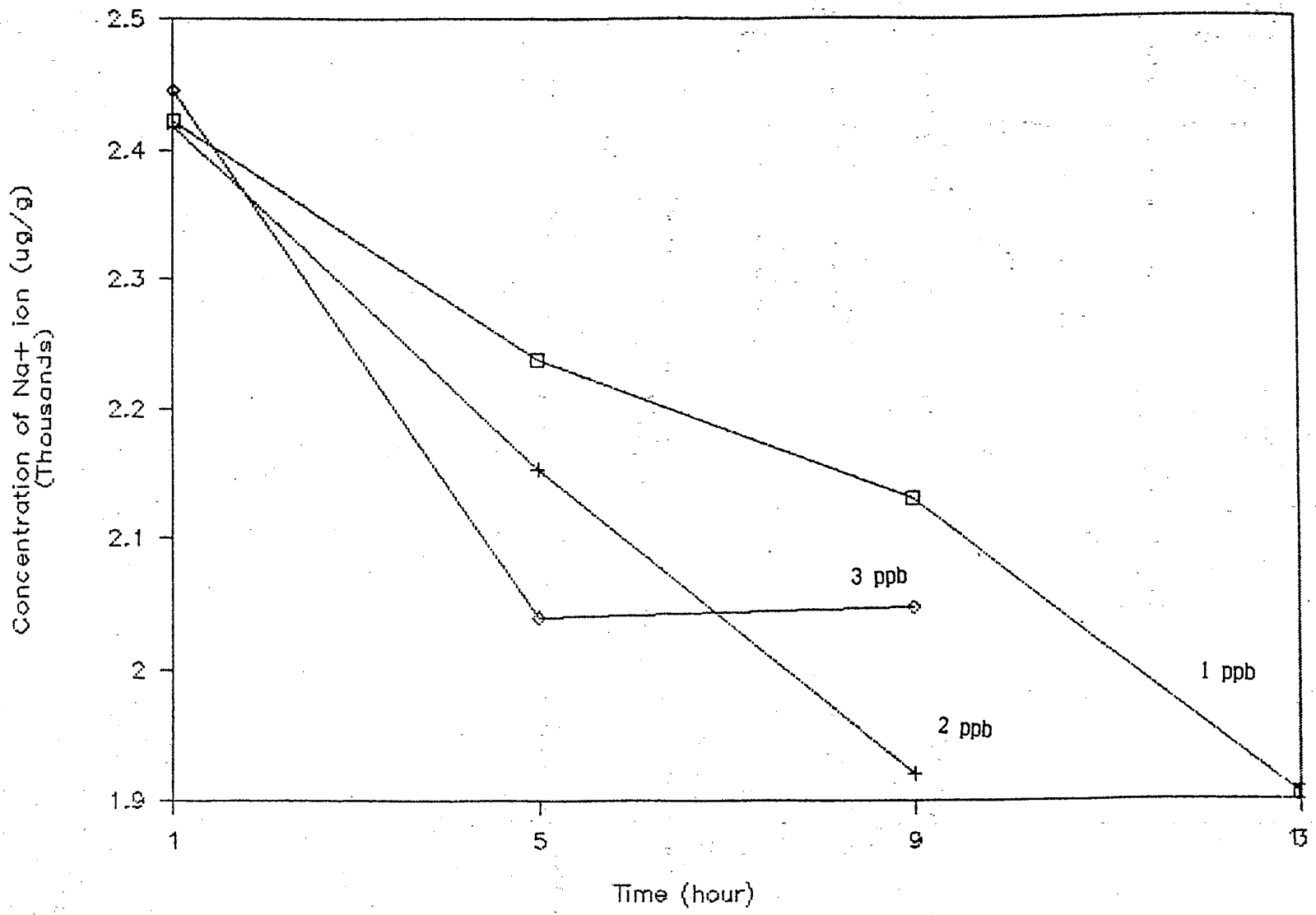


Figure 1. Concentration of Na^+ ion in the blood of grass carp after exposure to fenopropathrin.

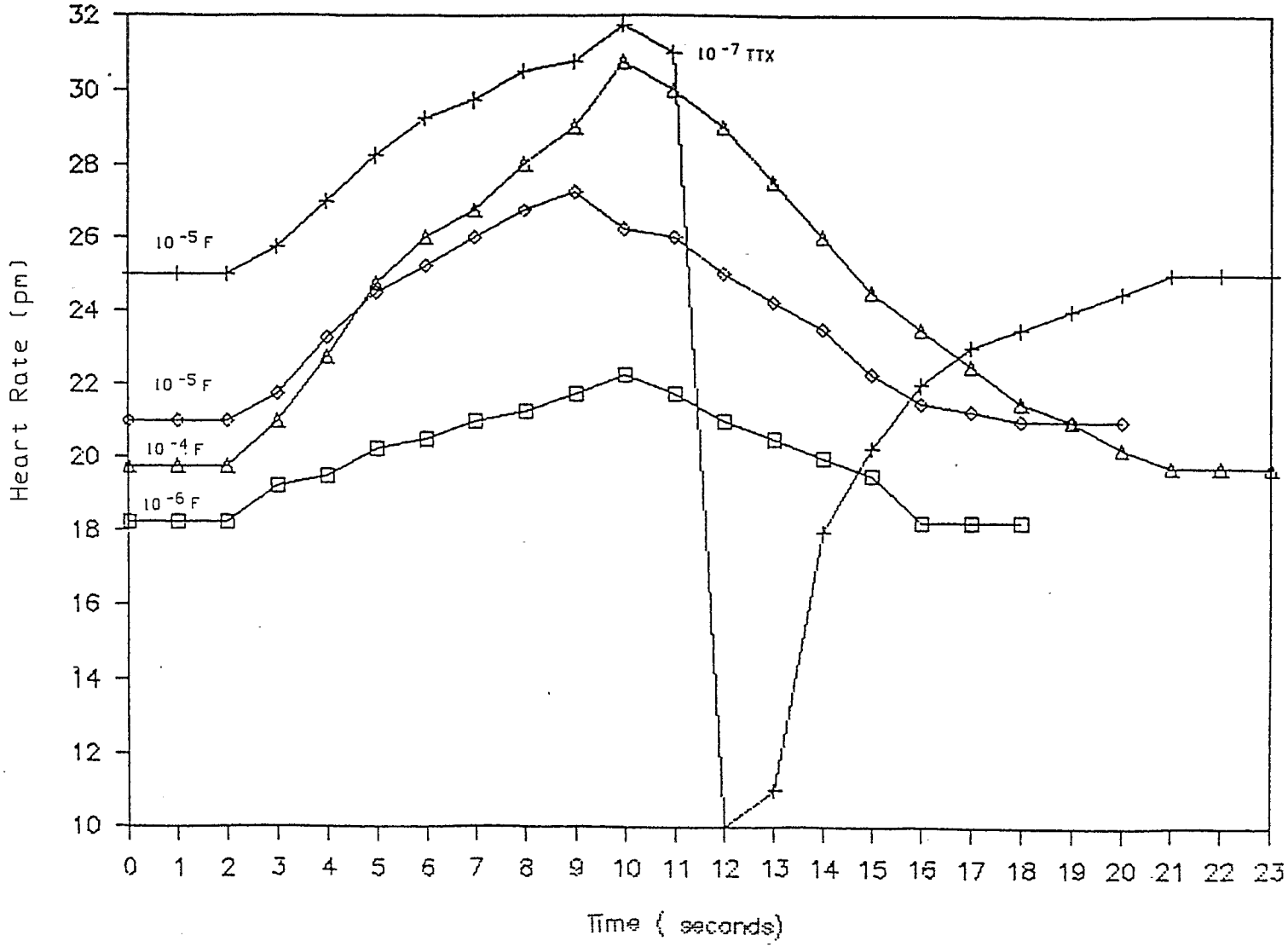


Figure 2. Effect of fenpropathrin and tetra dotoxia on heart rate of silver carp.

1984), modification of the sodium channel and imbalances in ion metabolism may also be involved in the lethal effect of fenpropathrin on fish.

D. Effect of Fenpropathrin on Gill Morphology of Silver Carp

Kumaragura et al. (1982) and Bradbury et al. (1987) have reported that fenvalerate may damage gill structure. Damage to the gill surface was characterized by local inflammation, increased mucous secretion and separation of adjacent lamellae from the connective tissue. Numerous necrotic cells were also found in these separated interlamella zones. These histological changes may alter respiratory function in addition to ion metabolism. The following study evaluated the effect of fenpropathrin on gill ultrastructure by histopathological examination of Silver carp gill tissue.. Silver carp fingerlings (10-15 cm in length) were exposed to 0.5, 0.75 and 1.0 ppb fenpropathrin for 24-96 hours. Another group was exposed to 5.0 ppb fenpropathrin for 5 hours. Gill tissue was removed after 5, 24, and 72 hr, , fixed in Bouins' fixative, sectioned in paraffin, and stained with H.E.. Results are shown in Figs. 3-6. Histopathological changes in the gill showed that the degree of damage was directly related to the duration of exposure and the concentration of fenpropathrin the fish were exposed to. The gill sections showed that lamellae were swollen, hyperplasic, and fused together. Mucous secretion was increased and some blood cells were free from the lamellae. Because of the importance of the gills in respiration, absorption, secretion and excretion in fish, injury to the gill may contribute to fish mortality. These changes also provide some histological basis for the functional alterations discussed above.

ACCUMULATION AND ELIMINATION OF FENPROPATHRIN IN FISH

There is little data on the toxicokinetics of fenpropathrin to date, although it has been reported that the octanol/water partition coefficient of fenpropathrin is 1070 (Coats and O'Donnell-Jeffery, 1979). It was therefore necessary to determine some toxicokinetic parameters of the pesticide in order to evaluate its effect on the aquatic ecosystem.

A routine shaking method (Coats and O'Donnell-Jeffery, 1979) was used to determine the partition coefficient of fenpropathrin, and the actual biological concentration factor (BCF) and elimination of the pesticide in the body of Silver carp were also determined. The average weight of the fingerlings was 10 g. The quantity of fenpropathrin was measured with a Hewlett-Packard 5890 A gas chromatograph (Wengzhong and Ying, unpublished).

The octanol/water partition coefficient of fenpropathrin was 1096.48, which corresponds closely to previously published results (Coats and O'Donnell-Jeffery, 1979). Accumulation and elimination of fenpropathrin in Silver carp carcasses are shown in Figs. 7 and 8. The concentration of fenpropathrin which accumulated in Silver carp reached a maximum after approximately 8 hours exposure (Fig. 7), at which time the BCF was 411.3. The fenpropathrin concentration then began to decrease. The half-life of fenpropathrin (Fig. 8) was 30.14 hours, and over 70% of the pesticide was eliminated after 60 hours. Accumulation therefore occurred much faster than elimination. The octanol/water partition coefficient of most pyrethroids ranges from 104 to 107 M, and the BCF lies generally in the range of several thousand in fish (Coats et al., 1989). Fenpropathrin has a much lower partition coefficient and BCF than most pyrethroids and can be eliminated rapidly, suggesting that the problem of bioconcentration may not be serious.

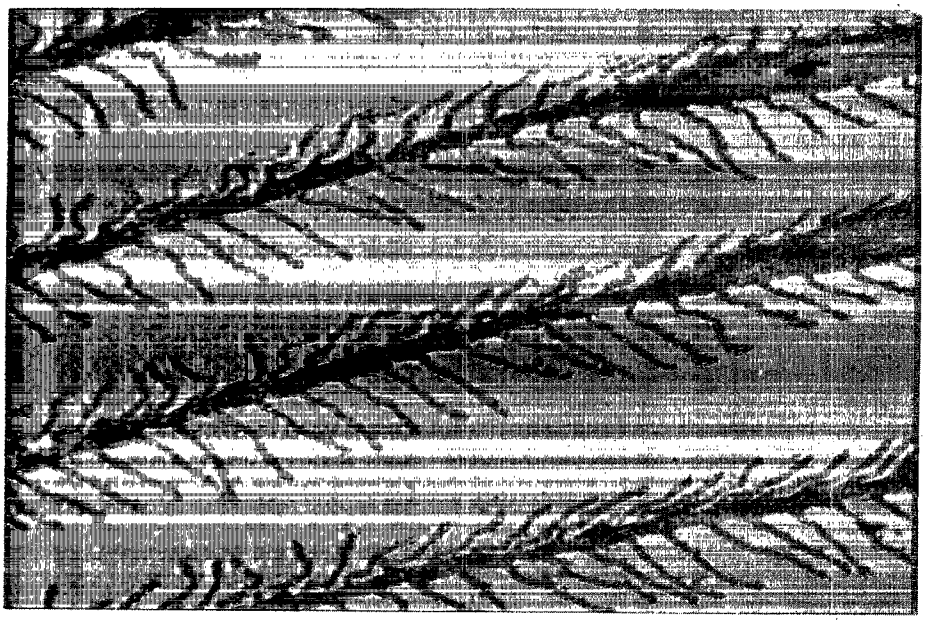


Figure 3. Control (x 100).

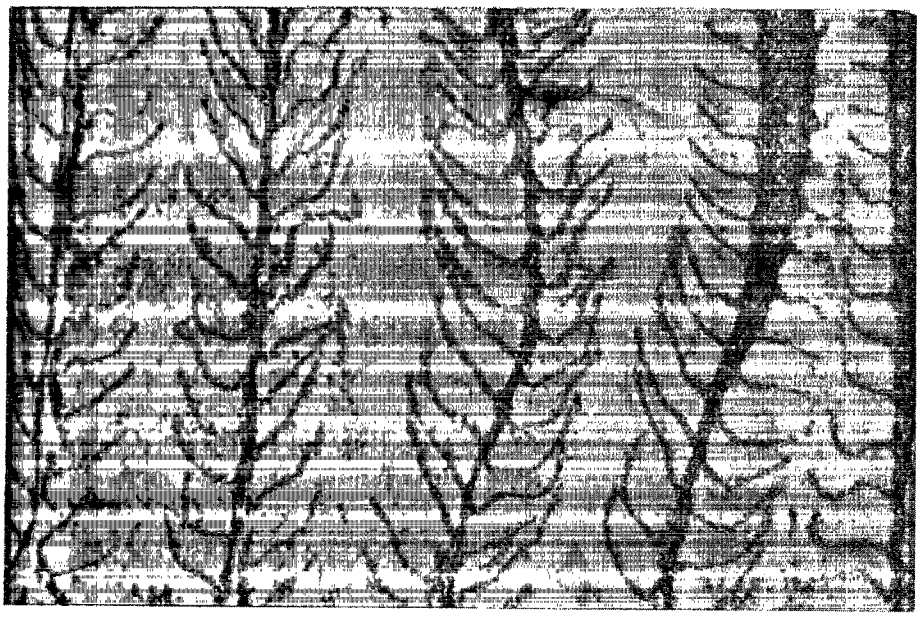


Figure 4. 5 ppb (x 100) after 5 hours exposed.

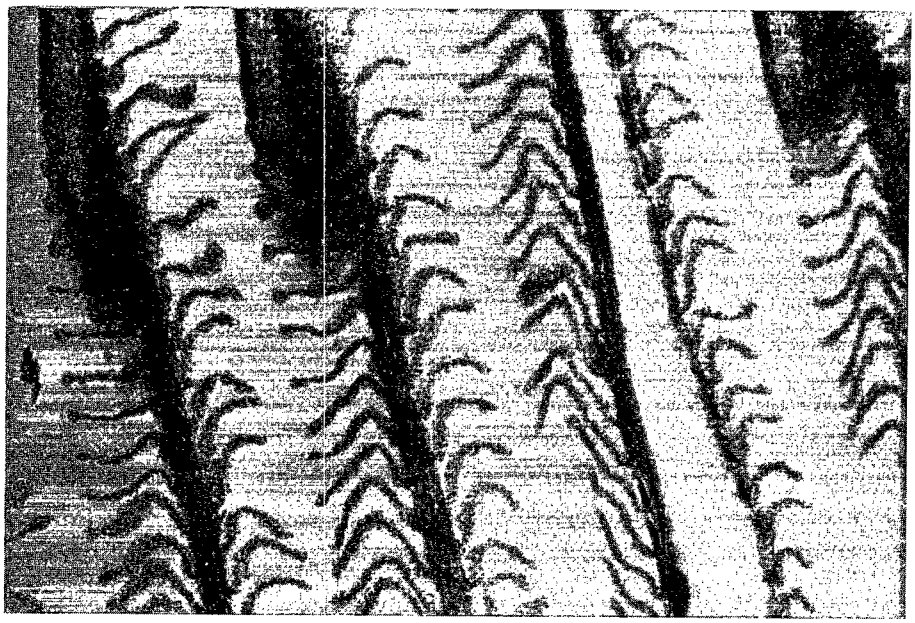


Figure 5. 0.75 ppb (x 100) after 24 hours exposed.

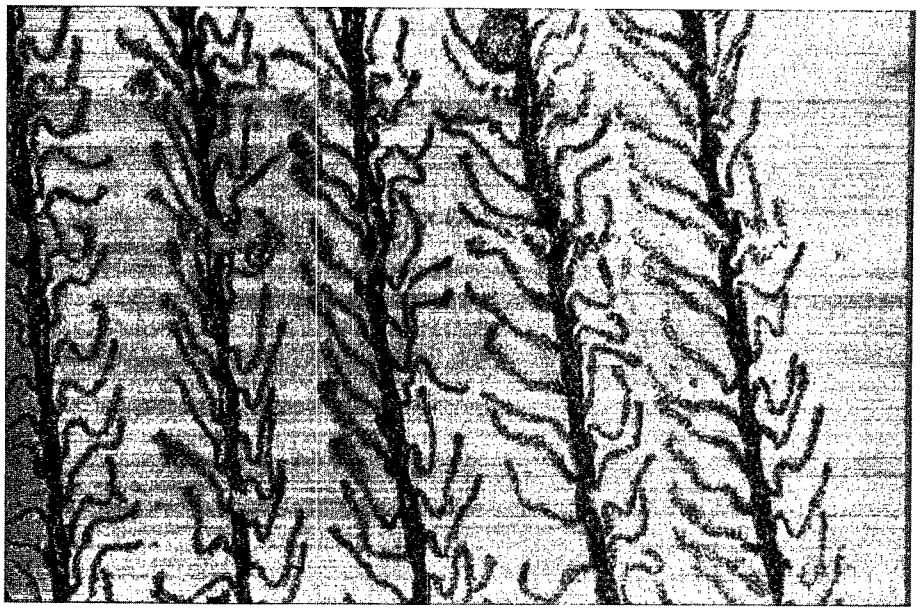


Figure 6. 0.75 ppb (x 100) after 72 hours exposed.

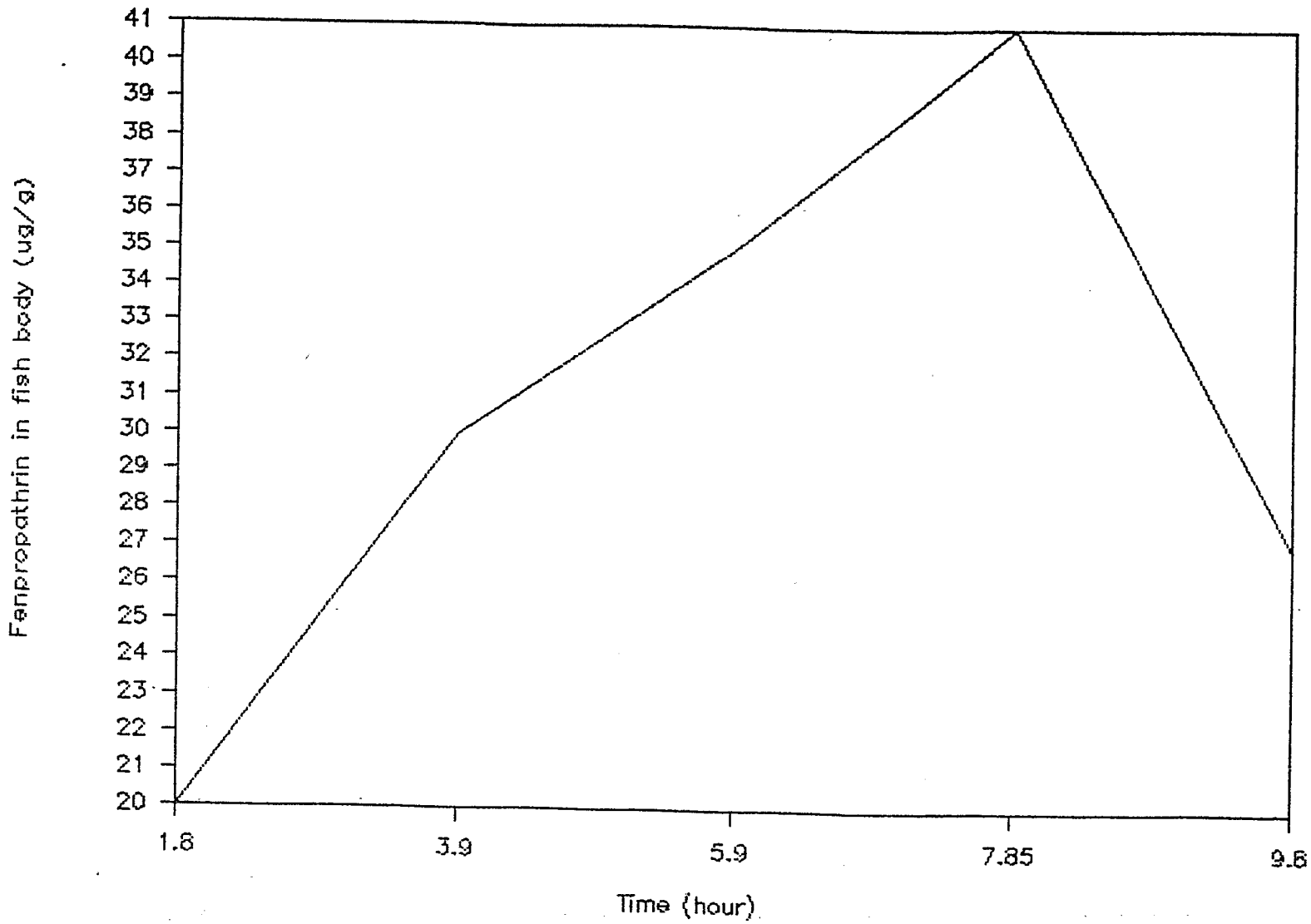


Figure 7. Accumulation of fenpropathrin in the body of silver carp.

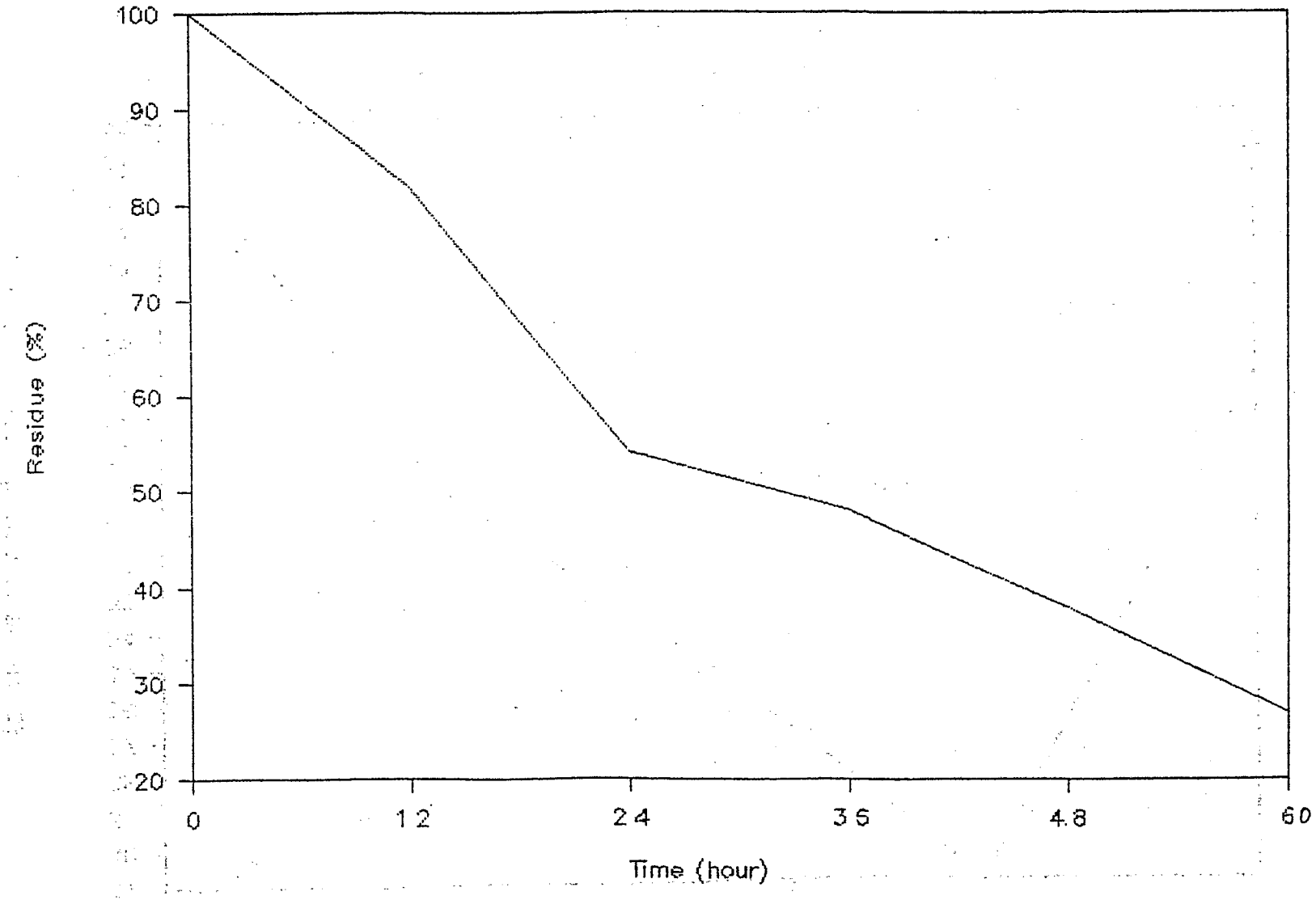


Figure 8. Depulation of fenpropathrin in the body of silver carp.

ELIMINATION AND TOXICITY OF FENPROPATHRIN IN A WATER-SEDIMENT SYSTEM

A detailed report concerning the degradation of fenpropathrin in soil was given by Su and Fan (1989), but there is no data on the fate or toxicity of the pesticide in water or sediment. The present study investigates the degradation and toxicity of fenpropathrin in a water-sediment system. Sediment was collected from the fish pond at the Institute of Hydrobiology, Chinese Academy of Science, Wuhan. The sediment was sifted through a 45 mesh/cm sifter, and allowed to settle overnight. Amounts of 1000 g of the settled sediment were added to 15 L dechlorinated tap water in 20 L glass aquaria. Fenpropathrin was added to the aquaria to make final concentrations of 1, 5, 10, 15, and 100 ppb. Seven hours after addition of the pesticide, samples of both the water and the sediment were taken. Both samples were analyzed for fenpropathrin with a gas chromatograph. Sediment samples were dried with a vacuum filter. Fenpropathrin was extracted with an acetone/hexane (10:3) solvent by agitation with an ultrasonic generator for 15 min. The extract was then filtered through a flouride clay and silica gel column, and further concentrated for quantification by gas chromatography.

An acute toxicity test was carried out when fenpropathrin was added to the tap water. Results are shown in Table 5.

Table 5. Acute toxicity of fenpropathrin on Daphnia magna (48 hr; water temperature 16-17 °C).

Concentration (ppb)	1	1.8	3.2	5.4	7.5
Mortality (%)	10	50	100	100	100

The elimination of fenpropathrin in water and sediment is shown in Figs. 9A and 9B. The fenpropathrin concentration in water decreased rapidly (Fig. 9B), but was rapidly absorbed at the same time by the sediment (Fig. 9A). After 7 hours, 3.5 ppb was measured in the water and 1800 ppb in the sediment. The half-life of fenpropathrin in water was between 3-7 days and no pesticide could be detected after 7 days. The decrease in fenpropathrin concentration corresponds with the mortality of Daphnia magna shown in Table 5. The half-life of fenpropathrin in sediment was between 7-14 days and no pesticide could be detected after 45 days. Furthermore, fenpropathrin residue in the sediment after 7 days was not found to be released into the water. It appears that the elimination of fenpropathrin in the aquatic ecosystem is more rapid than in soil (Su and Fan, 1989).

CONCLUSIONS

Fenpropathrin is acutely toxic to some common Chinese aquatic organisms. The toxic mechanism and lethal effect on fish may be related to inhibition of Na^+,K^+-ATPase, modification of the Na^+ channel in the nervous system, disruption of ion metabolism, and ultrastructural damage to the gills. However, this pesticide did not persistently accumulate in the body since it was rapidly eliminated into the water. Furthermore, the pesticide in the water was rapidly absorbed by the sediment. The residual fenpropathrin in the aquatic phase, and the pesticide absorbed by the sediment were both degraded within a few days. Despite the lack of persistence of fenpropathrin in the aquatic ecosystem, it is recommended that this pesticide be used cautiously in fisheries areas to avoid acute toxic effects.

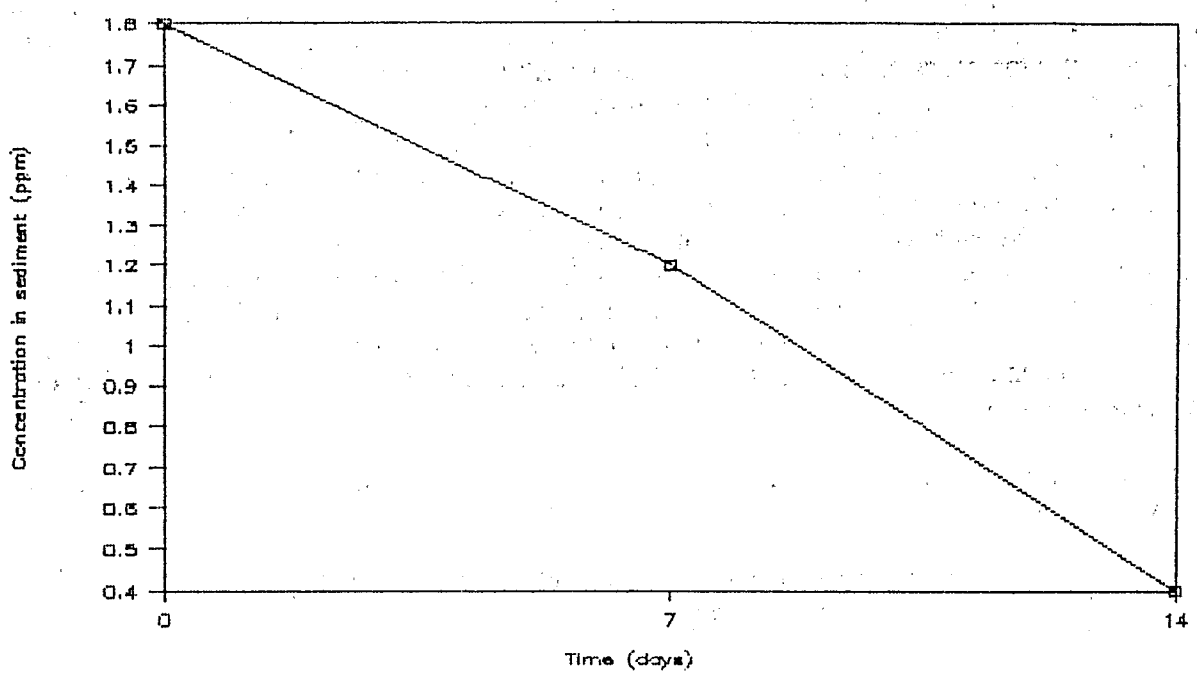
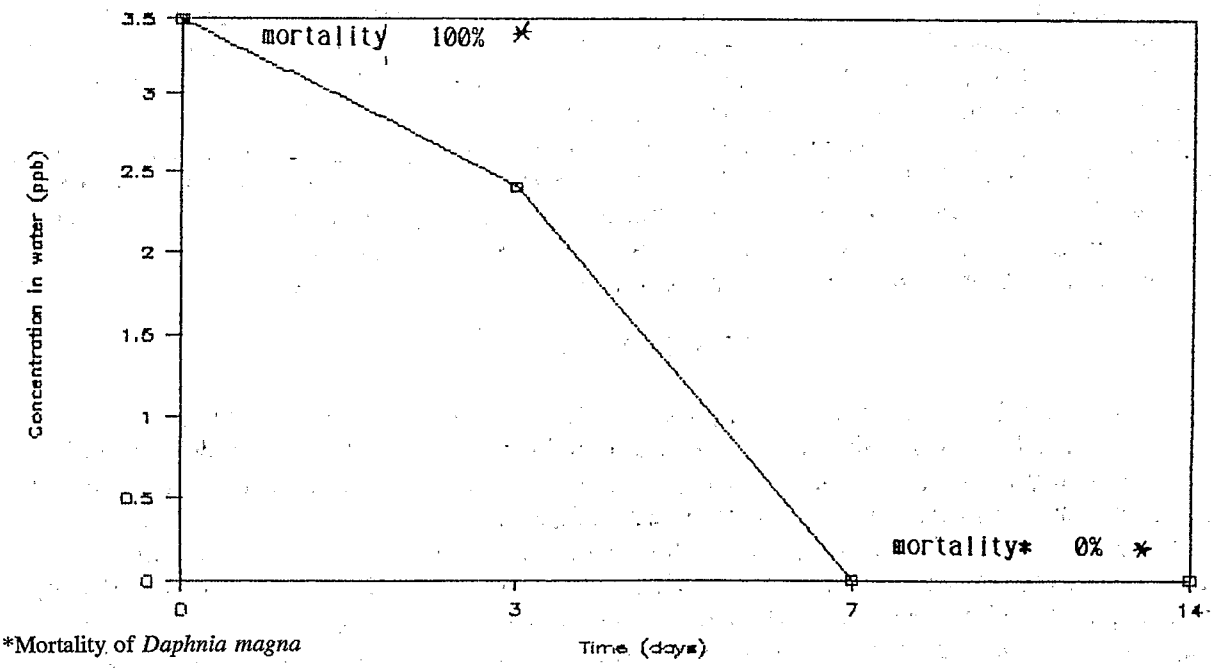


Figure 9A. Elimination of fenpropathrin in sediment.



*Mortality of *Daphnia magna*

Figure 9B. Elimination of fenpropathrin in water.

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BIOACCUMULATION OF ORGANIC CHEMICALS IN TISSUES OF FISHES

Robert V. Thurston[1] and John F. Neuman[1]
Colin J. Brauner[2] and David J. Randall[2]

INTRODUCTION

During the past 3 years we have been studying the rates of uptake by fishes of selected synthetic organic chemicals, foreign to natural systems, known as xenobiotics. We have conducted several tests using an automated respirometer in which we have exposed fish to 1,2,4,5-tetrachlorobenzene (TCB). Our principal test animal has been the rainbow trout (*Oncorhynchus mykiss*), although we have also tested other fish species to compare results. One of our long-range objectives is to determine to what extent uptake and depuration rates of xenobiotics by fishes may be related to their respiration rates, in the hope of developing a simple predictive model describing the rates of uptake and depuration of xenobiotics as a function of the oxygen uptake rate and chemical properties of the chemical in question. In this presentation we will describe our approach to this research and discuss some of our results.

METHODS

Test Conditions: Many of our preliminary tests have been conducted at Fisheries Bioassay Laboratory, Montana State University (MSU), and most of our respirometry experiments have been conducted in a modified Brett-type respirometer at the Zoology Department, University of British Columbia (UBC). This respirometer was designed and built specifically for our studies, and has been described in some detail by Gehrke et al. (1990). The respirometer can test fishes up to 2 kg in size and at swimming speeds as great as 2.5 m/second. Experiments can be conducted over several days with water velocity, temperature, pH, dissolved oxygen, and carbon dioxide all computer-controlled at predetermined levels, and data monitored continuously by the computer as well. For the tests at UBC described here, fish were sacrificed immediately after removal from the respirometer, and whole fish or fish tissues, and test water samples, were frozen and stored at -20C until shipment to MSU for chemical analysis; shipment was by overnight air carrier with samples packed in dry ice. Fish tested at MSU were prepared for analysis the same as those tested at UBC. All samples were stored at -30C at MSU prior to analysis.

TCB Analysis: Small whole fish (<10 g), subsamples of homogenates of larger fish, and individual organ tissues, were blended to a fine powder with the aid of dry ice and anhydrous Na_2SO_4 while still frozen. At least two separate samples were prepared from homogenates of larger fish. Only single samples were prepared from smaller fish and fish tissues because of limitations in sample quantity. TCB was soxhlet extracted (~>'8 hours) from each sample using hexane. Surrogate (1,2,3,4-TCB) in amount comparable to the amount anticipated for TCB results (usually 100 μg) was added at the initiation of the extraction step. Lipids were removed from portions of this extract using florisil column chromatography; TCB and surrogate were eluted with 5% methyl-t-butyl ether. Pentachlorobenzene (PCB) internal standard (0.25 μg) was added to the purified extract and component

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concentrations were determined by electron capture gas chromatography (ECD-GC). Packed GC columns (1.8 m x 2 mm i.d.) containing 3% SE-30 or 3% Carbowax on 100/120 Supelcoport were used. A multipoint calibration curve (0.5 - 10 µg/l) was employed during quantitation. Both standards and sample extracts were injected in duplicate. When plasma samples were required, the caudal peduncle was severed immediately after the fish was sacrificed and blood was collected from the caudal artery into heparinized capillary tubes. Plasma was separated by centrifugation and 5.0 µl of plasma was removed by means of a 10 µl glass syringe and transferred to a 10 ml volumetric flask containing 0.05 µg internal standard (PCB). This solution was made to volume with hexane, and the contents mixed for approximately 1 minute using a vortex mixer. TCB was extracted from test water samples using hexane, and internal standard was added to the diluted extracts. Both plasma and water samples were analyzed for TCB as described above.

TESTS CONDUCTED, RESULTS, AND DISCUSSION

In several of our initial experiments rainbow trout were exposed to TCB for different time periods up to 6 hours under either static test conditions at MSU or forced swimming conditions in the respirometer at UBC. After test, fish were sacrificed and different tissues analyzed to determine any apparent sequence among them in increase of toxicant concentration. Tissues analyzed were blood plasma, gills, liver, kidney, brain, spleen, heart, upper gut, lower gut, white muscle, pink muscle, and adipose. We also reviewed the data to determine if the rate of toxicant concentration increase in any one tissue might be representative of the fish as a whole. Because TCB is lipophilic, this presupposes one tissue might have a fat content representative of the fish as a whole, or at least with a fairly constant ratio to that of the fish as a whole. Standard deviations from the mean concentrations of TCB among the tissues analyzed were so great that we were unable to detect any sequential build-up among the tissues. Indeed, concentrations were not appreciably different between 1 and 6 hours except in the case of adipose tissue and possibly muscle (Table 1). We also looked at the tissue/blood plasma ratio of the test fish, and once again did not see any consistent ratio (Table 2).

It was apparent we would need to measure TCB concentrations in whole fish, and our next tests were designed to compare blood plasma TCB concentration against that of whole body over time. In our first experiment the concentrations of TCB in blood plasma and whole body were very similar after 2 hours of exposure, but blood plasma concentration at 6 hours had not increased, whereas whole body concentration had more than doubled (Figure 1). The obvious conclusion was that TCB equilibrium between exposure water and blood plasma was achieved within 2 hours or less, and after reaching saturation the blood was acting as a conduit to pass TCB from gills to body tissues. Thus, blood plasma could not be used to indicate total body content and therefore uptake rate of the toxicant.

In our next experiment 5-g rainbow trout were exposed to nominal TCB concentrations of 10, 50, and 100 µg/l in holding tanks for several days, during which they were transferred to a fresh solution every 24 hours. Four fish were removed from each tank at 1, 2, 4, and 8 days, sacrificed, and blood plasma and whole body TCB concentrations measured. A tight pattern of TCB increase over time emerged for both blood plasma and whole body, and these patterns were also correlated with exposure concentrations (Figure 2), but once again equilibrium between TCB in exposure water and whole body lagged behind TCB equilibrium reached in blood plasma.

Table 1. Fish tissue concentrations after exposure to TCB.

Exposure (Hours)	n	Plasma	Gills	Liver	Kidney	Spleen	U.Gut	L.Gut	W.Msc	P.Msc	Heart	Brain	Adipose
Test 118 500 - 25 µg/L TCB 0 BL/s													
0.5	2	5.73	4.68	8.70	8.10	<1.5	2.50	3.44	4.03	11.85	10.40	10.60	2.99
1.0	2	10.34	8.65	36.35	13.15	9.84	4.23	10.41	6.82	31.30	15.45	25.90	1.17
3.0	3	10.09	8.58	39.73	17.85	10.55	5.86	16.18	9.49	83.00	19.97	*42.00	20.97
6.0	3	8.39	5.20	14.87	14.20	7.64	7.28	6.19	7.26	63.33	16.00	15.43	23.83
Test 105 400 - 50 µg/L TCB 1.25 BL/s													
1.0	2	3.74	4.13	7.85	5.38	2.78	1.93	7.68	3.54	18.50	5.33	11.15	20.59
2.0	2	6.85	4.24	11.25	6.43	2.59	3.32	**7.34	5.20	18.00	6.13	11.29	8.22
6.0	2	3.17	2.96	10.78	9.29	2.21	4.22	no data	7.09	38.55	5.19	8.99	59.75

Plasma µg/ml, Tissue µg/g * = two values ** = one value

Table 2. Ratio of fish tissue concentrations to blood plasma after exposure to TCB.

Exposure (Hours)	n	Plasma µg/ml	Gills/ Plasma	Liver/ Plasma	Kidney/ Plasma	Spleen/ Plasma	U.Gut/ Plasma	L.Gut/ Plasma	W.Msc/ Plasma	P.Msc/ Plasma	Heart/ Plasma	Brain/ Plasma	Adipose/ Plasma
Test 118 500 - 25 µg/L TCB 0 BL/s													
0.5	2	5.73	0.816	1.52	1.41	0.0421	0.437	0.600	0.703	2.07	1.82	1.85	0.523
1.0	2	10.3	0.839	3.53	1.28	0.956	0.411	1.01	0.663	3.04	1.50	2.51	0.114
3.0	3	10.1	0.849	3.93	1.77	1.04	0.580	1.60	0.940	8.22	1.98	3.40	2.08
6.0	3	8.39	0.619	1.77	1.69	0.911	0.860	0.737	0.866	7.55	1.91	1.84	2.84
Test 105 400 - 50 µg/L TCB 1.25 BL/s													
1.0	2	3.87	1.07	2.03	1.39	0.720	0.499	1.98	0.915	4.78	1.38	2.88	5.32
2.0	2	6.85	0.619	1.64	0.939	0.378	0.484	1.05*	0.759	2.63	0.896	1.65	1.20
6.0	2	3.17	0.934	3.40	2.93	0.699	1.33	no data	2.24	12.2	1.64	2.83	18.8

* = two values

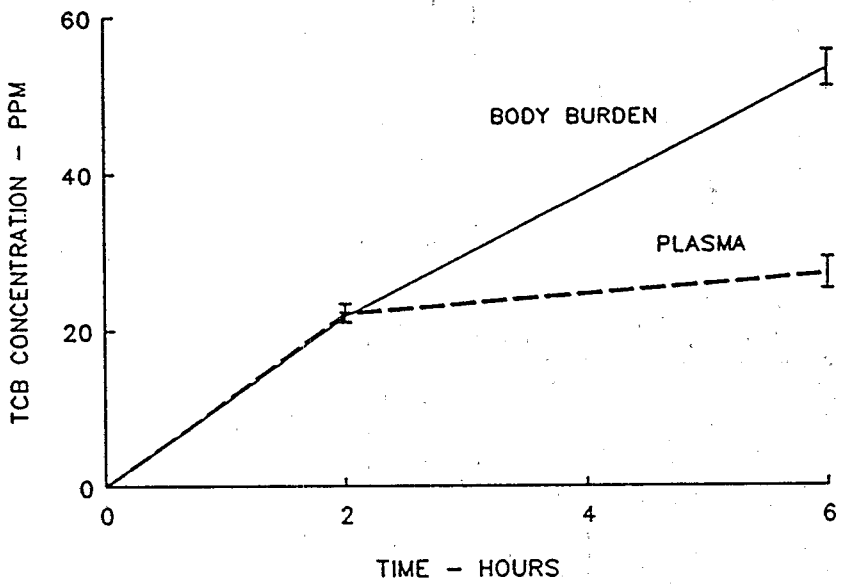


Figure 1. TCB plasma level vs whole body burden static conditions - water level 260 µg/l.

Earlier studies at MSU had shown the rate of uptake of some of the chemicals we might be working with can be extremely rapid, with equilibrium between whole body and water sometimes reached in just a few hours. Tischmak (1984) demonstrated that equilibrium for 2,4,6-trichloroaniline between fathead minnows (*Pimephales promelas*) and their water environment was achieved in less than a day (Figure 3), and although equilibrium for TCB required 6-8 days, it was also apparent that the rate of uptake of TCB decreased considerably between initial exposure and 24 hours into test (Figure 4).

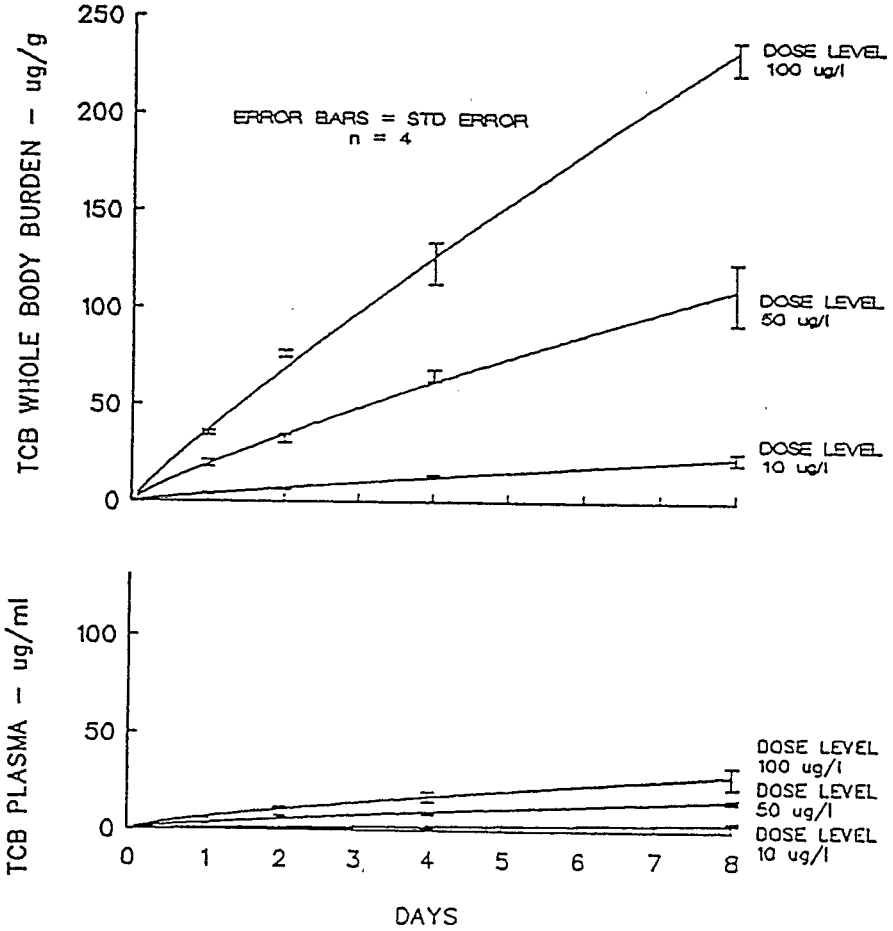
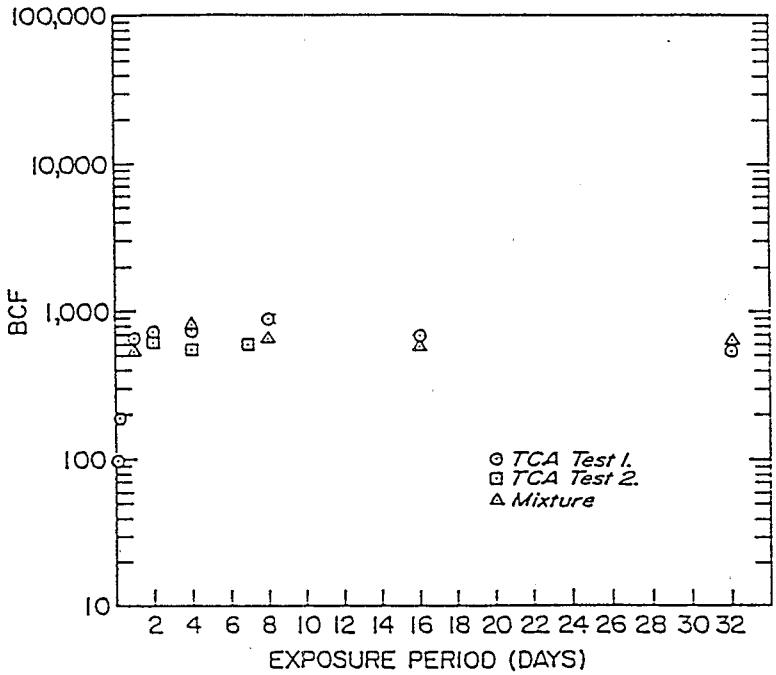


Figure 2. TCB plasma vs whole body burden static conditions with periodic dosing.

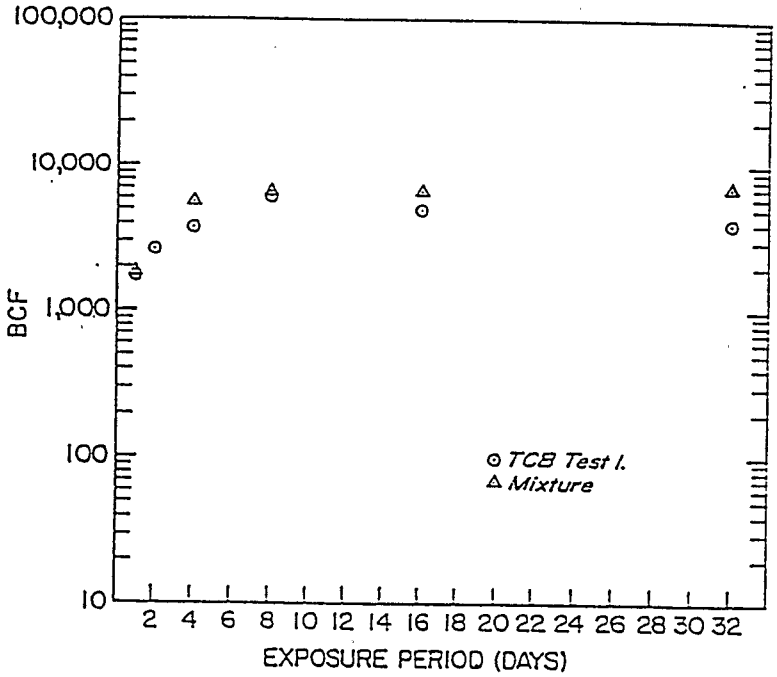


(From Tischmak, 1984)

Figure 3. TCA bioconcentrations profiles.

Our next step was to conduct a series of short term tests in which we exposed rainbow trout to TCB in the UBC respirometer to measure TCB uptake vs. oxygen consumed. We chose a 2-hour test period for several reasons, but principally because this was long enough to measure oxygen differences in the exposure water for as little as 100 g of test fish, and short enough so that rate of toxicant uptake by the test fish would not measurably vary between start and finish of the test period. The uptake of TCB by rainbow trout per gram of fish for three different weight classes is shown in Figure 5. We also looked at uptake of TCB in relation to oxygen consumption rate for each of the three different weight classes of rainbow trout, each tested at two different swimming speeds (Figure 6).

Separate from our chemical uptake studies, we have compiled a file of data from the published literature on respiratory oxygen requirements of fishes (Thurston and Gehrke, 1993). This file, called OXYREF, contains data from over 6800 individual laboratory tests in which oxygen consumption was measured. The data in our file include fish species, fish weight, certain test water conditions, measured respiratory oxygen requirements, and mode at which each fish was tested: "standard" (resting), "routine" (moving about), or "active" (measured swimming rate).



(From Tischmak, 1984)

Figure 4. TCB bioconcentration profiles.

From OXYREF, we plotted oxygen consumption vs. weight for fishes in an active swimming mode, which is that mode under which we tested our fish at UBC. If we now superimpose on this curve the data from our rainbow trout TCB uptake experiments, the species we tested in more than one weight range, one sees an excellent fit; the bulk of those data points from the higher swimming speed tests are slightly above the OXYREF curve, and the data points from the slower swimming speed tests are slightly below the curve (Figure 7).

There is a clear correlation between the rate of oxygen uptake and the rate of uptake of TCB among the freshwater fishes we have tested. Thus, if the oxygen uptake rate and the water concentration of a chemical to which a fish is exposed are known, it may be possible to predict uptake rates of that chemical during exposure. We are continuing to expand OXYREF as more data become available in the literature, but in the present laboratory study if we can establish correlations between oxygen consumption and toxicant uptake and depuration, then OXYREF can provide a powerful tool for the prediction of bioaccumulation by fishes of xenobiotics.

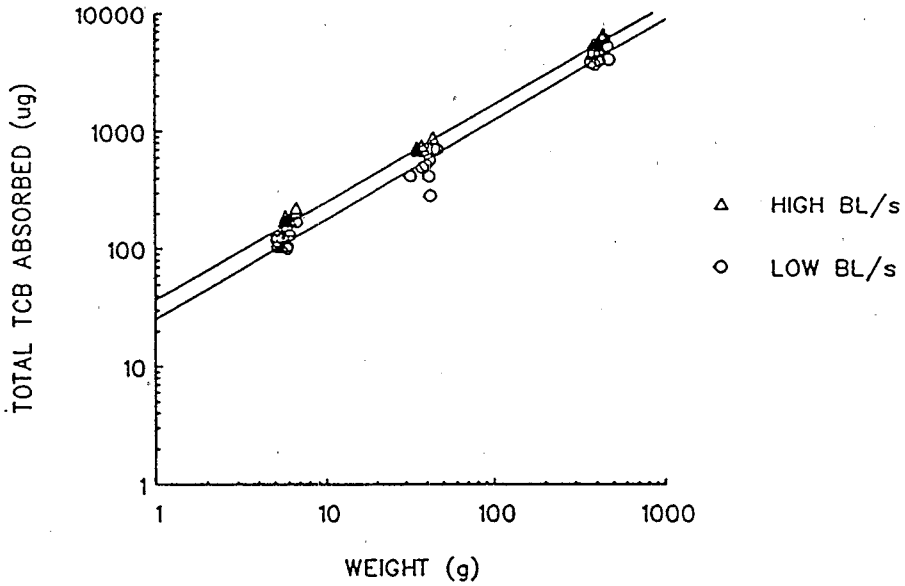


Figure 5. TCB absorbed vs weight rainbow trout.

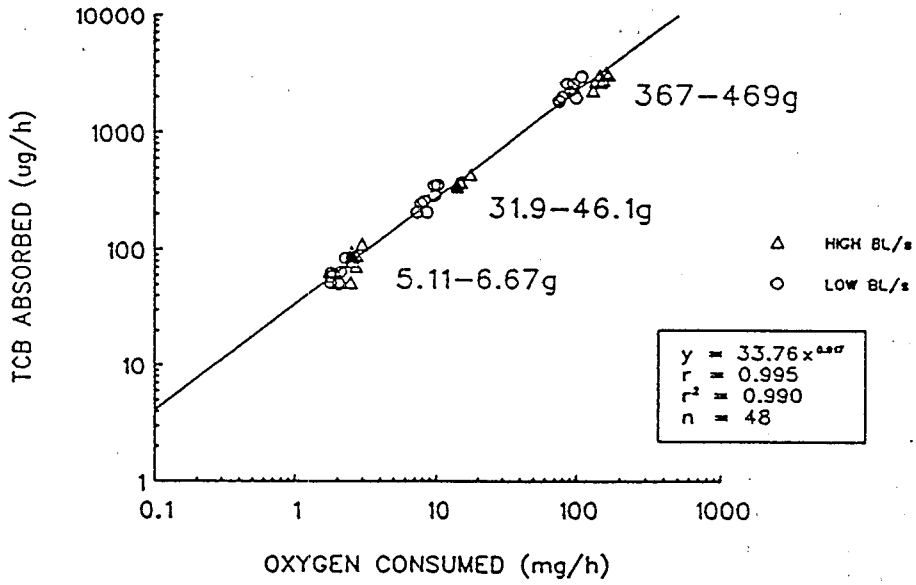


Figure 6. TCB absorbed vs oxygen consumed rainbow trout at three weight ranges.

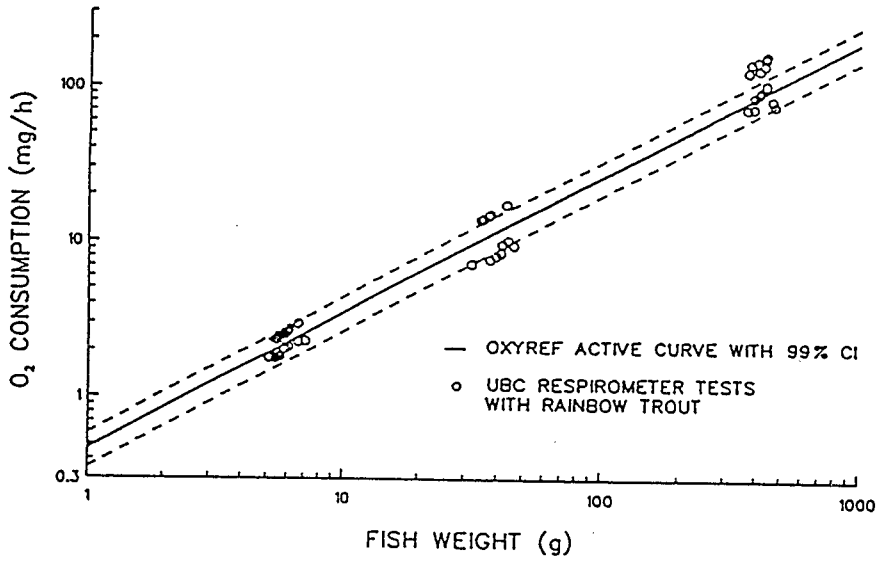


Figure 7. OXYREF data file active swimming regression curve vs rainbow trout oxygen uptake during UBC tests.

ACKNOWLEDGEMENT

This research was funded by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia, through Cooperative Agreements CR 813424, CR 816369, and CR 816778.

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THE RELATIONSHIP BETWEEN THE UPTAKE OF ORGANIC CHEMICALS AND OXYGEN CONSUMPTION IN FISH

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Robert V. Thurston[2], and David J. Randall[1]

ABSTRACT

Physiological models used to predict toxicant uptake are dependent on parameters such as epithelial thickness, perfused area of the gills, gill blood and water flow rates and water boundary layer thickness. These parameters are difficult to measure and are species specific. In addition, they are adjusted in accordance with metabolic rate to ensure adequate oxygen delivery to the tissues. Oxygen is a lipid soluble molecule and crosses the gills transcellularly as do most lipid soluble xenobiotics. Thus, conditions for oxygen transfer may be indicative of those for toxicant transfer.

In five species of teleost fish similar in size (3 to 11 g) and for rainbow trout weighing 40 and 400 g, the uptake rate of a model xenobiotic, 1,2,4,5-tetrachlorobenzene (TCB, log K_{ow} of 5.0), is dependent upon external TCB concentration and oxygen consumption rate of the fish. In 3-5 g fish, the elevation of the regression line describing TCB uptake relative to oxygen consumption rate is significantly greater than in fish larger than 11g. The slope of the regression lines; however, are not significantly different, supporting the concept of using a general coefficient to predict toxicant uptake as a function of consumption rate (MO_2) in fish. The greater elevation of the regression line in the small fish data is likely due to TCB binding to the body surface and reflects the relationship between surface area and volume.

The coefficient relating the initial uptake of TCB with MO_2 in fish has been used to predict the uptake of tetrachloroguaiacol in 400 g rainbow trout and 700 g large scale suckers. Tetrachloroguaiacol has a similar log K_{ow} (4.41) to TCB and the predicted levels approximate the measured values.

We have created a large oxygen data base OXYREF which can be used to estimate the oxygen consumption rate of a fish based on fish size and activity level. Potentially, the coefficient relating toxicant uptake with MO_2 can be multiplied by the MO_2 value retrieved from OXYREF to predict chemical uptake rates. The coefficient can be corrected to account for the uptake of different chemicals provided log K_{ow}, weak acid dissociation constant, water pH and freely dissolved concentration of the chemical are known.

INTRODUCTION

With the advancement of technology there is a massive production of chemicals synthesized to meet the demands of industry. Most of these chemicals are foreign to the body (xenobiotics) and eventually enter the environment where they may be available for uptake by organisms. Once within the body many xenobiotics are extremely toxic; particularly the lipid soluble, chlorinated organics which are not susceptible to oxidation by the multi-function oxidases. The mode of toxicity is often specific to each xenobiotic and may be localized to a specific target site or more likely it may act at multiple sites. Thus, a chemicals mode of toxicity is difficult to quantify, let alone predict. Most xenobiotics, however, have one thing in common; they must enter the animal before they can exert their toxic effect(s). Uptake can

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occur through biomagnification in the food chain or by direct uptake from the environment across the respiratory surface or skin. This discussion is concerned with direct uptake of xenobiotics from water by fish.

Generally, the gills comprise the major surface area of the body in fish. In addition, this boundary between the animal and the environment is only a few cells thick (1-10 μ) and is highly perfused with blood which flows counter currently to water. These conditions ensure adequate gas exchange across the gills but also make the gills an ideal organ for xenobiotic uptake. The gills are often thought to be the major site for xenobiotic uptake (Neely, 1979, Gobas, Opperhuizen and Hutzinger, 1986, Randall and Brauner, 1993); however, uptake across the skin can not be neglected, particularly in small fish when the body surface area can make up a large proportion of the animals total surface area.

The uptake of xenobiotics by fish directly from water is determined by numerous factors, the most dominant of which are the physico-chemical properties of the compound and the physiological processes which occur at the gills.

PHYSICO-CHEMICAL CHARACTERISTICS WHICH INFLUENCE UPTAKE

The physico-chemical characteristics which most strongly influence the flux of xenobiotics across biological membranes are: water and lipid solubility, molecular weight and volume, "active concentration", tendency to ionize, and susceptibility to metabolic transformation. The amount of toxicant which can be carried in the inhalant water is determined by the chemicals water solubility. The rate at which the chemical can diffuse across the lipid membrane of the respiratory epithelium from inhalant water will in turn depend on lipid solubility. Subsequent distribution of the chemical across the lipid and aqueous barriers of neighbouring cells will be determined by a compromise between lipid and water solubility. The importance of a ratio between lipid and water solubility to toxicant uptake has long been recognized and is the basis for the well established octanol:water partition coefficient (K_{ow}). Octanol was chosen to represent "biological lipids" and K_{ow} became the standard for quantifying the partitioning characteristics of compounds (Connell, 1990).

Xenobiotics diffuse across membranes transcellularly and thus diffusion depends largely on lipid solubility but molecular weight and volume of a compound cannot be ignored. In general, the diffusion coefficient of a chemical decreases as molecular weight increases. Saito et al. (1990) used electron microscopy to qualitatively demonstrate that compounds greater than 2000 in molecular weight are not absorbed across the gills in carp.
Zitko and Hutzinger (1976) have proposed that the upper molecular weight limit for xenobiotic uptake is approximately 600.

The driving force for diffusion is supplied by the "active concentration" gradient between the environment and the fish. Most xenobiotics are extremely hydrophobic and consequently will bind to organic matter (Black and McCarthy, 1988) or become oriented into small, hydrophilic droplets called micelles. In both situations, the chemical is not aqueously dissolved and therefore not available for uptake by diffusion. The uptake rate of a xenobiotic to which a fish has not previously been exposed is directly proportional to the aqueously dissolved concentration of the compound in the environment. Thus, it is crucial to know the "active concentration" or the concentration of the chemical available for uptake.

Lipids are nonpolar and thus noncharged molecules diffuse across lipid membranes much more easily than charged molecules. This is of significance in the uptake of compounds which dissociate into weak acids because the membrane diffusivity of the dissociated form of the compound is very low relative to the non-dissociated form. The dissociated proportion of a compound is dependent upon the pH of the

medium in which it exists and its tendency to dissociate which is quantified by the weak acid dissociation constant (pK_a). The non-dissociated, aqueously dissolved concentration of the compound in water can be considered the "active concentration" which is available for uptake by the fish. This concentration can be calculated if water pH and the pK_a of the compound are known. The pH required for this calculation is that of the water in the interlamellar channels of the gills (see Randall this volume). Saarikoski et al. 1987 attempted to predict uptake of a variety of weak acids in guppies by taking into account the water pH and compound pK_a but were only partially successful.

The final physico-chemical characteristic mentioned above is the resistance of the chemical to the action of multi-function oxidases (MFO). These enzymes convert compounds to a less hydrophobic form which reduces the toxicity of the xenobiotic and facilitates excretion of the compound. Multi-function oxidase activity in fish has been demonstrated in many tissues but exists predominantly in the liver and gills (Barron et al. 1989). If xenobiotics are oxidized at the gills before they enter the fish or are converted to a less toxic form at the same rate that they are taken up, the compounds will not bioaccumulate. Unfortunately, the most harmful xenobiotics are often resistant to metabolic transformation which contributes to the toxicity of the compound.

All the characteristics described above can effect uptake of xenobiotics. However, in general, the most important physico-chemical characteristics in predicting toxicant movement across membranes are the K_{ow} and the "active concentration" of the compound which encompass both the concentration of the aqueously dissolved compound and the concentration of the non-dissociated form if the chemical is a weak acid.

XENOBIOTIC UPTAKE ACROSS FISH GILLS

There has been considerable effort to model the uptake of xenobiotics using a variety of different types of models which range from simple, one compartment models to complex pharmacokinetic models (Barron et al. 1990). Hayton and Barron (1990) have recently proposed a physiological model to describe xenobiotic uptake across the gills in fish:

$$\text{Uptake rate} = (C_w - C_f)[(d(D_m^{-1})\ A\ K_m)+(h(D_a^{-1})A)+V_b(K_b^{-1}+V_w^{-1}]^{-1} \qquad \text{eq.(1)}$$

This model consists of three components: 1.) the concentration gradient between the environment and the fish, where C_w and C_f are the concentration of the xenobiotic in the external water and the plasma water respectively, 2.) physiological and anatomical characteristics of the gills, where d is epithelial thickness, h is aqueous stagnant layer thickness, A is gill surface area, V_b is effective gill blood flow, V_w effective water flow, and 3.) physical constants specific to the compound, where D_m is diffusion coefficient in epithelium, D_a is diffusion coefficient in water, K_m is epithelium/water distribution coefficient, and K_b is the blood/water distribution coefficient.

During initial exposure to a xenobiotic, the first component, the concentration gradient between the environment and the fish, will be equal to the concentration of the xenobiotic in the environment. This will persist for some time because immediately after entering the blood the lipophilic compounds will bind extensively with blood lipid and proteins (Schmeider and Henry, 1988) keeping the aqueously dissolved concentration of the xenobiotic in the plasma water very low.

The second component describing the physiological and anatomical characteristics of the gills are not easy to quantify. For example, it is very difficult to estimate the surface area of the gill and even if an anatomical value is derived it probably does not reflect the functional surface area of the gills because in resting fish, only 60% of the gill is perfused with blood (Booth, 1978). The thickness of the respiratory

epithelium ranges from 0.5 to 11 μm depending upon the species (Hughes, 1984) and the thickness is probably not constant from the proximal to distal portion of the secondary lamellae. To further complicate matters many model parameters are not constant and are influenced by the metabolic rate of the fish. For instance during exercise, there is an increase in cardiac output and ventral aortic blood pressure (Kiceniuk and Jones, 1977) which increases the perfused gill surface area and gill blood flow, and reduces the thickness of the respiratory epithelium (Jones and Randall, 1978). The thickness of the aqueous stagnant layer or boundary layer will also be greatly influenced by the velocity of water flow over the gills. Thus, all of these parameters are difficult to measure, they are species specific but most importantly they are all adjusted according to the metabolic demand of the animal to ensure adequate gas exchange at the gills.

Oxygen is a lipid soluble molecule as are most xenobiotics. Therefore, conditions for oxygen transfer may be indicative of those for toxicant transfer. The development of a coefficient (λ) describing xenobiotic uptake as a function of oxygen consumption rate can be used to replace the physiological and anatomical parameters of the gills in equation 1. This coefficient can then be multiplied by oxygen consumption rate (MO_2) and be incorporated into a general model to predict xenobiotic uptake. Oxygen consumption rate is easy to measure or it can be retrieved from an oxygen data bank (OXYREF) compiled by Thurston and Gehrke (1990) (see Thurston et al, this volume). This bank contains over 15 000 measurements of oxygen consumption rate in over 300 species of fish exposed to different temperatures at rest and during exercise. Analyses of these data indicate that the main determinant of oxygen consumption rate in fish is body weight.

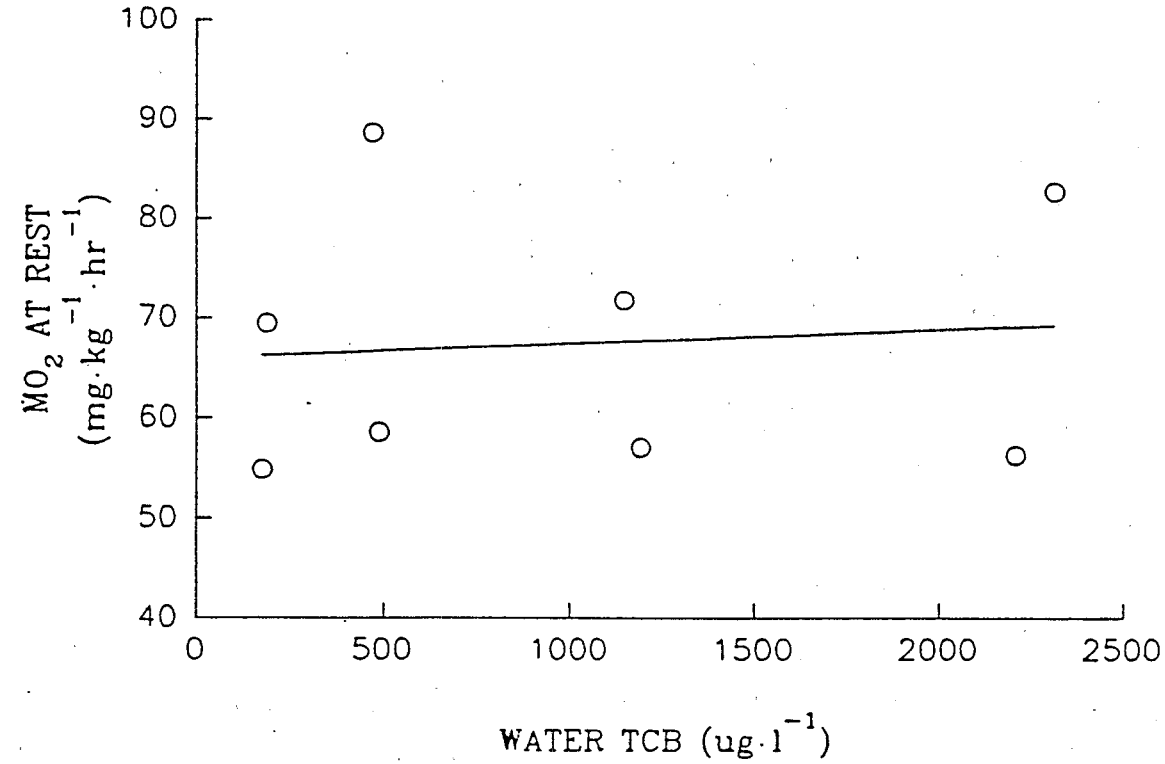
The third component in equation 1 has to do with characteristics specific to the xenobiotic to which the fish is exposed. As mentioned above the most important physico-chemical characteristic which determines xenobiotic uptake rate is K_{ow}. Both K_m and K_b can be calculated from the K_{ow} of the xenobiotic. Saarikoski et al. (1987) have demonstrated in guppies that the logarithm of chemical uptake rate increases linearly with log K_{ow} between 1 and 4.3 for a variety of compounds. The uptake rate is approximately constant for compounds with log K_{ow} between 4.3 and 6 and decreases with log K_{ow} greater than 6 (McKim, Scheider and Veith, 1985). We chose 1,2,4,5 tetrachlorobenzene (TCB) as our model toxicant to calculate λ because it does not dissociate, is not easily metabolized and has a log K_{ow} of 4.97 which is in the range where K_{ow} does not effect maximum uptake rate (Saarikoski et al. 1987). The data of Saarikoski et al. (1987) may be used to derive a coefficient (K_{ow} coeff.) which can be multiplied by λ to predict the uptake of xenobiotics which differ in log K_{ow} from TCB. Thus, during initial exposure to a compound, equation (1) can be simplified to:

$$\text{Uptake rate} = (C_w)\ \lambda\ (K_{ow}\ \text{coeff.}) \qquad \text{eq. (2)}$$

where λ is a coefficient describing xenobiotic uptake as a function of oxygen consumption rate, and K_{ow} coeff. is the coefficient used to account for uptake in xenobiotics which differ in log K_{ow} from TCB. For xenobiotics with log K_{ow} between 4.3 and 6, this coefficient is 1, for those between 1 and 4.3 this coefficient is $10^{0.429\log Kow-1.842}$ (Randall and Brauner, 1993 calculated from Saarikoski et al. 1987).

DERIVATION OF λ

One of the requirements of using λ to predict the uptake of xenobiotics in fish based upon MO_2 values retrieved from OXYREF, is that there is no influence of TCB exposure on MO_2. In rainbow trout exposed to a large range of TCB concentrations there is no significant effect of toxicant exposure on oxygen consumption rate in resting and active fish (Fig. 1a and 1b). Thus, there are no direct effects of TCB exposure to complicate the relationship between toxicant uptake and oxygen consumption rate in fish.



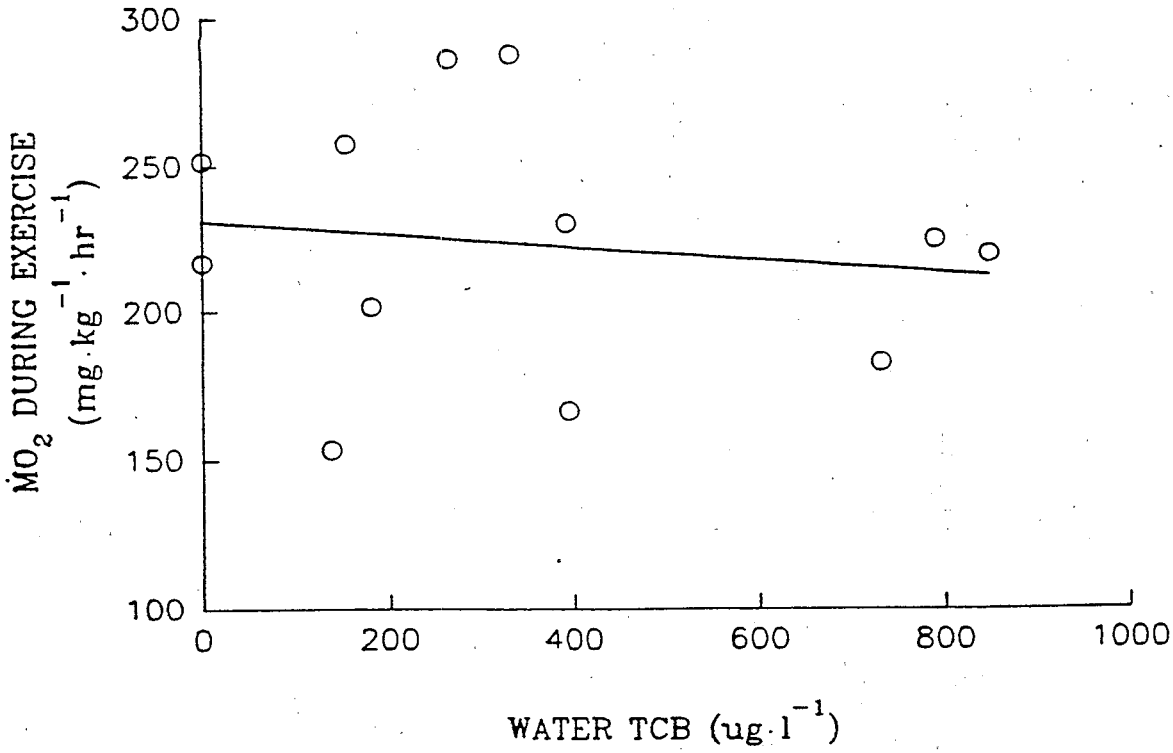


Figure 1. A) The effect of water 1,2,4,5 tetrachlorobenzene (TCB) concentration on the oxygen consumption rate of adult rainbow trout at rest (r^2=0.01); or B) swimming at 1.25 Body lengths per second (Bls^{-1}) (r^2=0.02).

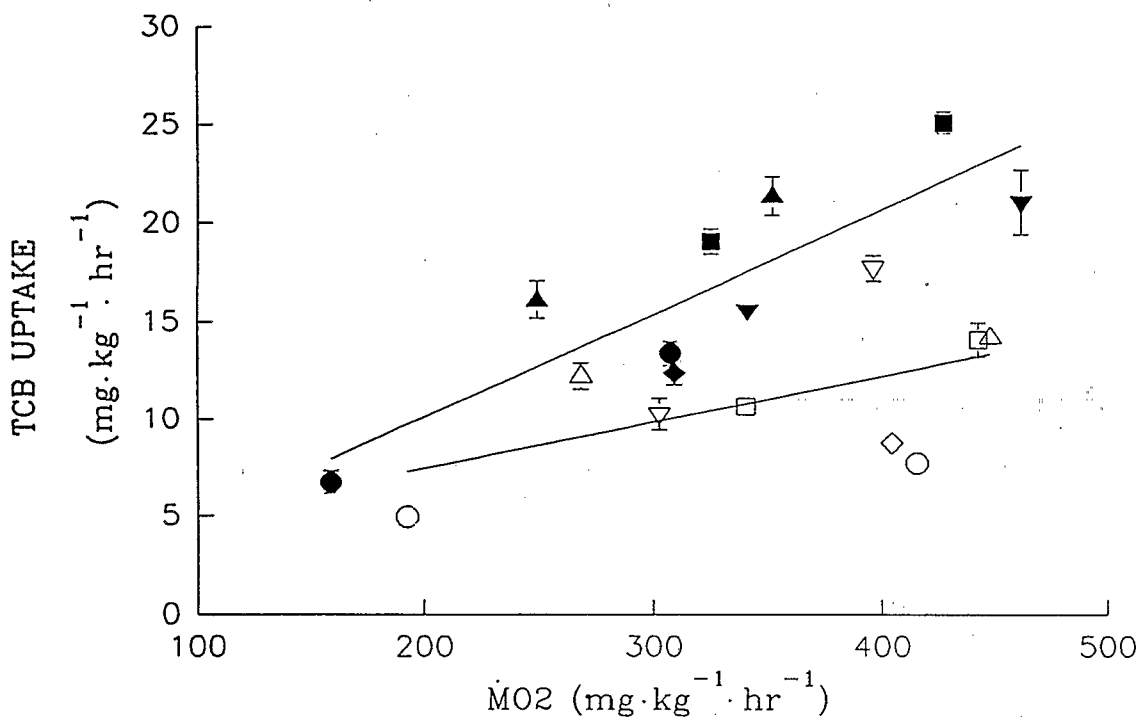


Figure 2. The effect of oxygen consumption rate on TCB uptake rate ($mg \cdot kg^{-1} \cdot hr^{-1}$) in five species of fish exposed to one of two external TCB concentrations (260, open symbols, or 780 $\mu g \cdot l^{-1}$ TCB, closed symbols) at a water velocity of 2.25 or 3.75 Bls^{-1} for 2h.(circle, goldfish; diamond, largemouth bass; upright triangle, channel catfish; inverted triangle, fathead minnow; and square, rainbow trout) (n=8, vertical lines represent 1 standard error, r^2=0.72 at the high TCB concentration, and r^2=0.29 at the low concentration).

The relationship between MO_2 and TCB uptake rate was examined in five species of fish similar in size, and for several different weight classes of rainbow trout. Forced exercise was chosen as the means to influence oxygen consumption rate because during exercise, fish can increase MO_2 relative to resting rates by up to 10 fold (Brett, 1964). In all tests, fish were introduced into a 130 l Brett-type respirometer at the University of British Columbia (described by Gehrke et al. 1989) for two hours at a water velocity of 18 $cm \cdot s^{-1}$. Tetrachlorobenzene was added to the respirometer, a water sample was taken and fish were forced to swim for 2 h at either a low or a high water velocity (less than 80% of maximal swimming velocity) while MO_2 was measured. At the end of the test, a water sample was taken and the fish were removed from the respirometer, killed and immediately frozen. The fish and water samples were kept frozen at -80°C and then transported to Montana State University in dry ice for TCB analyses. The entire fish was homogenized, TCB from the tissue was concentrated in hexane by Soxhlet extraction and total body and water TCB concentration was measured by Gas Chromatography.

Five species of fish: goldfish (*Carrasius auratus*, 11.43 ± 0.99 g), large mouth bass (*Micropterus salmoides*, 4.8 ± 0.18 g), channel catfish (*Ictalurus punctatus*, 3.7 ± 0.06 g), fathead minnow (*Pimephales promelas*, 3.92 ± 0.34 g) and rainbow trout (*Oncorhynchus mykiss*, 5.02 ± 0.07g) were exposed to water

TCB concentrations of 260 or 780 μl^{-1} at low or high swimming velocity with exception of the large mouth bass which were only capable of swimming at the low velocity. There is a significant correlation between TCB uptake rate and MO_2 at both the low TCB concentration (r^2= 0.29) and the high TCB concentration (r^2= 0.79) (Fig. 2). The rate of toxicant uptake at a given oxygen consumption rate is dependent upon water TCB concentration (Fig. 2). During initial exposure to a toxicant, the gradient for toxicant uptake is equal to the toxicant concentration in the water and uptake is proportional to the external concentration (Spacie and Hamelink, 1982).

In an attempt to standardize for external TCB concentration and generate one regression equation describing toxicant uptake relative to MO_2, the measured TCB uptake rate was divided by the mean water TCB concentration over the exposure period (Fig. 3). It is apparent; however, that two distinct relationships exist, one for each TCB exposure concentration. One explanation for this is that the measured TCB concentration in the water is an overestimation of the aqueous concentration. The high water TCB concentration (760 $\mu g \cdot l^{-1}$) is near to the maximal water solubility for TCB and it is possible that TCB is binding to organic matter or forming micelles. Thus, the measured content of TCB in the water is greater than the aqueously dissolved concentration. An overestimation of the aqueous TCB concentration in the high TCB treatment would result in a lower placement of the regression line.

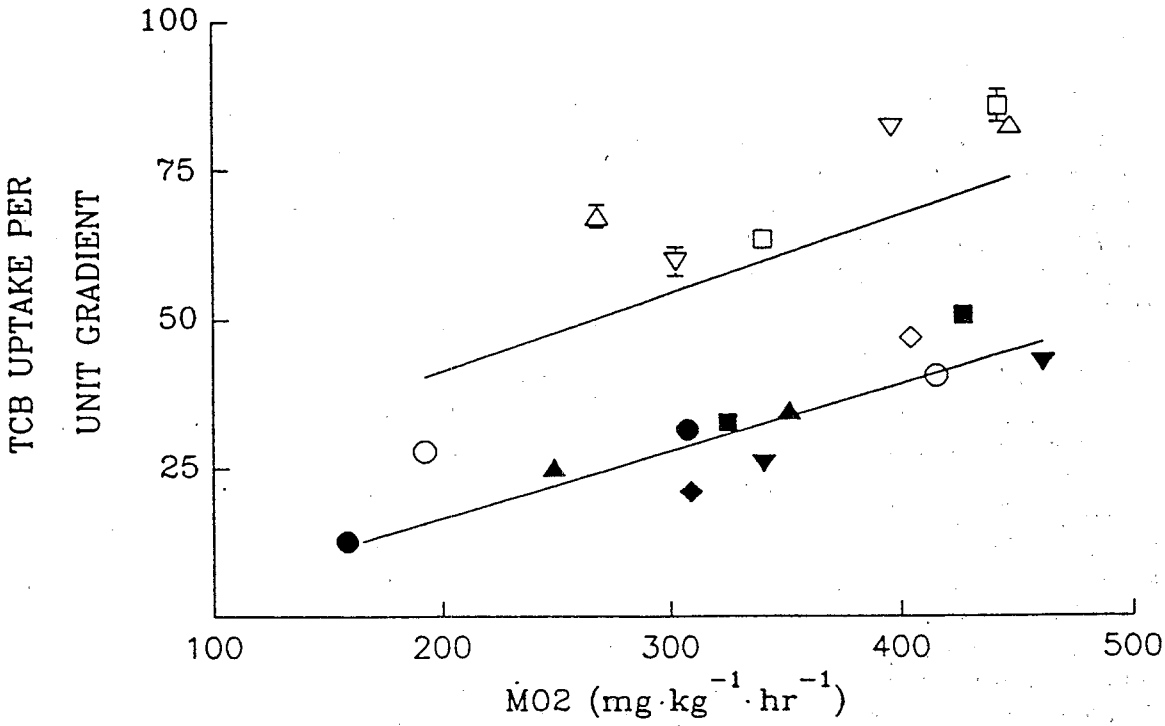


Figure 3. The effect of oxygen consumption rate on the TCB uptake ($mg \cdot kg^{-1} \cdot hr^{-1}$) per unit gradient TCB ($mg \cdot l^{-1}$) in five species of fish. See Fig. 2 for further explanation. (n=8, r^2=0.79 at the high TCB concentration, and r^2=0.317 at the low concentration).

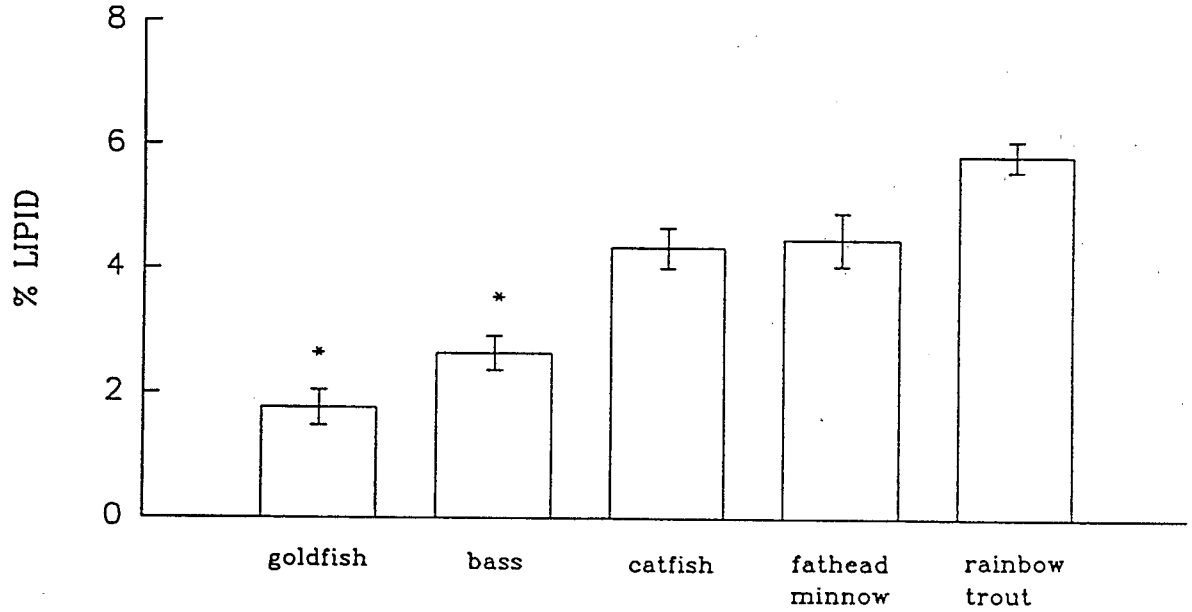


Figure 4. The percent body lipid determined for five species of fish. * indicates statistically different from those without symbol. (n=8)

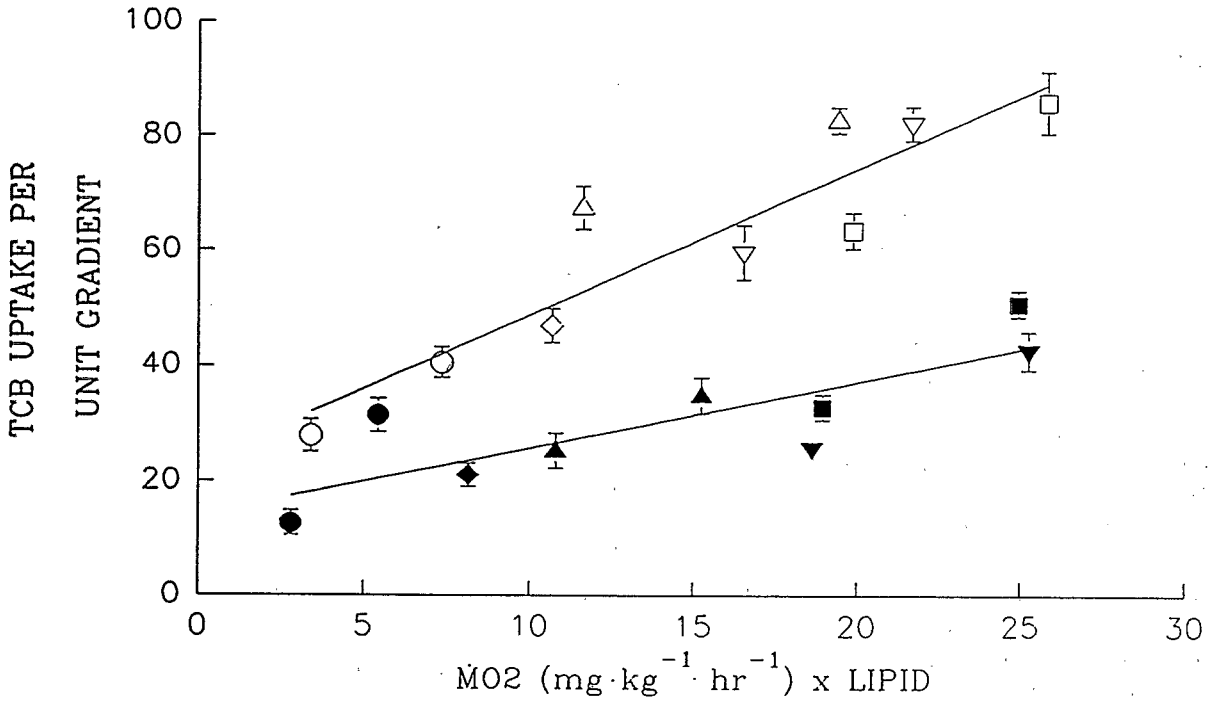


Figure 5. The relationship between TCB uptake per unit gradient and the product of oxygen consumption rate and proportion of body lipid, in five species of fish. See Fig. 2 for further explanation. (n=8, r^2=0.69 at the high TCB concentration and r^2=0.85 at the low concentration).

Lipid content in fish is important during long term exposure to xenobiotics particularly as fish approach equilibrium with the environment (Connell, 1990, Geyer et al. 1985) but the importance of lipid content to toxicant uptake during initial exposure is not known. The lipid content of the five species was measured and significant differences were found between goldfish and bass relative to channel catfish, fathead minnow and rainbow trout (Fig. 4). The inclusion of lipid in the relationship describing toxicant uptake as a function of MO_2 significantly improved the coefficient of determination at the low TCB concentration (r^2=0.85) and only marginally reduced the coefficient in the high TCB concentration (from 0.79 in fig. 3 to 0.68) (Fig. 5). These results indicate that the body lipid content in fish may be an important determinant of toxicant uptake during initial exposure to TCB; however this requires further investigation. The data presented so far indicates that for five species of fish weighing between 3 and 11g, there is a significant relationship between TCB uptake rate and MO_2 during initial exposure to TCB. Does this relationship exist for fish over a range of body masses?

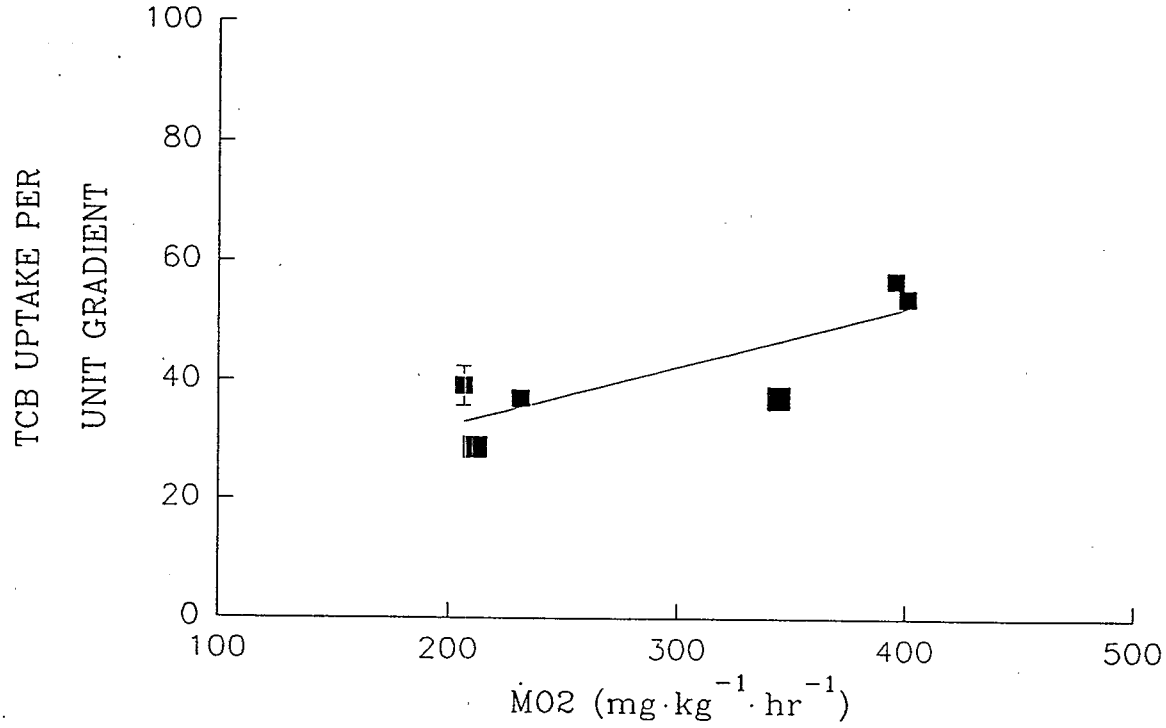


Figure 6. The effect of oxygen consumption rate on the TCB uptake ($mg \cdot kg^{-1} \cdot hr^{-1}$) per unit gradient TCB ($mg \cdot l^{-1}$) in medium (39 g, small solid square, n=4) and large (412 g, large solid square, n=8) rainbow trout exposed to 260 $\mu g \cdot l^{-1}$ TCB (r^2=0.70).

Medium (38.6 ± 0.96) and large (412.5 ± 6.9) rainbow trout were forced to swim at one of two water velocities during exposure to an aqueous TCB concentration of 260 μl^{-1}. Toxicant uptake rate was divided by the mean TCB concentration during the exposure duration and when regressed against MO_2 the coefficient of determination is significant (r^2=0.59)(Fig. 6). However, the slope of this regression line differs from that for the five species of small fish exposed to the low TCB concentration (Fig. 3). In Fig. 3, the low coefficient of determination (r^2=0.32) for the five species of small fish exposed to the low TCB concentration is predominantly due to the low TCB uptake rate measured in goldfish and large mouth bass. The goldfish are more than twice the size of the other fish in this group and when the goldfish data are combined with the large rainbow trout data, there are still two distinct regression lines but only the

elevations differ significantly, the slopes do not (Fig. 6). The constant slope of the regression lines indicates that TCB uptake rate relative to MO_2 is the same for all fish sizes and justifies the use of MO_2 as a general tool in the prediction of xenobiotic uptake. The point at which the regression lines intersect the abscissa (when MO_2 is zero) likely reflects TCB binding to the body surface. The proportion of whole animal TCB uptake rate which is determined by TCB binding to the skin will not be great in large fish but may be quite large in small fish where the surface area to body mass ratio is large. The significant difference between regression line elevations probably reflects this difference in surface area to body mass ratios between the two groups. These data indicate that in fish greater than 11g there is very little effect of fish size on the uptake rate of TCB due to TCB binding to the skin, but there is a large effect in fish less than 11g. Therefore, in fish greater than 11g, TCB uptake during initial exposure in fish can be predicted for a variety of fish species using equation (2) where $\lambda = 15.63 + 0.0820MO_2$.

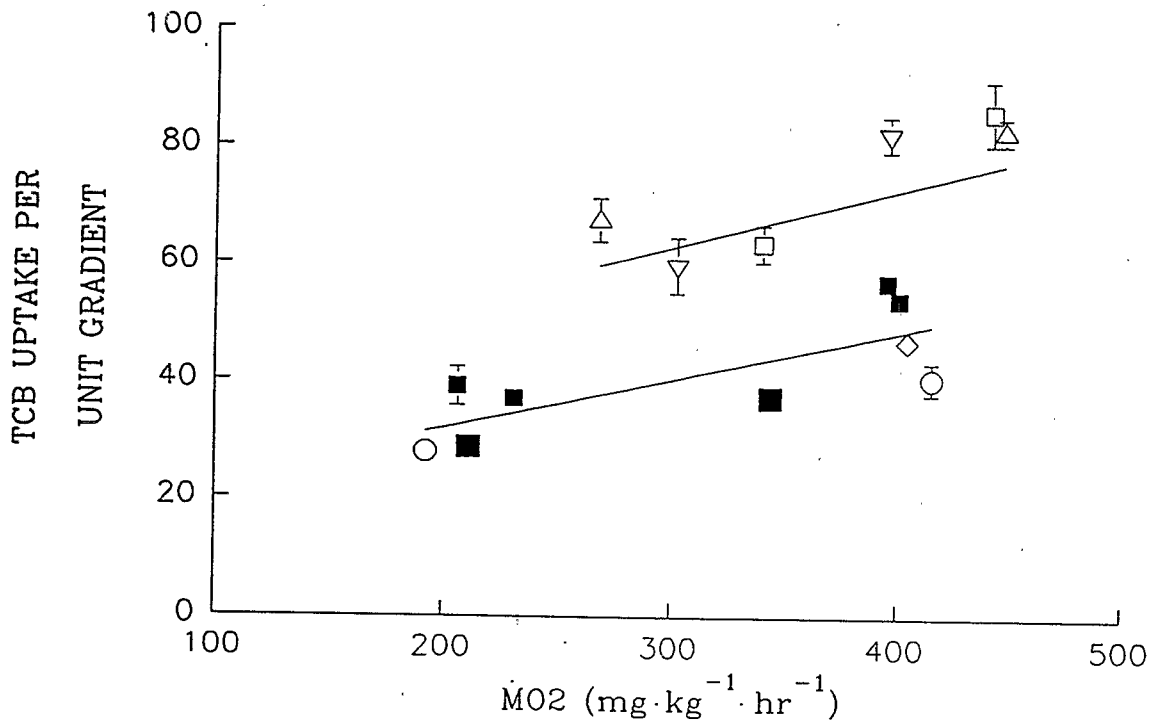


Figure 7. The effect of oxygen consumption rate on the TCB uptake ($mg \cdot kg^{-1} \cdot hr^{-1}$) per unit gradient TCB ($mg \cdot l^{-1}$) where open symbols are data for small fish from Fig. 3 and closed symbols are data for medium and large rainbow trout from Fig 6. The goldfish data (open circles) are grouped with the medium to large rainbow trout data (solid squares) (r^2=0.59, equation of regression line is Y=15.63+0.0820X). The remaining fish are grouped into second regression (r^2=0.22, equation of regression line is Y=33.46+0.0975X).

As discussed earlier, there is very little effect of log K_{ow} on log uptake rate for xenobiotics with log K_{ow} between 4.3 and 6 (Saarikoski et al. 1987). Tetrachloroguaiacol (TCG) has a log K_{ow} of 4.41 (Xie and Dryssen, 1984) and was chosen to determine whether the relationship between TCB uptake rate and MO_2 can be applied to other xenobiotics. Rainbow trout (409.5 ± 0.02 g) were exposed to 260 μl^{-1} TCG at low or high swimming speed for 2 h and large scale suckers (*Catastomus macroheilus*) (706 ± 0.05 g), which could not be forced to swim at a high velocity, were exposed to 260 μl^{-1} TCG at two different

temperatures (9 or 18°C). Tetrachloroguaiacol dissociates and the concentration of the non-dissociated form was calculated from water pH and the pK_a for TCG (6.19, Xie and Dryssen, 1984). The uptake rate of TCG was divided by the concentration of non-dissociated TCG and is significantly correlated with MO_2 (r_2=0.97)(Fig. 8). The regression line (and 95% confidence interval) describing TCB uptake rate relative to MO_2 in fish greater than 11g (from Fig. 7) is also plotted in figure 8 and approximates the TCG data. Thus, the equation of the regression line describing TCB uptake rate relative to MO_2 can be used to predict the uptake of TCG and likely other xenobiotics similar in log K_{ow}.

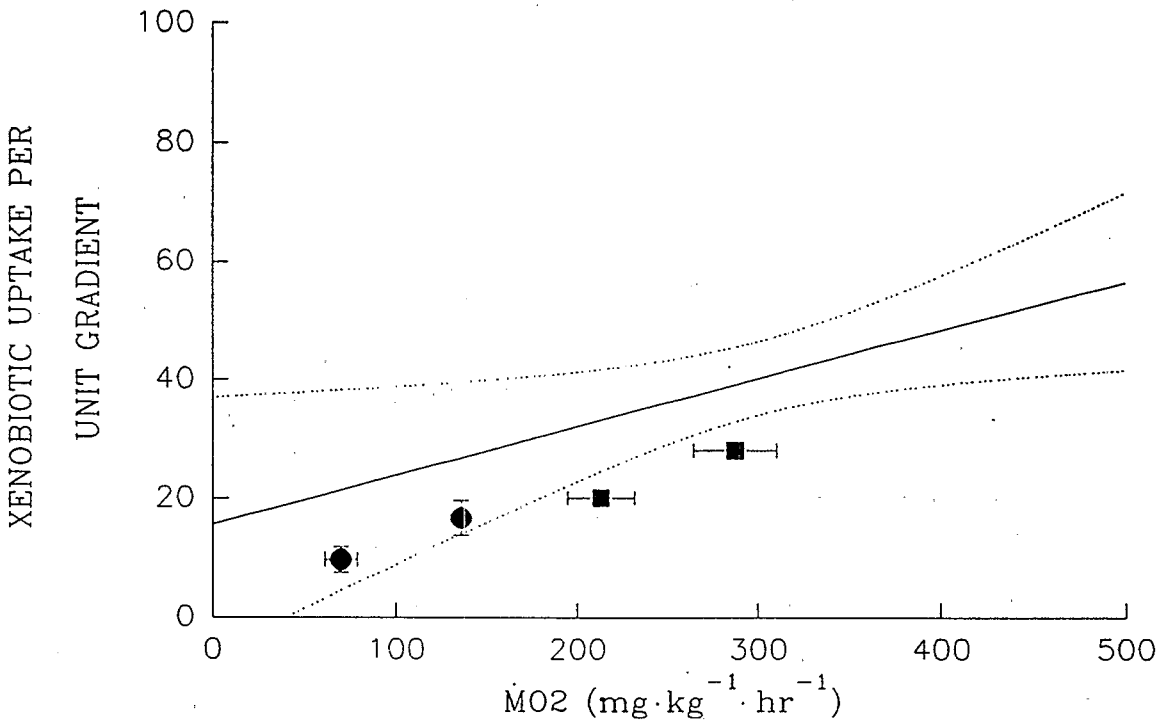


Figure 8. The effect of oxygen consumption rate on tetrachloroguaiacol (TCG) uptake ($mg \cdot kg^{-1} \cdot hr^{-1}$) per unit gradient TCB ($mg \cdot l^{-1}$) in rainbow trout 410 g (solid squares, n=8) and large scale suckers 706 g (n=8)(r^2=0.97). The regression line and 95% confidence interval is for TCB uptake per unit gradient for goldfish, medium and large rainbow trout (the lower regression line) in Fig. 7.

The model proposed above at present is restricted to conditions of normoxia, during initial exposure to xenobiotics. It is simple, and requires limited information about the xenobiotic to which a fish is exposed (log K_{ow} and pK_a if it is a weak acid) and the metabolic rate of the fish. The metabolic rate of the fish can be measured directly or estimated from the oxygen database OXYREF. The use of this model to predict the uptake of xenobiotics over the log K_{ow} range of 1 to 4.3 remains to be tested. We are presently testing and expanding this model to predict xenobiotic uptake over a longer duration of exposure as the animal reaches equilibrium with the environment.

ACKNOWLEDGEMENT

This work was supported in part by Cooperative Agreement CR816369 from The U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia.

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TOXIC EFFECTS OF MEOTHRIN ON THE GILL ULTRASTRUCTURE IN GRASS CARP, *CTENOPARYNGODON IDELLUS*

Bingsheng Zhou[1], Yongyan Zhang[1] and Ying Xu[1]

ABSTRACT

Fingerling Grass carp, *Ctenopharyngodon idellus*, were exposed to two different concentrations of meothrin close to the 24 hr LC50, and gill morphology subsequently examined by light and electron microscopy. Fish exposed to meothrin experienced convulsions, coughing, ataxia, intermittent paralysis and death. Post-mortem examination revealed significant morphological alterations in exposed fish compared to control fish. In both exposed groups, short exposure to meothrin resulted in hypertrophy of the secondary lamellae, which degraded to local telangiectasia (fusion between secondary lamellae) upon longer exposure. Ultrastructural changes in the gills included collapse of the pillar cell system which resulted in large non-tissue spaces which were invaded by leucocytes. Myelin figures and electron-dense deposits were accumulated in the cytoplasm of the epithelial cells. Chloride cells appeared severely damaged, showing degenerated mitochondria and ruptured nuclear membranes. The higher concentration of meothrin caused a loss of adhesion between the epithelial cells, accompanied by collapse of the structural integrity of the primary lamellae and degeneration of epithelial and chloride cells.

INTRODUCTION

Meothrin or fenpropathrin ((s)-a-cyano-3-phenoxybenzyl(s)-a-(4-chlorophenyl)-3-methylbutylate), a synthetic pyrethroidal insecticide, is used to control insects and mites on grain and cotton crops, fruit trees, vegetables, flowers and other crops. Meothrin is therefore wildly distributed in the surrounding environment. There have been many reports of cypermethrin uptake, depuration, metabolism and toxicology in fish and aquatic animals (Coats et al., 1979; Mclease, 1980). Although some reports of meothrin hydrolysis in water and its metabolism in plants have been published (Mikami et al., 1985), there are only a few systematic descriptions of the toxic syndromes associated with exposure to the pesticide. Such descriptions are vital to an understanding of the mechanisms of action of the pesticide. Meothrin is highly toxic to fish, with a 24 hr LC50 of 2.2 ppb. No detailed histological studies of the effects of meothrin on aquatic animals are available, and the exact mechanism of meothrin poisoning in fish has not been ascertained. The gill, which serves as a major organ for respiration and osmotic regulation, is easily damaged by even low concentrations of numerous pollutants (Skidmore & Tovell, 1972; Papathanossiou & King, 1983; Lars et al., 1983; Karlsson et al., 1985; Waster et al., 1988) and pesticides (Drewett & Abel, 1983). Zinc, copper, cadmium, sodium bromide (Waster et al., 1988), as well as lindane and DDT, have been reported to cause histopathological changes in fish gill and other organ structures (Mathur, 1962). Gill damage can, of course, be severely debilitating to fish in many ways, including impairing oxygen uptake (Lars et al., 1983; Waster et al., 1988). The purpose of this paper is to identify the histopathological effects of meothrin on the gill structure of the Grass carp using light and electron microscopy, and to evaluate potential mechanisms involved in the reaction of gill tissue to this pesticide.

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MATERIALS AND METHODS

EXPOSURE OF FISH TO MEOTHRIN

Seventy Grass carp, with a wet weight of 9.5-10.5 g, and a total length of 10-11.5 cm, were obtained from the fish pond of the Institute. The fish were acclimated for 1 week at 23±1 °C in aquaria filled with tap water filtered by active carbon. A stock solution of 99.9% pure meothrin (Sumitomo, Japan) was used in all experiments. Test solutions of 1 and 3 ppb were prepared by adding the appropriate quantity of meothrin acetone solution to water to make a final volume of 10 L. Control solutions contained the same concentration of acetone as the test solutions. Test solutions were completely replaced with a fresh solution every 12 hr in the 1 ppb test group and every 2 hr in the 3 ppb test group by siphoning out the old solution and replacing it with fresh solution.

PREPARATION OF THE SAMPLES

Fish gills were removed from experimental animals after 6, 12, 24, 48, and 72 hr of exposure in the 1 ppb test group and 1, 2, 4, 6, 8, and 10 hr in the 3 ppb test group. In order to avoid changes in the gill filaments before chemical fixation, in situ fixation with 0.1 M PBS buffered (pH 7.2) 2.5% glutaraldehyde was used to minimize shrinkage. Gill filaments were then dissected out, fixed in the same glutaraldehyde solution as above for at least 4 hr, and then post-fixed with osmium tetroxide (1% in the same buffer) for 2 hr. After acetone dehydration, specimens were embedded in Epon 812 resin and polymerized in an oven at 60°C. Semi-thin sections were obtained with a LKB ultratome and stained with 1% toluidine blue in 1% Borax. A number of these sections were used for light photomicrography. Ultra-thin sections were cut with glass knives, stained with a saturated solution of uranyl acetate in 50% alcohol, post-stained with lead citrate, and examined under a JEM-100CX transmission electron microscope.

RESULTS

TOXICITY

Behavioural symptoms of a toxic reaction were observed among the Grass carp after immersion in meothrin. The affected fish in 1 ppb meothrin frequently burst into bouts of rapid swimming and often attempted to burrow into the bottom of the tank after 6 hr exposure. Some fish experienced coughing and convulsions. Affected fish in 3 ppb meothrin suffered coughing and convulsions after 2 hr of immersion. These fish subsequently showed symptoms of paralysis and ataxia and eventually died. No mortality was recorded for up to 10 hr in the remaining 3 ppb groups. No abnormal behaviour or mortality was recorded in the control group.

LIGHT MICROSCOPY EXAMINATION

In the 1 ppb meothrin test group, light microscopy examination of the gills revealed disarray of the secondary lamellae and a slight curl-over as compared with the control fish gills (Fig. 1) so that the maximum length from the lamellar margin to the gill filaments was reduced. The lesions of the secondary lamellae included hypertrophy of the secondary lamellar epithelium, and collapse of the pillar cell system (Fig. 2). Some of the secondary lamellae were fused, and occasionally a group of secondary lamellae fused so completely that there were no conspicuous interlamellar spaces.
In the 3 ppb meothrin test group, the secondary lamellae were severely degenerated compared with the lamellae of the 1 ppb test group. After 2 hr exposure to meothrin, approximately 50% of the secondary lamellae were grossly curled over. After 4 hr exposure, the secondary lamellar epithelium

was completely separated from the pillar cell system to form a large non-tissue space. Some secondary lamellae were clogged with a large accumulation of blood cells at the distal ends as a result of telangiectasia (Fig. 3). Following 8 hr immersion in meothrin, the secondary lamellae showed a significant increase in the number of chloride cells and hypertrophy of the epithelial cells. The apical epithelial tissue of the secondary lamellae had increased in thickness to more than 1 cell layer.

TRANSMISSION ELECTRON MICROSCOPY EXAMINATION

The normal epithelium of secondary lamellae in the Grass carp gill is simple, consisting of a thin, single or double cell sheet separated by the pillar cell system and blood lacunae (Fig. 4). The epithelia of the primary lamellae have chloride cells, mucous cells and accessory cells interposed between the epithelial cells. After immersion in 1 ppb meothrin, the apical portion of the secondary lamellae became enlarged. Lesions of the secondary lamellae were mainly characterized by an increased diffusion distance, due to the swelling of the epithelia. Necrotic cells, pillar cells, myelin figures and leucocytes were frequently found in these spaces (Fig. 5). The epithelial cell membrane was irregular in shape and protruded in the ambient water (Fig. 6). Degenerated and necrotic epithelial cells were often torn from the epithelium and many epithelial cells had lost their typical flat shape and had become rounded. The cytoplasm of the epithelial cells was vacuolated and showed accumulation of myelin figures (Fig. 7), organelles and electron-dense deposits (Fig. 8). The pillar cell, which is dominated by the nucleus and thin cell flanges, were irregular in shape and microridges protruded into the blood vessels associated with the epithelium. Some sections of the secondary lamellar pillar cell system had severe ultrastructural damage and formed extensive clubbings with blood cells (Fig. 3).

The most pronounced change compared to control gill tissue was the hypertrophy of the secondary lamellae, and the increased number of epithelial and chloride cells in this part of the gill. The central lamellar blood vessels were markedly reduced in size and in some cases were not easily identified (Fig. 6). The tips of the secondary lamellae were more heterogeneous than normal, containing accumulations of chloride and mucous cells (Fig. 5). Chloride cells were often degenerated, showing loss of cytoplasm and loss of contact with neighbouring epithelial cells, which resulted in large intercellular gaps. Chloride cell mitochondria were severely damaged with decreased cristae (Fig. 9) and swollen or ruptured nuclear membranes (Fig. 10).

Gills from the 3 ppb test group were similar to that of the 1 ppb test group. Again, the most pronounced change in the secondary lamellae was an increase in the number of chloride and mucous cells, and the vacuolation and cytoplasmic protrusion of the epithelium (Fig. 11). Surface extensions of these rounded epithelial cells were longer and more numerous than extensions of epithelial cells from control gills. Loss of adhesion between the epithelial cells and chloride cells, and rupture of the basement membrane, resulted in dispersion and degeneration of cell contents with a resultant decrease in staining quality (Fig. 12). Some cells were almost completely disconnected from the lamellae.

DISCUSSION

The secondary lamellae, where the major portion of gas exchange takes place, are a very sensitive part of the respiratory system (Karlsson, 1985). The most overt syndromes of meothrin poisoning in fish gills, i.e. hypertrophy and telangiectasia, are similar to the structural changes observed in the gills of other fish species exposed to other pollutants (Smart, 1976; Temmink et al., 1983; Stoker et al., 1985). Telangiectasia was observed in all test fish in this study. This type of structural damage shows a close similarity to lesions caused by Cd (Karlsson et al., 1985), Zn (Skidmore & Tovel, 1972), and ammonia (Smart, 1976). Hypertrophy of the second lamellae also

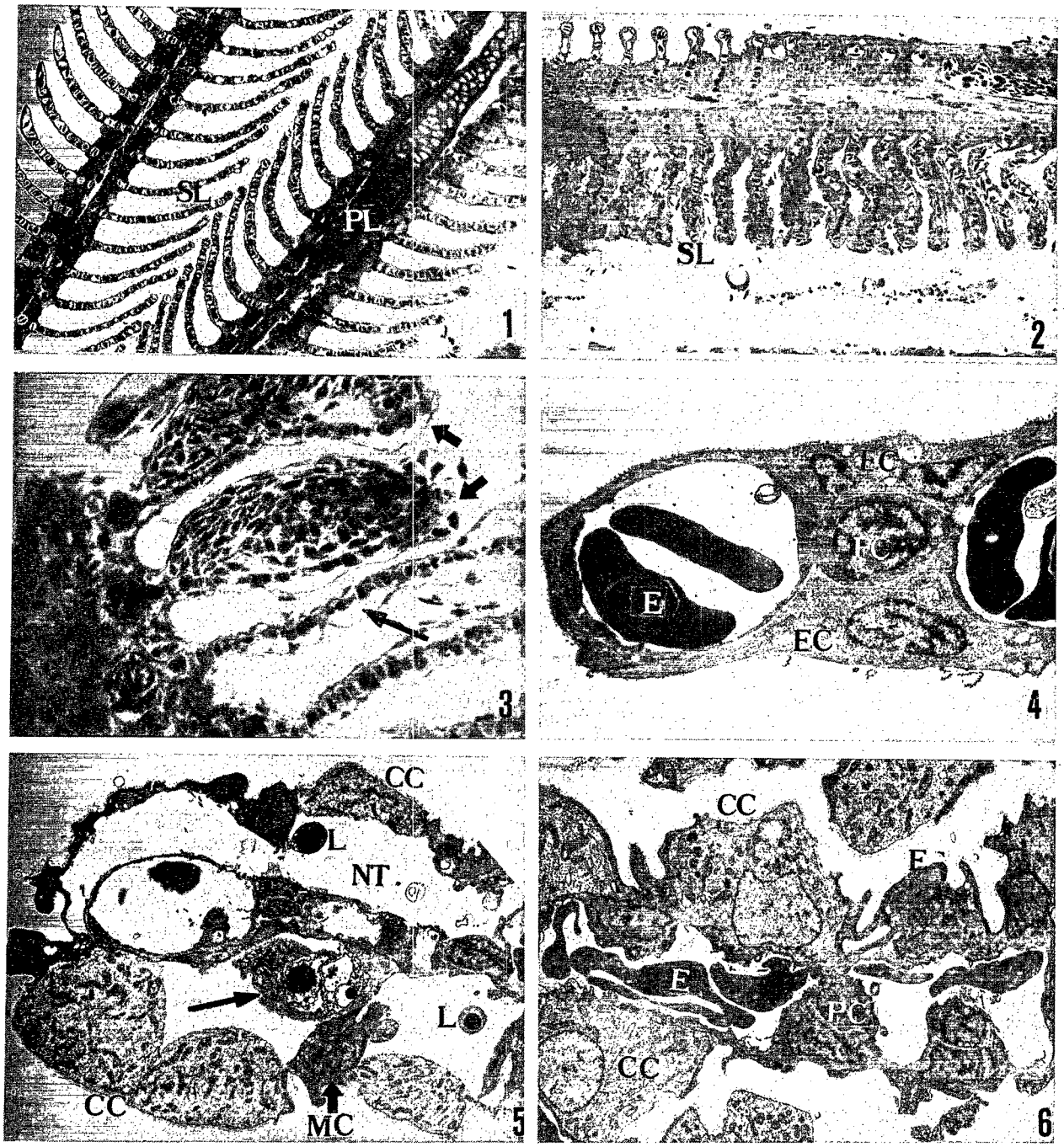

Figure 1. Section through the gill filament of control fish, showing the longer secondary lamellae (x360). **Figure 2**. Section through gill filament of fish exposed to 1 ppb meothrin for 6 hr. Hypertrophy of the secondary lamellae can be seen (x250). **Figure 3**. Local extensive clubbing with blood (short arrow) and large non-tissue spaces (long arrow) of secondary lamellae in fish exposed to 3 ppb meothrin for 10 hr (x575). **Figure 4**. The tip of a normal secondary lamella from a Grass carp gill. The double later of epithelial cells and a pillar cell are seen (x4,550). **Figure 5**. The tip of a secondary lamella of a Grass carp gill after exposure to 1 ppb meothrin for 12 hr. Note the epithelial layer is more heterogenous than normal, containing an increased fraction of mucous cells, chloride cells, and large non-tissue spaces which are invaded by leucocytes. A necrotic epithelial cell can also be seen (arrow) (x2,400). **Figure 6**. The centre part of a secondary lamella after exposure to 1 ppb meothrin for 12 hr. The epithelial cells have become rounded and hypertrophied, and irregular erythrocytes can also be seen (x3,500). *Abbreviations on following page.*

Abbreviations: SL, secondary lamella; PC, pillar cell; EC, epithelial cell; NT, non-tissue space; MC, mucous cell; N, nucleus; PL, primary lamella; L, leucocyte; CC, chloride cell; E, erythrocyte; M, mitochondria; MF, myelin figures

Figure 7. An epithelial cell after exposure to 1 ppb meothrin for 12 hr containing many myelin figures and cell inclusions (arrow) which were composed of degenerating mitochondria (x8,450).

Figure 8. A degenerating epithelial cell after exposure to 1 ppb meothrin for 12 hr containing electron-dense inclusions (x7,200).

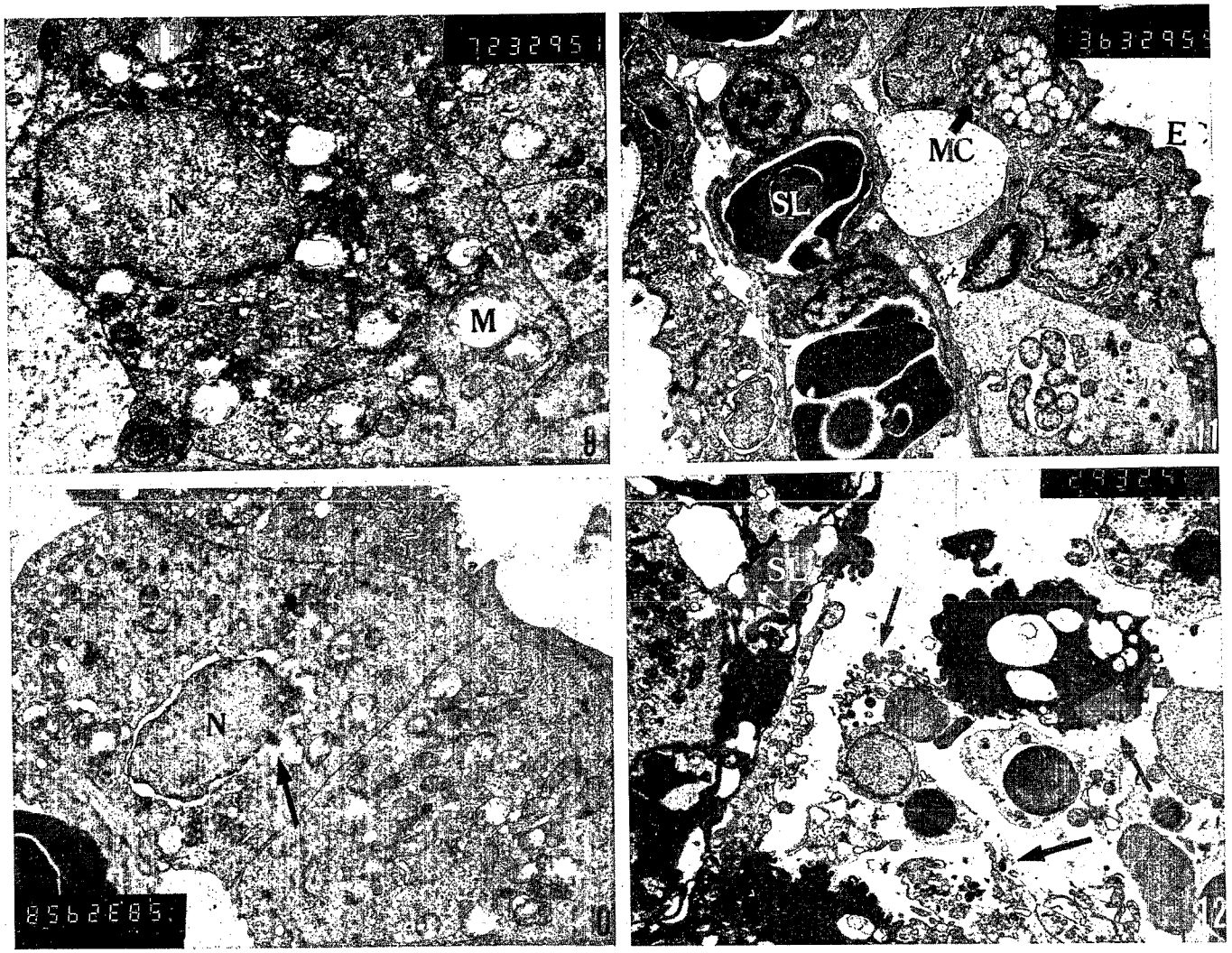

Figure 9. A chloride cell in the primary epithelium of a grass carp after exposure to 1 ppb meothrin for 12 hr. Note all mitochondria are damaged, and many of them no longer have cristae (x8,450).

Figure 10. Chloride cells in the primary epithelium of a Grass carp gill after exposure to 1 ppb meothrin for 12 hr. Note the collapsed nuclear membrane (arrow) and degenerating mitochondria (x7,100).

Figure 11. The central part of a secondary lamellae after exposure 3 ppb meothrin for 8 hr (x4,800).

Figure 12. Primary epithelium of a Grass carp gill after exposure 3 ppb meothrin for 6 hr, showing epithelial cells lost their contact with neighbouring cells and lost their capability of staining (arrow) (x4,100). *Abbreviations on previous page.*

occurred in all test fish and is closely comparable to the damage caused by tributyltin compounds (Holm et al., 1991) and lindane (Drewett & Abel, 1983). Fusion of the secondary lamellae may be a secondary result of gill hypertrophy, and has been reported in the gills of fish exposed to chromate. The results suggest that hypertrophy and telangiectasia of secondary lamellae are the primary symptoms of exposure to toxic pollutants. The secondary lamellar epithelium can vary in thickness between one or two cell layers, and is usually thinnest adjacent to the blood vessels. The nuclei of the epithelial cells frequently align with the pillar cell nucleus to minimize the gas exchange distance. The epithelial cells are sometimes separated by an intercellular space, which has been suggested to contain lymph (Hughes & Morgan, 1973). Such a lymphatic space may have an important function in the regulation of respiratory or osmotic exchange. Since swelling of these spaces and infiltration by leucocytes is a common tissue reaction to toxic exposure, the increased non-tissue space could reduce the diffusion capacity and result in reduced gas exchange. The accumulation of leucocytes is probably a defense reaction by the immunological system to the toxin (Temmink et al., 1983).

The plasma membrane of the epithelial cells normally forms microvilli-like structures. Such folding may increase the functional lamellar area and produce microturbulence, enhancing the effectiveness of the exchange processes over the surface epithelial (Karlsson et al., 1985). Reduction of these structures and other changes in the epithelial cell surface could diminish the capacity for gas exchange. The loss of normal epithelial cell shape, and the appearance of microridges and cytoplasmic protrusions after exposure to meothrin may be due to osmotic perturbation and resultant swelling of the secondary lamellae. The increased number of degenerating and necrotic epithelial cells are indicative of the reduced life span of these cells after exposure to the toxin. Such gill lesion combined with the increased number of myelin bodies in the secondary lamellar epithelial cells could affect blood circulation, and further reduce respiratory function.

Most chloride cells are found in the stratified epithelium separating the bases of successive lamellae, although some chloride cells are also found in the secondary lamellae. Accumulation of chloride cells in the secondary lamellae was detected only following acute, experimental, heavy metal contamination and other severe environmental changes. Chloride cells are easily identified partly due to their dense population of mitochondria, and the abundance of granular endoplasmic reticulum. The structure and function of chloride cells have been studied by many authors (Karlsson, 1983; Cioni et al., 1991). The basal lateral cell surface has been shown to be the site of the K-dependent, ouabain-sensitive phosphatase component of the Na-K-ATPase enzyme complex (Karnaky et al., 1976), and many reports suggest that chloride cells are involved in the osmoregulatory mechanism of fish gills (Karlsson, 1983; Karnaky, 1986). The most pronounced effect of meothrin on chloride cells was the degeneration of the mitochondria, which has seldom been reported in fish gills exposed to other toxins. Such damage of the mitochondria may be caused by the meothrin itself or by changes in osmolarity, and is likely to severely affect the functioning of the chloride cells. The swelling and degeneration of the chloride cell nuclear membrane may have been the result of changes in osmolarity. Lesions of mitochondria can cause severe inhibition of Na-K-ATPase, enzymes which are vital for energetic processes such as oxidative phosphorylation (Cionic et al., 1991). Chloride cell mitochondria are the first organelles to have shown alteration and signs of degeneration implying dysfunction due to exposure to toxins. Several models of the mechanistic action of pollutants such as tributyltin (Holm et al., 1991) and cadmium (Papathanassiou & King, 1983) have been proposed, and include the inhibition of mitochondrial oxidative phosphorylation. The present study confirms the usefulness of morphology in the identification of gill damage. Possible effects on energy metabolism and on the brain and nervous system should be investigated through biochemical, histological and physiological studies.

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CHEMICAL AND BIOLOGICAL CONTROLS ON THE BIOACCUMULATION OF HYDROPHOBIC ORGANIC COMPOUNDS IN FOODWEBS

Deborah L. Swackhamer[1]

INTRODUCTION

The accumulation of hydrophobic organic compounds (HOCs) in fish is of grave concern due to the possible adverse health effects on aquatic organisms as well as potential human health effects in fish-eating populations. In the Laurentian Great Lakes of the United States and Canada, the lack of reproduction of lake trout has been attributed to contamination by chlorinated organic contaminants such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzodioxins and furans (PCDDs and PCDFs) (Mac et al., 1991). Deformaties in several colonies of different species of fish-eating birds correlate with concentrations of organochlorine contaminants (Giesy et al., 1994). Also, developmental deficiencies have been observed in children born to women who consume large amounts of Great Lakes fish (e.g. Jacobson and Jacobson, 1984) raising concerns about trans-generated effects in humans due to chronic exposures to chlorinated organic compounds.

Fish accumulate contaminants from water exposure and also from the food web. In the Great Lakes, top predators such as lake trout are thought to accumulate more than 90% of their body burden of PCBs from food consumption, making transfer across the gill a minimal exposure route. This species has an extensive foodweb consisting of four to five trophic levels. Thus to fully understand the process of bioaccumulation of contaminants in fish, one must understand bioaccumulation in each step of the food web. This paper focuses on the bioaccumulation process in the primary trophic level of foodwebs.

The primary trophic level plays an inportant role in determining the fate and transport of HOCs in aquatic systems. HOCs preferentially associate with phytoplankton because of their high lipid content of 10-40%. The particulate-associated HOCs can then be transferred to the foodweb by grazing, or be transported to bottom sediments by settling.

It follows that the accumulation of HOCs by phytoplankton should be dependent on both the physical-chemical properties of the HOC, as well as biological characteristics of the phytoplankton. The chemical properties include solubility, octanol-water partition coefficient (Kow; a measure of lipophilicity), molecular weight and size, and molecular configuration. The phytoplankton characteristics that should be considered are lipid content differences among species, lipid composition, surface area, and surface type, and the growth rate of the population. While previous research has focused on the chemical controls affecting the association of HOCs with phytoplankton, our premise is that biological controls are as, if not more, important in regulating the bioaccumulation process.

The chemicals of concern in the Great Lakes ecosystem are typical of aquatic systems impacted by both long-range atmospheric transport and point source inputs. Organic chemicals that bioaccumulate in fish include PCBs, DDT and metabolites, dieldrin, chlordane, nonachlor, oxychlordane, PCDDs and

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PCDFs, hexachlorobenzene, toxaphene, and mirex. These chemicals share certain properties, generally imparted by the presence of chlorine substituents: they are low in aqueous solubility, they are low in reactivity and thus persistent, they are hydrophobic (log Kow > 4), and they exhibit toxicity.

Mathematical models have been constructed that predict HOC concentrations in fish from known water concentrations, by making assumptions about the uptake of chemical at each step of the foodweb. These so-called "foodchain" models typically begin by assuming that the primary trophic level is the first step in the pelagic food web, and that the HOC reaches an equilibrium partitioning between the water and the phytoplankton lipids (e.g. Thomann and Connolly, 1984). Implicit in this approach is that HOCs have a similar solubility in lipids as they do in octanol. Thus it is assumed that the lipid-normalized bioaccumulation factor (BAF) is equal to the Kow of the HOC:

$$BAF = [HOC]_{phytoplankton}/[HOC]_{water}$$

and

$$BAF/\text{fraction lipid} = Kow$$

Several researchers have demonstrated the validity of this assumption in fish. The BAFs for fathead minnows determined in lab experiments for a wide range of chemicals were correlated to the chemicals' Kows resulting in a relationship having a slope near to one (Veith et al., 1979; Mackay, 1982). The slopes near unity support the above assumptions to a first order approximation. As a result, current phytoplankton bioaccumulation models assume that the process is a thermodynamic partitioning to lipids that occurs quickly relative to other processes (i.e. equilibrium is assumed).

This paper will report on laboratory experimental results that evaluate the effect of biological controls on bioaccumulation and call into question the conventional modeling approach described above.

METHODS

The methods used on our studies have been described in detail elsewhere (Swackhamer and Skoglund, 1991; Swackhamer and Skoglund, 1993; Skoglund and Swackhamer, 1993; Stange and Swackhamer, 1994), and will be summarized here. Uni-algal cultures of phytoplankton (obtained from the Starr Collection, University of Texas at Austin) were incubated in batch experiments with a mixture of 40 polychlorinated biphenyl (PCB) congeners. These 40 congeners were chosen as representative HOCs having a wide range of known physical-chemical properties. Under specified growth conditions, experiments were maintained for 20-30 days and samples taken at specified time points. The PCB concentrations were measured in the biomass and media to calculate a BAF for each compound at each time point, and the results correlated to controlled changes in the experimental variables. Experiments were conducted at two different growth rates, "low" (0.3-0.9 day^{-1}) and "active" (0.13 day^{-1}), and for four genus of algae, Scenedesmus, Selenastrum, Synedra, and Anabena. Cultures were maintained with 16 hours light/8 hours dark, and growth was regulated by temperature (10-12 C for low, 20-21 C for active). Bold-Allen media (Nichols and Bold, 1965; Allen, 1968) made in filtered Lake Superior water was used in all experiments. Total PCB concentration was approximately 7 ng/mL.

Phytoplankton biomass was Soxhlet extracted in 1:1 hexane-acetone, and the media phase batch-extracted with hexane. Extracts were cleaned with alumina and silica gel column chromatography, and analyzed by gas chromatography with electron capture detection using a 60 m DB-5 column by the internal standard method.

Lipid content and composition were determined using a micro-extraction technique after Gardener et al. (1985) as described in Stange and Swackhamer 1994).

RESULTS AND DISCUSSION

We have shown from these experiments and have discussed elsewhere the dramatic effect of growth rate on the uptake and magnitude of PCB bioaccumulation by algae (Swackhamer and Skoglund 1993, Skoglund and Swackhamer 1993). We found that under limited or "low" growth conditions, the uptake of PCB congeners with log Kow < 6 was related to the congener's Kow indicating that the process is a thermodynamic partitioning into lipophilic materials. However, we observed the process to be much slower than assumed, with equilibrium not fully achieved after 20 days of exposure. The uptake for congeners with log Kow > 6 was not correlated to Kow, indicating that the uptake of these very hydrophobic compounds was strongly limited by the kinetics of uptake, or physically inhibited by the cell membrane in some way. This will be discussed further below.

The uptake of PCB congeners under "active" growth conditions was not related to Kow. We interpreted this as a dilution phenomena caused by the biomass increase during growth. The uptake rate is slow relative to growth rate, such that equilibrium is never reached during active growth. Thus concentrations of PCBs were lower in actively growing cultures compared to dormant cultures, and could not be predicted by Kow. These experiments are summarized in Figure 1 The implication of these experiments is that the conventional approach to modeling phytoplankton bioaccumulation of HOCs is invalid for actively growing blooms, leading to large uncertainties in fish bioaccumulation models. While the physical-chemical properties of the compound control bioaccumulation when growth is minimal, it is the biological control of growth rate that influences bioaccumulation in active populations.

These results also indicated that bioaccumulation was a two-step process, conceptually seen as a relatively quick sorption to the surface of the cell followed by a much slower transport across the lipid bilayer membrane into the cell. Thus given enough time, equilibrium is reached with cell internal lipids; in short time, the compounds are surface-associated and do not reach equilibrium. We have modeled the effect of growth by assuming this two step process and using rate constants for uptake and loss from the literature (Connolly and Pedersen, 1988) to demonstrate the effect of growth rate and Kow on the magnitude of bioaccumulation (Figure 2). When growth is zero, BAF has a direct and linear relationship to Kow. At higher growth rates, BAF is sharply decreased for the more hydrophobic compounds, and is independent of Kow. For more highly hydrophobic compounds, the BAF in phytoplankton can vary by 3 orders of magnitude for growth rates varying from 0 to 1 day^{-1}. Thus the effect of growth on BAF and the subsequent effect on contaminant concentrations at higher trophic levels cannot be ignored.

We repeated the "low" growth experiments across different species to further evaluate the biological controls on uptake. We chose a representative green, blue-green, and diatom species to compare differences in lipid content, lipid composition, and surface area. The results from these experiments were similar to those described above. The uptake was slow, and was proportional to Kow. However, the rates of uptake and the magnitude of uptake differed among the different species. Differences were not related to the surface area of the different species. Normalization of the BAFs to total lipid content slightly decreased the differences among species. To further elucidate the effect of lipids, the BAFs were normalized to one of three different lipid classes: glycolipids (internal lipids), neutral lipids (storage lipids), and phospholipids (membrane structural lipids). The results are shown in Figure 3. Interestingly, differences among species for congeners with log Kow < 6.5 were minimized by normalization to glycolipids, whereas differences among species for congeners with log Kow > 6.5

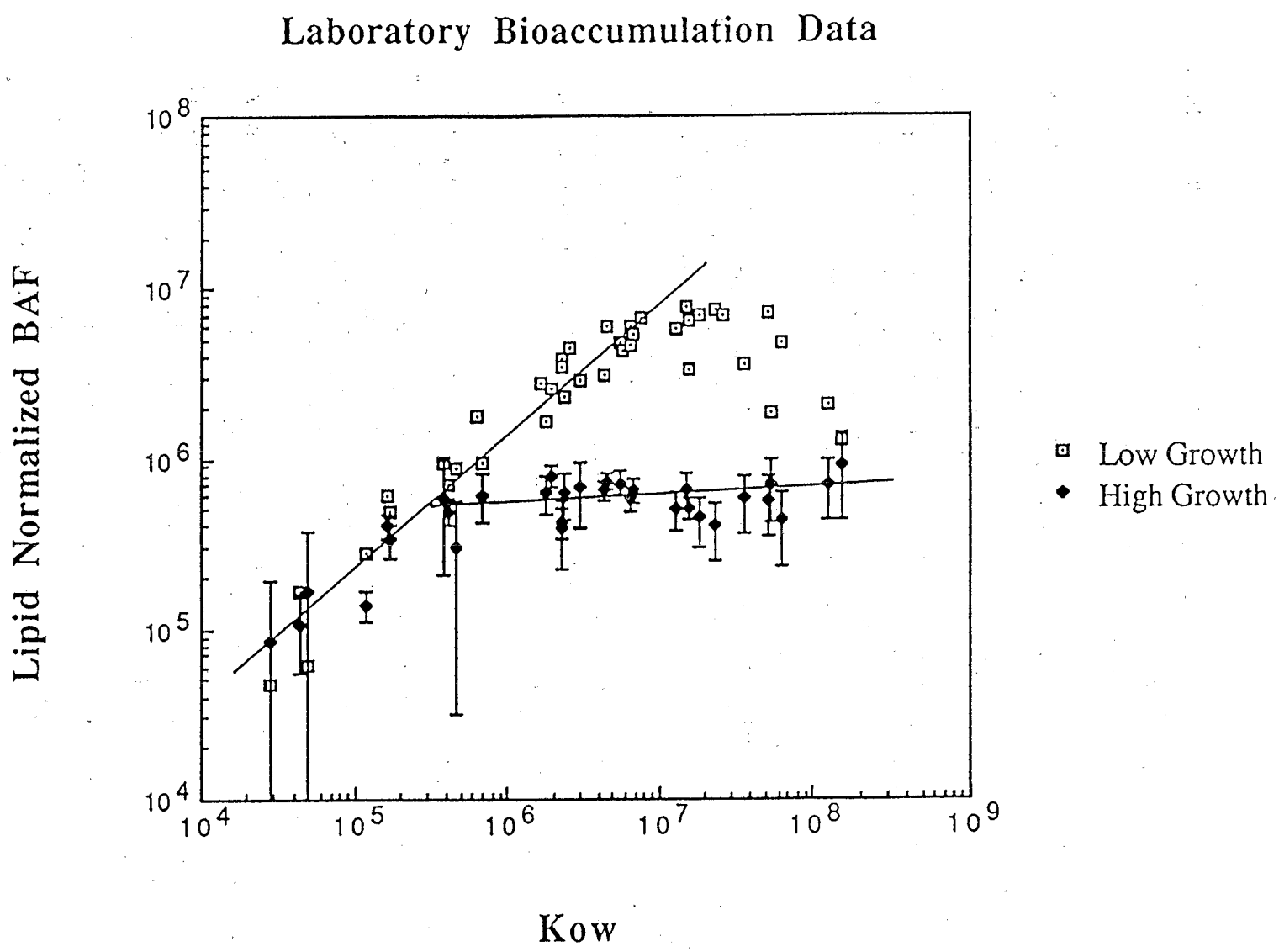

Figure 1. Log of the observed BAF normalized to lipid fraction from two laboratory experiments for 40 PCB congeners vs. the log Kow of the congener. Growth rates in low growth and high growth experiments were 0.03 and 0.16 day $^{-1}$, respectively. Algae was Scenedesmas sp. Regression line for high growth experiment not significantly different from zero. (from Swackhamer and Skoglund, 1993).

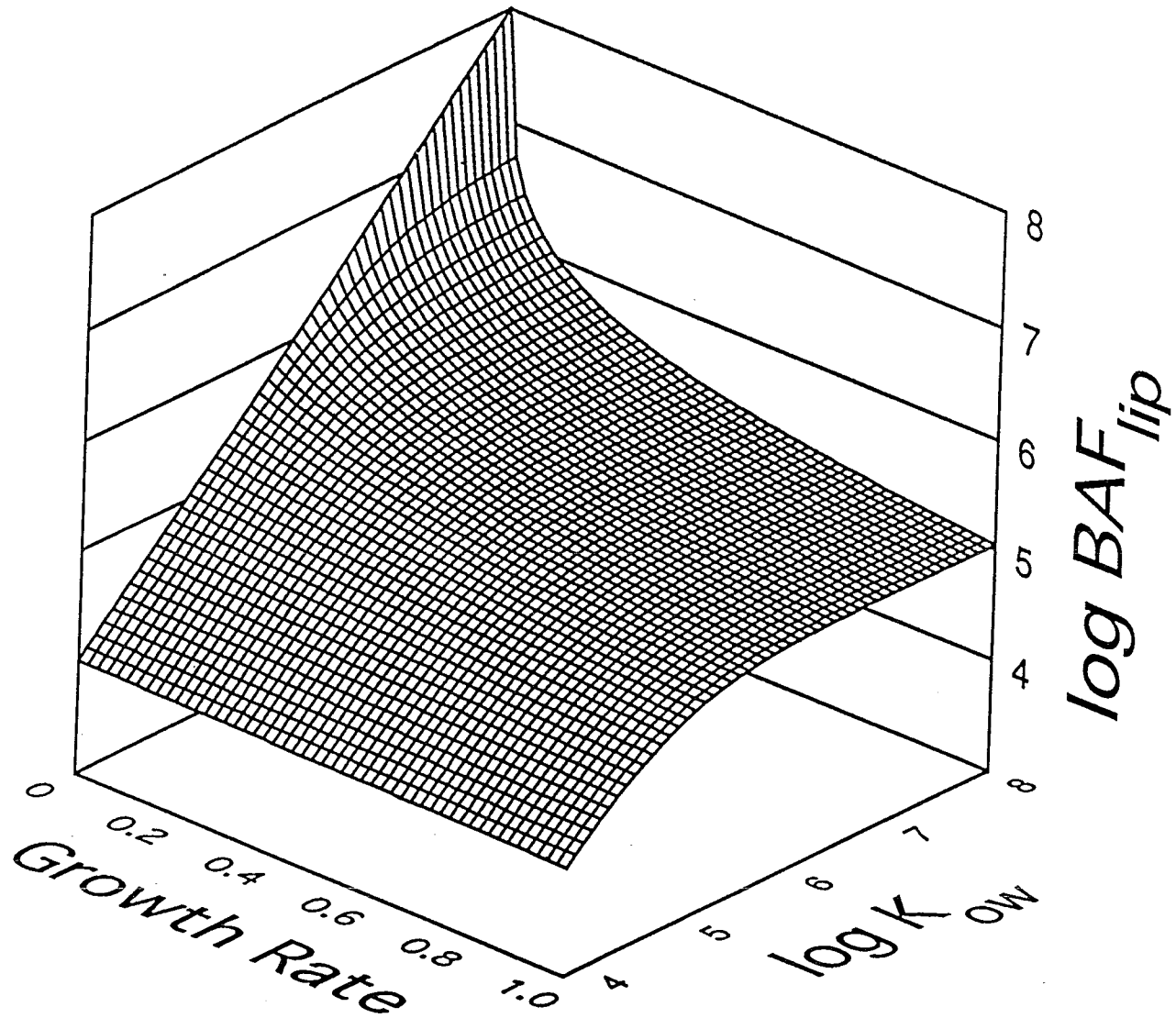

Figure 2. Theoretical relationship of the Log BAF normalized to lipid to growth rate of phytoplankton and the log Kow of the compound. (from Skoglund and Swackhamer, 1994).

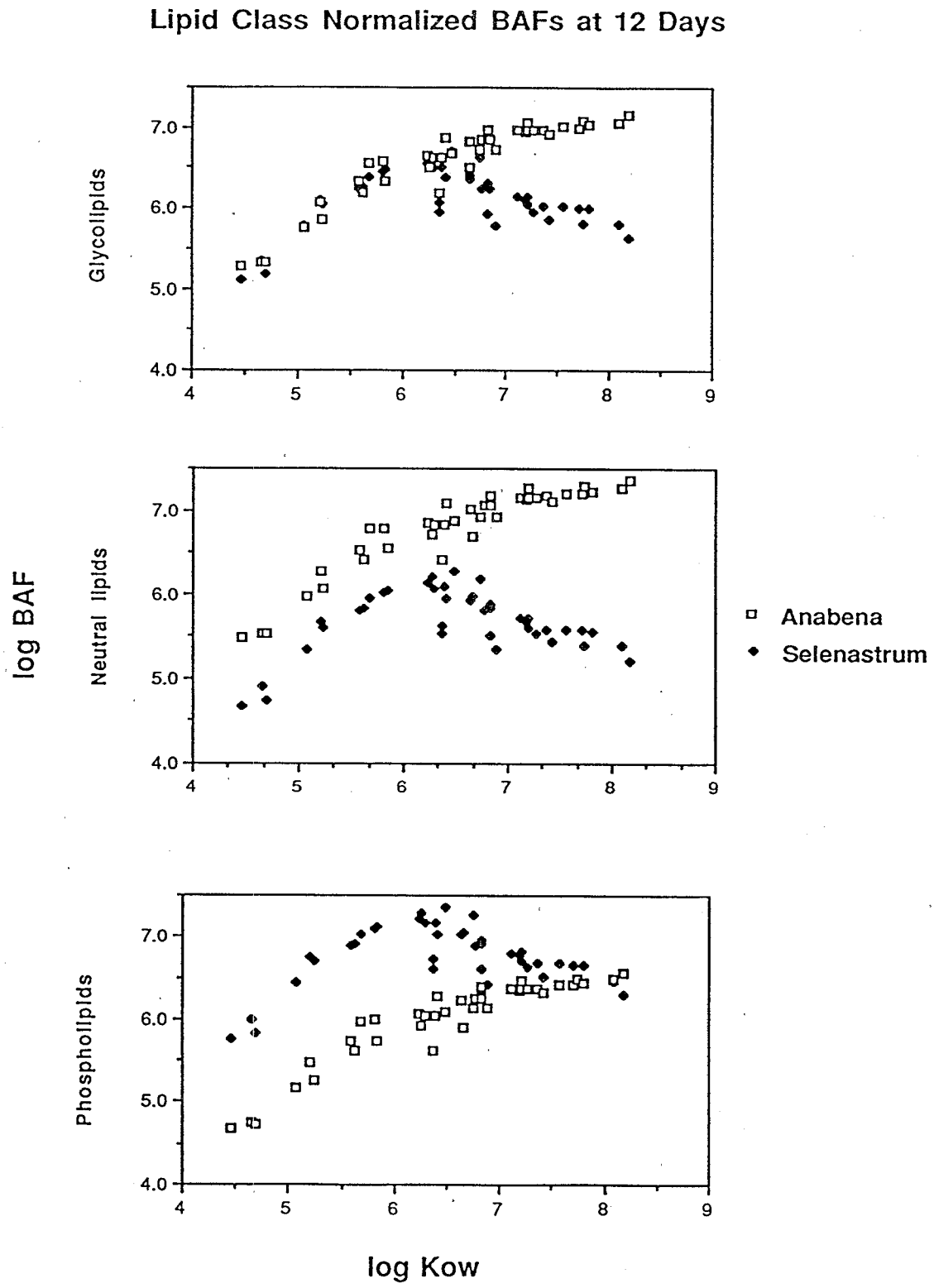

Figure 3. Observed BAFs for 40 PCB congeners in laboratory experiments where growth rate was minimized for two species of algae vs. log Kow of the congener. The BAFs are normalized to three different lipid classes.

were minimized by normalization to phospholipids. This provides support for our earlier interpretation that uptake for the medium hydrophobic compounds is a thermodynamic partitioning to internal cellular lipids (albiet a slow process). Thus species differences in BAF are minimized by normalizing to that pool of lipids. However, the very hydrophobic compounds appear to associate most strongly with the phospholipid membrane, supporting the concept that uptake of these compounds is physically restricted rather than limited by the kinetics of transport.

CONCLUSIONS

The results presented here underscore the importance of physical-chemical properties of contaminants as well as the biological properties of phytoplankton in controlling the bioaccumulation of contaminants in the primary trophic level. The hydrophobicity of the chemical, expressed as Kow, plays an inportant role in determining the magnitude of accumulation, and is linearly related to the BAF at equilibrium for compounds with $4 < \log Kow < 6$. These compounds appear to associate with the glycolipid fraction of total lipid, representative of the internal lipids of the phytoplankton. In contrast, the more highly hydrophobic compounds with $\log Kow > 6$ seem to accumulate at the lipid bilayer and do not move into the cell, as indicated by their association with phospholipids. However, when phytoplankton populations are actively growing the increase in biomass occurs more quickly than the uptake of contaminants, thus preventing equilibrium from being achieved. Thus modeling frameworks describing the accumulaion of HOCs by phytoplankton must incorporate both chemical and biological processes, and include Kow, growth rate, and lipid content and class as variables.

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ECOTOXICOLOGICAL MONITORING OF MAJOR INDUSTRIAL EFFLUENTS IN NANJING, CHINA

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ABSTRACT

Physico-chemical analysis is at present the only method employed to supervise and manage the levels of industrial discharges in China. It is important to demonstrate that biological assays should be incorporated in the studies that are used to determine industrial effluent discharge limits. By using toxicity testing on aquatic organisms, this study evaluated the effects of effluents discharged from 24 factories, to determine which effluents were toxic and required control and regulation, and examines the efficiency of effluent treatments. Twenty-four effluents form twelve types of industries were tested. The biological toxicity data from these effluents did not entirely agree with the physico-chemical data gathered during the monitoring of these effluents.

Acute toxicity tests were conducted with two species of fish and one species of daphnids. Fourteen treated effluents, based on their chemical compostion, contained toxic chemicals in concentration which exceeded National discharge standards. Some of these effluents were toxic to the aquatic life. Ten treated effluents met National discharge standards, but were still highly toxic. The concentration-based acute toxicity measurements of the effluents were translated into acute toxicity units (TUa), and the annual discharge (AD) data of the effluents were tabulated. The Annual Discharge-Acute Toxicity Unit (AD-TUa) is recommended to assess the environmental impact of effluents, and to establish effluent priority with regard to toxicity.

Three high priority effluents were then studied in a 21 day chronic toxicity test using daphnids. Reproductions rate was the most sensitive indicator to these effluents, and the no observable effect concentation (NOEC) and lowest observable effect concentration (LOEC) of each effluent to *Daphnia magna* were derived. The range of acute and chronic ratios for these effluents was found to be from 10 to 100. Acute toxicity tests were performed on fish and daphnids using three selected effluents both before and after treatment. It was determined that the effectiveness of effluent treatment could not be monitored adequately by physico-chemical testing alone. The toxicity of one chemical effluent was tested downstream from the effluent outlet at three stations, and the water downstream was found to be lethal to *D. magna* with a short time.

The supervision of toxic effluent discharge must be strengthened by means of biological toxicity assays on the effluents. Effluent toxicity limits should be established and incorporated into discharge permits to prohibit the discharge of pollutants in toxic amounts.

INTRODUCTION

The impact of industrial effluents on the aquatic ecosystem is increasing with the development of modern industry in China. This impact has drawn the attention of both the public and environmental agencies. Pollution control is of urgent concern world-wide, and in the West, biological

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assays have become an essential test in the monitoring of toxic effluents (Thomas, 1988; Zwart, 1988; USEPA, 1991). Physico-chemical analysis is at present the only method used in the supervision and management of industrial discharge in China, and it appears to be less reliable in establishing effective guidelines especially when the effluent is a complex mixture of toxic pollutants or a mixture with unknown constituents. As control over conventional pollutants is strengthened in China, emphasis should be placed on the reduction of toxic chemical discharge. In order to control toxic discharge via water quality criteria, it is important to understand that the results of biological assays must be incorporated into the criteria to be used to set industrial effluent discharge limits. Nanjing is one of the major petrochemical industrial bases in China, and there are many factories along the Yangtze River. By using toxicity tests on aquatic organisms, this study aims to evaluate the ecological effects of effluents discharged from 24 factories in order to determine which are the most toxic, and to examine the efficiency of effluent treatment. Further studies and the application of ecotoxicological monitoring are recommended to control toxic discharge levels in China.

MATERIALS AND METHODS

TEST EFFLUENTS

Effluents were sampled directly from 24 factories in Nanjing representing 12 different kinds of industries (chemical, metallurgy, gas-making, pharmaceutical, chemical fiber, textile, hide-processing, electronic, instrument and meter-making, engine manufacturing, paper, and food plants). Both composite and grab samples were used. The chemical parameters of the effluents were measured at the Nanjing Municipal Environmental Monitoring Center according to the methods recommended by the NEPA of China (China NEPA, 1989). The ambient toxicity of one of the chemical effluents was tested at 3 different sites downstream from the outlet of the discharge station. Effluent samples were diluted with tap water aerated for 3 days prior to use. The dilution water had a pH of 7.2-8.5, dissolved oxygen of 6.9-8.1 mg/L, COD of 1.08 mg/L, conductivity of 294 μs/cm, and a hardness of 1.082 me/L.

TEST ORGANISMS

Juvenile Crucian carp (*Carassius auratus*) and Silver carp (*Hypophthalmichtys molitrix*) were obtained from the Freshwater Fisheries Institute of Jiangsu province, Nanjing. The fish were kept in holding tanks at a water temperature of 22±2 °C and a 12 h light:12 h dark photoperiod. The fish were last fed 24 h before experimentation. Daphnids (*Daphnia magna*) were obtained from the Laboratory of the Department of Environmental Sciences at Nanjing University.

TEST PROCEDURES

The testing procedures followed the methods recommended by the relevant agencies (APHA-AWWA-WPCF, 1985; China NEPA, 1986).

1. Acute Toxicity Tests

Renewal tests with fish were conducted in 40 L glass aquaria, while artificial stream tests were conducted in artificial laboratory streams measuring 31 L in volume, 11 cm in depth and with a flow rate of 100 ml/min. Each vessel contained 20 juvenile fish. Daphnia were kept in 250 ml glass beakers, with 10-20 daphnia per beaker. At least 5 test concentrations and a dilution water control were used in each experiment. The water temperature and photoperiod were maintained as in the

holding tanks. Observations were made after 24, 48 and 96 h of exposure. The end-point of testing was 96 h LC50 for both the fish and daphnids.

2. Chronic Toxicity Tests

Chronic tests with daphnids were conducted in glass beakers fitted with a side opening covered with #25 silk screen for overflow of test solution. The test liquid was delivered by a peristaltic pump and maintained at a volume of 120 ml per beaker. Each test concentration and control were run in triplicate. Five daphnids (12 h age) were placed in each beaker. The tests were run for 21 days at 22±1 °C and the test animals fed with concentrated green algae in medium. Mortality, reproduction rate and growth rate were recorded every day.

STATISTICAL ANALYSIS

The LC50 and 95% confidence intervals for the acute toxicity data were determined by the Trimmed Spearman-Karber method (Hamilton et al., 1978). One-way analysis of variance was performed on the chronic toxicity data.

RESULTS

The annual amount of discharge and the chemical characteristics of each effluent were determined from the Nanjing Municipal Environmental Monitoring Center. It could therefore be concluded whether or not a treated effluent corresponded to the requirements of the national discharge limits. Table 1 shows the types, the over-discharge situation, acute toxicity and AD-TUa data of the 24 effluents. For the fish tests, the lower LC50s were selected and presented in the table.

The degree or grade of chemical toxicity to aquatic life may be classified in a variety of ways, but one of the most useful of these was proposed by an international group on the basis of acute toxicity threshold values of chemicals (Cairns and Dickson, 1980). There is apparently no publication on the classification of industrial effluent toxicity, but it follows from Table 1 that some effluents (see chemical and metallurgical effluents) even after treatment may be considered to be toxic or very toxic to aquatic life. Table 1 also indicates that most of the effluents ater treatment still exceeded chemical discharge limits, especially the chemical effluents C-1, C-5 and C-7 which were also very toxic according to the biological assay. Although some effluents with no over-discharge met the discharge standards, some (such as chemical effluent C-6) were still highly toxic. The concentration-based toxicity measurements can be translated into toxic units (TUs), i.e. the acute toxic unit (TUa) and the chronic toxic unit (TUc). TUs are a simple way to quantify acceptable in-stream toxicity levels (Thomas, 1988). The impact of any toxic effluent is of course dependent on the dilution factor and other conditions. The Annual Discharge-Acute Toxicity Unit (AD-TUa) could be recommended to assess the impacts of effluents and to screen the toxic priority of effluents. The lowest LC50 for both the fish and daphnid species should be used to calculate such values. By using the AD-TUa data listed in Table 1, the priority rating of effluents in Nanjing can be established. The chemical effluents C-1, C-2, C-3 and C-5, and the metallurgical effluent M-1 would probably cause significant impacts on the aquatic ecosystem. Some of the effluents such as F-2, EM, TE-1, TE-2, and TE-3 would have a lesser impact on the environment.

Table 1. Annual discharge and acute toxicity data from effluents from 24 factories.

Industry Type	Effluent	Annual Discharge (10^4T/yr)	Over-discharge (Yes/No)	96h LC50† for Fish	48h LC50† for Daphnids	TUa	AD-TUa (T.TUa*10^4
CHEMICAL	C-1	2655.0	Yes	1.02 (0.95-1.10) c. carp, FT	0.31 (0.25-0.40)	322.6	856,503
	C-2	1571.54	Yes	13.33 (11.63-15.28) c. carp, R	2.22 (1.60-3.06)	45.0	70,719
	C-3	1400.20	Yes	26.99 (25.4-28.64) c. carp, R	7.50 (6.48-8.76)	13.3	18,671
	C-4	1095.64	No	19.98 (18.40-21.69) c. carp, R	21.32 (17.95-25.32)	5.0	5,484
	C-5	34.02	Yes	0.18 (0.16-0.20) s. carp, R	0.36	555.6	18,900
	C-6	18.42	No	0.74 (0.67-0.81) s. carp, R	39.43 (33.44-46.60)	135.1	2,489
	C-7	0.36	Yes	NAT s. carp, FT	0.11 (0.09-0.15)	263.2	327
METALLURGY	H-1	72.26	Yes	1.71 (1.51-1.93) c. carp, R	0.38 (0.24-0.59)	263.2	19,016
	H-2	607.0	No	10% death caused by 100% effluent	34.86 (21.47-56.95)	2.9	1,741
GAS	G	248.4	Yes	11.41 (10.38-12.53) s. carp, R	11.32 (8.56-14.97)	8.8	2,194
PHARMACY	P-1	600.0	Yes	39.73 (34.90-45.23) c. carp, R	51.50 (45.93-57.84)	2.5	1,510
	P-2	106.3	No	---	30.00 (22.56-39.9)	3.3	354
CHEMICAL FIBRE	CF	1,191.25	Yes	NAT c. carp, R	43.37 (39.45-47.7)	2.3	2,747

Continued...

Table 1 (continued)

Industry Type	Effluent	Annual Discharge (10^4T/yr)	Over-discharge (Yes/No)	96h LC50+ for Fish	48h LC50+ for Daphnids	TUA	AD-TUa (T.TUa*10^4)
TEXTILE	TE-1	48.18	No	46.75 (41.93-49.76) c. carp, R.	48.00 (37.64-61.21)	2.1	103
	TE-2	7.62	Yes	14.37 (13.44-15.38) s. carp, R.	5.95 (4.76-7.54)	16.8	128
	TE-3	15.72	Yes	15.77 (15.08-16.48) s. carp, R.	39.85 (35.09-45.26)	6.3	100
TANNING	TA	33.5	Yes	12.93 (11.26-14.86) c. carp, R.	84.29 (75.60-93.97)	7.7	259
ELECTRONIC	E-1	50.91	Yes	17.42 (14.21-21.36) c. carp, R.	1.74 (1.16-2.64)	56.5	2,926
	E-2	82.97	No	NAT c. carp, R.	61.38 (52.32-72.0)	1.6	135
INSTRUMENT AND METER	IN	61.30	No	NAT s. carp, R.	18.85 (15.26-23.28)	5.3	325
ENGINE MANUFAC-TURING	EN	3.85	Yes	NAT s. carp, R.	12.5	8	30.8
PAPER MAKING	PM	169.55	No	90.03 (78.26-100.0) s. carp, R.	57.99 (47.83-70.30)	1.7	292
FOOD	F-1	56.10	No	14.40 (12.63-16.43) c. carp, R.	22.86 (19.23-26.89)	6.9	390
	F-2	8.33	No	30% death by 100% effluent s. carp, R.	47.48 (38.55-55.48)	2.1	18

+ = % Effluent V/V
C. carp refers to crucian carp; s. carp refers to silver carp
FT refers to Flow Through test; R. refers to Renewal test

Table 2. Toxic effect of three effluents on the reproductive rate of *D. magna*.

	C-1	C-5	M-1
NOEC*	0.0125	0.0056	0.032
LOEC*	0.025	0.032	0.056

* % effluent V/V

Table 3. Toxicity of chemical effluent C-1 downstream from the discharge outlet.

Distance from the Outlet (M)	0	200	400	1500
Lethal Toxicity (Lethal Time)	LT100 6 min	LT100 25 min	LT100 8 h	LT40 24 h

Table 4. Main chemical parameters and toxicity of selected effluents before and after treatment.

		Chemical Effluent	Textile Effluent	Electronic Effluent
UNTREATED	Chemical Parameters (mg/l)	COD 479 Nitro-Compounds 4.8 Amino-Compounds 13.2 Phenol 2.8	COD 172 Phenol 0.011	Cr^{6+} 26.0
	48 h LC50# for *O. magna*	19.84 (16.29-24.17)#	13.43 (7.05-25.54)	0.84 (0.68-1.04)
	96 LC50 for Silver Carp	6.13 (5.76-6.54)	44.05 (39.32-49.32)	22.39 (20.41-24.55)
TREATED	Chemical Parameters (mg/l)	COD 25.4 Nitro-Compounds in trace Amino-Compounds in trace Phenol in trace	COD 52.1 Phenol 0.009	Cr^{6+} 0.12
	48h LC50 for *O. magna*	NAT	48.0 (37.64-61.21)	2.40 (1.95-2.96)
	96h LC50 for Silver Carp	NAT	46.75 (43.93-49.76)	14.09 (12.68-15.65)

* Effluent V/V;
# 95% Confidence Interval
NAT = No Acute Toxicity

The results of the acute toxicity tests demonstrated that *Daphnia magna* was usually the most sensitive species to the effluents, which agrees with previous reports (Thomas, 1988). Three of the high-priority effluents, chemical effluents C-1 and C-5 and metallurgical effluent M-1, were selected in the chronic toxicity tests with daphnids. The test concentrations of each effluent were set on the basis of the acute toxicity data. The end-points of chronic toxicity involved survival, and growth and reproduction rates. The results showed that the reproduction rate was the most sensitive indicator of toxicity. The NOEC and LOEC of each effluent to *D. magna* is listed in Table 2. On the basis of acute and chronic toxicity data, the range of acute and chronic ratios (ACR) of the selected effluents was found to be from 10 to 100. This parameter could also be used to evaluate the acceptable in-stream concentrations of other effluents based on acute toxicity data alone.

The ambient toxicity of chemical effluent C-1 was tested at 3 sites downstream from the discharge outlet. The results showed that the water downstream from the outlet was lethal to *Daphnia magna* within a short period of time (Table 3).

In order to examine the efficiency of effluent treatments, acute toxicity tests using 3 selected effluents before and after treatments were conducted with 2 species of aquatic animals. The effluents chosen were from chemical, textile and electronic industries, and tests were conducted with *D. magna* and Silver carp. According to physico-chemical parameters, biological treatment removed 99% of pollutants in the chemical effluent, and there was no mortality in the acute toxicity tests (Table 4). The efficiency of electrolytic treatment on the textile effluent was very good, but the toxicity of the effluent to fish was not obviously reduced. Removal of chromium by ion-exchange from the electronic effluent reached 99.2%, but while the toxicity to daphnids was decreased, the toxicity to fish seemed to be increased (Table 4).

DISCUSSION

Management of industrial discharges in China today relies on physico-chemical monitoring since it reveals the primary constituents of the effluents. However, the results of this study demonstrate that such tests do not detect all chemicals in industrial effluents, nor do they measure possible interactions of chemicals or their impact on the environment. Ecotoxicological monitoring of whole effluents through toxicity testing on aquatic organisms (biological assay) can reflect the overall impact of industrial discharges on an aquatic system although it does not identify the actual toxic pollutants in waste water. In the present study, the acute toxicity tests were employed to establish a toxic order among the effluents tested, but the impact of an effluent depends not only on the effluent toxicity but also on the discharge rate. Therefore, the AD-TUa of effluents was recommended as a measure for discharge management, and, based on this unit, a toxic priority of industrial effluents in Nanjing was established (see Table 1).

According to the LC50 and NOEL values of selected effluents (Tables 1 and 2), the Application Factor (AF) for protection of aquatic life from toxic effluents ranged from 0.01 to 0.10. However, the possible interactions between the chemistry of the real receiving water (the Yangtze River) and the effluents were not measured in this study, so the range of safe concentrations for other effluents for which chronic testing has not been done can only be evaluated by using a proper AF value.

Most of the factories involved in this study are located along the Yangtze River into which the effluents are directly or indirectly discharged. A river can have a large flow and therefore a tremendous dilution capacity, but the prevalence of riverside industrial discharge does damage the

ecosystem at the edge of the river. Large-scale fish mortality caused by discharge of industrial effluents into small water bodies or the polluted zone of large water bodies does occasionally occur in China. These constitute a warning that must be taken seriously. Despite dilution, the results of this study demonstrated that the water downstream from the outlet of chemical effluent C-3 was still lethal (Table 3).

The results of this study showed that the physico-chemical data did not entirely tally with the results of the bioassays (Table 1). The treatment of the effluents was insufficient according to the National Discharge Standards, and some of the treated effluents were still very toxic to aquatic life. Some of the major constituents of the effluents might include aromatic compounds, benzene and its homologues and derivatives, as well as some unknown chemicals. Ten of the fourteen treated effluents were considered to have met the requirements of the National Standards, but one of them, chemical effluent C-6, was still highly toxic based on the bioassay results. Obviously, the results of bioassays can not be predicted or substituted for by physico-chemical monitoring. The effluents may contain some toxic substances not detectable at present. Enforceable discharge limits in China exist for only a few of the thousands of chemicals in use, and the routine monitoring of effluents in China includes only a few chemical parameters, while other toxins are ignored. There is undoubtedly additivity and/or synergism of toxic effects in multi-chemical effluents. The use of more chemicals in treatment procedures may enhance interactions and actually increase the toxicity of effluents to aquatic life. Clearly, the efficiency of effluent treatments can not be effectively examined only by means of physico-chemical monitoring, and further studies on the identity of the toxic substances in industrial effluents need to be done to control toxic discharges.

In an attempt to control industrial discharge, the China NEPA has developed a Waste Load Allocation and created a discharge permit system. The parameters used in development of permits usually include BOD/COD, S2-, oil and other general chemical indicators, but no biological toxicity limits. The system of fines for exceeding discharge limits is based on Waste Load Allocation and enforced by environmental agencies, but appears unable to protect aquatic life against chronic damage. The whole-effluent toxicity limitation concept involves using acute and chronic toxicity tests to measure the biological toxicity of waste waters. Toxicity is a useful parameter to protect water quality and resources, and ecotoxicological monitoring of whole effluents is cost effective. In the United States, the toxicity of effluents has been measured since the late 1970s and discharge permits have ncluded toxicity limits since the early 1980s. The increased use of toxicity testing has identified substantial numbers of unacceptably toxic effluents (USEPA, 1991). In China, the management of toxic effluents must be improved and strengthened. In order to reduce toxic chemical release, especially from petrochemical factories, biological assaying must be developed, and effluent toxicity limits should be established and incorporated into discharge permits. A national policy for development of water quality-based permit limitations for toxic pollutants should be established, and the policy should recommend the use of whole effluent toxicity testing to achieve a standard that prohibits the discharge of waste materials in toxic amounts.

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INVESTIGATION INTO THE CAUSE OF THE DEATH OF FISH OCCURRING IN LAKE XUANWU (IV).

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ABSTRACT

The cause of recurring incidents of large-scale fish mortality in Lake Xuanwu was investigated. The pathogenic bacteria, *Aeromonas hydrophila*, was isolated from silver and variegated carp recovered from the lake, and is believed to be the responsible pathogen. Population changes of *A. hydrophila* in relation to phytoplankton succession and changes in redox shielding level in Lake Xuanwu are also discussed.

INTRODUCTION

In recent years, large-scale fish mortality in Lake Xuanwu has been observed to be a recurring phenomenon (Xu 1991). Despite numerous previous investigations (Pal and Tripathi 1978, Yu and Mao 1991, Chen 1992), controversy over the exact cause of death still exists. Since 1988 there have been three additional outbreaks of fish mortality, which prompted this study.
The water quality of Lake Xuanwu was tested during one such incident of fish mortality. Levels of un-ionized ammonia (0.12 mg/l), oil (0.41 mg/l), and BOD (14.4 mg/l) were found to slightly exceed the Chinese National Standard water quality for fisheries (GB11607-89). Other pollutants, however, were almost never above the National Standard, and only trace amounts of pesticide were found.

Despite the measurement of water contaminent levels during a fish epidemic that exceeded those of the National Standard, experiments found that these amounts, and their additive effects, were far below lethal levels. Furthermore, tests performed on water taken from the river feeding into the lake found no indication of the water being toxic to fish. Therefore, it is reasonable to conclude that the water quality itself is not directly responsible. This paper reports our experimental results that identify *Aeromonas hydrophila* to be the pathogen involved in the large-scale mortality of fish in Lake Xuanwu.

MATERIALS AND METHODS

SYMPTOMS OF FISH INFECTION

In August of 1992, an infectious epidemic was responsible for the death and injury of a large number of fish in Lake Xuanwu. Numerous red dots were found on the skin of fish, particularly in the abdominal area. The mouth, eyes, fins and anus also typically turned red.

ISOLATION AND IDENTIFICATION OF THE PATHOGENIC BACTERIA

Silver carp and Variegated carp were chosen for isolation of the bacteria. Fish used for the study were all visibly infected and included both dead and living specimens.

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Samples of blood and dis-coloured skin were first placed into a salt water solution. The mixture was then ground and stirred under sterile conditions. A 1 ml sample of the blood-skin mixture was extracted and used for a plate culture. Owing to the identification of *Aeromonas hydrophila* as a pathogen common to silver and variegated carp, only those micro-organisms having the following characteristics were used for subsequent isolation and identification:

a. Sphere shaped, slightly medially convex
b. Smooth skin
c. opaque white
d. Diameter 1 - 1.3 mm (after culture for 24 h)

STANDARD BACTERIA STRAIN USED FOR COMPARISON TESTS

A standard strain of *A. hydrophila* was purchased from the Shanghai sanitation and antiepidemic station.

THE FISH USED FOR GERM VIRULENCY AND COMPARISON TESTS

The fish used for the tests described below were purchased from a local supplier at the market (mean length 35.0±2.0 cm; mean weight 0.7±0.06 kg). The fish appeared healthy and showed no signs of being infected with any form of disease.

GERM VIRULENCY TESTS

Once *Aeromonas hydrophila* was isolated and identified, it was cultivated in meat soup and protein peptone. Standard germs and isolated germs were cultivated in large scale. The virulency of the bacteria was tested using both body cavity injection and immersion methods.

i. Body cavity injection.

The body cavity of healthy fish were injected with 10 ml of isolated *A. hydrophila* solution or standard germ solution at a concentration of 1.8 x 10/8 germs/ml. The fish were returned to a tank for observation (water temperature 27-31 °C, dissolved oxygen content (DO) = 5.0-7.0 mg/l).

ii. Immersion method.

The b odies of healthy fish were scratched at 10 different points with a small needle prior to being introduced into a tank containing *A. hydrophila* at a concentration of 107 germs/ml. The water in the tank was maintained at 30±2 °C with a DO of 6.6±1.0 mg/l.

RESULTS

THE ISOLATION AND IDENTIFICATION OF THE PATHOGENIC BACTERIA

i. Morphological features of the isolated pathogenic bacteria.

Following 20 hours of cultivation, the isolated bacteria was observed to be composed of short, rod-shaped cells found singly or in pairs. The cells were 0.6-0.8 μm in diameter and 1.1-2.2 μm in length.

ii. The physiological and biochemical properties of the isolated bacteria.

The bacteria isolated from the silver and variegated carp were successfully grown in the semi-solid cultivation medium. Comparison of them to data outlined by Buchonan and Gibbons (1984) in "Bergeys Manual of Determinative Bacteriology", the isolated bacteria was identified as *A. hydrophila.* The bacteria have a positive Voges-Prokauer test, and have the ability to oxidize glycol and adversely effect the health of fish.

VIRULENCY ASSESSMENT OF THE BACTERIA

A virulency test was performed on healthy carp through comparative exposure to the isolated strain and the standard *A. hydrophila* strain. Following infection with the bacteria, the following symptoms appeared chronologically: red spots appeared around the mouth, then on the anus; discharge of blood from the anus was observed; red spots appeared on the skin, eyes, and operculum. Upon necropsy, blood was found within the intestine.

The symptoms of infection were found to be greater among fish that had been directly injected with the bacteria over those that were exposed to it through immersion. The virulency of bacteria was found to be higher in bacteria isolated from blood samples than from skin samples.

DISCUSSION

1) The bacteria *A. hydrophila* was isolated from living and dead infected fish taken from Lake Xuanwu. The results indicate that this bacteria is the pathogen responsible for the periodic large-scale fish mortality observed in this lake. This bacteria acts to destroy the blood circulation system of fish.

2) The isolated bacteria were found to readily infect healthy, Variegated and Silver carp with strongly adverse effects. Specimens injected with the bacteria expired within 48 hours even when maintained in water with a high DO (6.5 mg/l). Although those fish that were immersed in water containing *A. hydrophila* did not die after 48 hours, they clearly showed symptoms of disease. While the test fish immersed in water containing *A. hydrophila* and a DO of 6.6±1.0 mg/l remained alive after 96 hours, fish exposed to the same conditions except for the DO (which was controlled at 2.5 mg/l), expired within 3 hours. These fish showed symptoms indicative of bacteria infection and not low dissolved oxygen. Healthy fish were found to survive in water with a low DO (2.5 mg/l) for at least 6 hours.

3) Jiang (1992) indicated that environmental conditions were the underlying cause of the mortality of the Lake Xuanwu fish. Poor water quality, harmful organic chemicals and low dissolved oxygen levels have combined with increased temperatures in summer to allow for accelerated growth of bacteria. *Aeromonas hydrophila* acts to destroy the circulatory system of fish by attacking red blood cells. Ni (1979) reported that this bacteria is conditionally pathogenic in that it is only lethal to fish that are stressed by poor water conditions. Injection of the bacteria directly into the body cavity of fish resulted in rapid growth of the bacteria because of favorable conditions, and led to the death of the fish shortly thereafter. The infection of fish exposed through immersion in bacteria-laden water was reduced owing to the high quality of the water, which slowed growth of the bacteria. In this circumstance, the immune system of the fish was able to successfully combat the bacteria taken in by the fish when feeding. When the immune system of the fish is stressed through poor water quality (such as low dissolved oxygen), the rate of infection occurs much more rapidly. During the summer, the water quality is compromised by organic and other pollutants and elevated water temperatures

(27-32 °C). Additionally, there are numerous other germ carriers found in Lake Xuanwu (including Myxobolus sp., Sinergasilus polywplus, Lernaea polymorpha, and Dactylogyrus sp.). Finally, the dissolved oxygen concentration of the lake during summer nights tends to be very low (the mean DO between 2-7 a.m. is 2.53 mg/l). These factors combine to facilitate growth of the bacteria and hence, the infection and death of fish within the lake.

4) The dynamic changes in pathogenic bacteria.

i. The effect of changes within the aquatic biome upon pathogenic bacteria.
Variations in the population structure of several aquatic species within Lake Xuanwu occurred over the period involving an outbreak of *A. hydrophila*. The *Crytomonas* population was found to decrease dramatically in contrast to an increase in the numbers of *Merismopedia*. At the same time, the total bacteria population in the lake increased sharply. It should be noted that *Crytomonas* feed on bacteria, while *Merismopedia* is a substrate for numerous pathogenic micro-organisms, including *A. hydrophila*. Ingestion of *Merismopedia* by fish has led to infection by *A. hydrophila*.

ii. The effect of redox shielding level changes upon pathogenic bacteria.
The redox shielding level of Lake Xuanwu is found 5.8 cm below the sediment during the winter season, and 0.6 cm below the sediment during the summer. The density of bacteria has been found to be highest in the redox shielding level (Saxena et al., 1992, Doran 1982). *Aeromonas* sp. in particular, are common at this level. Therefore, during the summer when the redox shielding level is close to the surface of the sediment, the introduction of bacteria from the sediment into the water column occurs readily.

CONCLUSIONS

a) The water quality of Lake Xuanwu has been seriously degraded through eutrophication and exposure to organic pollutants. While pollutants are below lethal concentrations, their presence is believed to compromise the immuno-response systems of fish within the lake.

b) The growth of *A. hydrophila* is encouraged by the phytoplankton succession resulting from eutrophication and also by the upward movement of the redox shielding level.

c) The agent responsible for the recurring incidents of large-scale fish mortality in Lake Xuanwu is the conditional bacteria, *Aeromonas hydrophila*.

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FLOODPLAIN REHABILITATION ALONG STREAMS DEGRADED BY MINING AND MILLING WASTES

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ABSTRACT

Acid generating wastes associated with metal mining and processing have been accumulating in Butte, Montana (U.S.A.) for over 125 years. In the past, these materials were stored behind temporary embankments. Periodic precipitation events breached the dams and flushed the tailings or other wastes down the local stream (Silver Bow Creek). The banks of this stream are lined to various extents and depths with wastes from the erratic floods. The release of wastes from Butte has been corrected, but snowmelt or heavy thunderstorms still wash surficial waste deposits back into Silver Bow Creek and into its receiving stream, the Clark Fork River. The fishery resource of the upper Clark Fork River has periodically been devastated by these flushes of salts and metals. These metals also pose a threat to humans living along the stream or river and using ground or surface water supplies.

The Reclamation Research Unit evaluated the use of soil amendments, various incorporation techniques, and selected species of plants as potential remedial strategies to correct these pollution problems. Over 35 potential amendments were evaluated for their ability to correct waste pH and reduce metal solubility. These amendments ranged from industrial wastes to locally mined limestone. The most effective amendments ($CaCO_3$ and $Ca(OH)_2$) were utilized in replicated greenhouse trials of numerous grasses and legumes. These amendments and selected plant species were combined into a replicated field trial of different amendment incorporation techniques.

The amendments, plant species, and incorporation techniques were evaluated on the basis of plant cover and production, depth of root penetration, soil neutralization, vadose zone metal levels, and quality and quantity of surface runoff. The data support coversoils as most effective. In the area of this study, however, coversoils are not available in quantities adequate for the rehabilitation of all of the disturbances, therefore, other remedial activities were evaluated. One of these "other remedial activities", the pressure injection of lime slurry into the wastes did not provide an adequate rootzone for long-term plant growth. Agricultural tilling provided only 15 cm of adequate rootzone materials. A plow capable of mixing waste to a depth of 122 cm provided an amended rootzone that varied from 30 to 60 cm deep. Plant cover and production were greater on deep plowed plots than on plots prepared by the other two incorporation techniques. Deeper root penetration was also found in the deep plowed plots than in the plots treated by the agricultural plow or the injection technique.

Soil pore water quality appeared to be slightly improved under each of the treatments, but the data are not consistent. Surface runoff was reduced and surface water quality improved by all treatments but preliminary analyses indicate that the deep plow improved infiltration more than the other treatments.

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INTRODUCTION

The Reclamation Research Unit is a division of the Animal and Range Sciences Department of Montana State University. Our research efforts are concerned entirely with the rehabilitation of violently disturbed lands. The Streambank Tailings and Revegetation Studies ("STARS" as they are euphemistically called) are part of an ongoing investigation of the mine waste pollution problems associated with toxic streambank deposits along Silver Bow Creek.

These streambank deposits are a direct result of the erosion of tailings and mine wastes originally located in and around the city of Butte, Montana. Dams on tailings ponds have burst and wastes have been carried down Silver Bow Creek by heavy precipitation events or snowmelt numerous times over the past 100 years. The materials have been transported to the Clark Fork River and on down the river to the Milltown Dam over 160 km away.

The area that concerns us extends from Butte to the Warm Springs settling ponds, a distance of over 40 km. Several areas along this stream are locally well known for the depth and extent of mine and mill wastes and the severity of pollution. Various areas of these waste reveal deposits ranging from centimeters to meters thick and from less than a hectare to 100's of hectares in extent. Waste pH levels range from 2.3 to 7.4; salinity as indicated by electrical conductivity ranges from 0.3 to 7.5 dS/m or dS m^{-1} (Table 1). Particle size distributions range from 25 to 95% sand, 6 to 65% silt, and 3 to 30% clay. Elements found in excess at various sites include aluminum, arsenic, cadmium, copper, iron, lead, manganese, mercury, and zinc.

Because of the large quantities and areal extent of tailings and mine wastes present within the Silver Bow Creek drainage, it was decided to explore new and innovative technologies for remediating these wastes as opposed to the traditional method of removal and burial. The technologies under consideration are insitu neutralization, revegetation, and stabilization. These STARS technologies were evaluated for their effectiveness in significantly reducing the toxicity and mobility of contaminants present in the wastes, protecting human health, and for cost effectiveness.

OBJECTIVE

The primary objective of the STARS project was:

- to field test and evaluate remedial technologies for the mitigation of environmental impacts from streambank tailings or mine wastes. These studies incorporated such concepts as microencapsulation, deep incorporation of amendments, coversoil isolation, and/or enhanced evapotranspiration techniques.

The project team collected performance data on depth of waste neutralization, plant cover and production, pore water contamination, and surface water runoff quality and quantity. These data were necessary to develop alternatives for consideration in the study of waste neutralization.

Table 1. Range of chemical properties of wastes.

pH	2.3 to 7.4
Conductivity	0.3 to 7.5 dS/m or dS m^{-1}
Total Aluminum	230 to 19,700 mg/kg
Total Arsenic	20 to 3,100 mg/kg
Total Cadmium	3 to 110 mg/kg
Total Copper	200 to 11,200 mg/kg
Total Iron	3,900 to 106,000 mg/kg
Total Lead	83 to 6,500 mg/kg
Total Manganese	17 to 13,300 mg/kg
Total Mercury	0.1 to 61 mg/kg
Total Zinc	19 to 22,000 mg/kg

INVESTIGATION APPROACH AND RESULTS

The STARS investigation is divided into three components: 1) a bench-scale treatability study (Phase I); 2) a pilot-scale treatability study (Phase II); and 3) the pilot-scale monitoring program (Phase III). Phase I was conducted in the laboratory and greenhouse facilities at Montana State University. Phase II was a field demonstration of the results obtained during Phase I and was conducted onsite in the Silver Bow Creek drainage. Phase III was the monitoring and evaluation of the plots implemented during Phase II.

PHASE I: BENCH-SCALE NEUTRALIZATION STUDIES: LABORATORY AND GREENHOUSE INVESTIGATIONS

Phase I was designed to develop and test, in the controlled environment of the laboratory and greenhouse, materials that would ameliorate streambank tailings sufficiently to allow them to support vegetation. In addition, the amendments were selected on their ability to reduce acid and metal contamination of surface and vadose zone water.

Existing data concerning tailings and contaminated soils along Silver Bow Creek were reviewed by the project team. This information guided the selection of 35 contaminated sites within the Silver Bow Creek ecosystems. These sites were representative of the wastes along the 40 km of this stream. From each of these 35 sites, a 225 kg bulk soil or waste sample was collected. These samples were dried, sieved, mixed, and subsampled for analysis of key chemical and physical characteristics. Six primary "kinds" or groups of waste types were identified on the basis of this chemical and physical characterization. These six sites were representative of the full spectrum of contamination in the Silver Bow Creek area. A representative example of each group or kind of waste was selected for further analyses in the laboratory and greenhouse.

Concurrent with the selection of mine wastes or tailings for further study both traditional and innovative amendment materials were evaluated for possible use in the greenhouse and field studies. Candidate amendments were evaluated in terms of their ability to raise waste pH, reduce the solubility of heavy metals, availability of the amendment, and cost. Materials evaluated included calcium carbonate ($CaCO_3$) (agricultural lime), cement kiln dust, calcium chloride ($CaCl_2$), calcium hydroxide ($Ca(OH)_2$), calcium oxide (CaO), power plant flyash, sugarbeet waste, gypsum, zeolite, triple superphosphate, fluoro-phospho silicate, phosphogypsum ferric sulfate ($FeSO_4$), phosphate ore, and various organic amendments. The chemical and physical characteristics of these amendment materials were determined. The amendments were mixed with the tailings or waste materials in specially designed reaction flasks (Tempe Cells) and their ameliorative properties in controlled and replicated laboratory column studies determined (Reclamation Research Unit et al. 1989). Quantities of the amendments necessary to neutralize various wastes were measured.

Effluents from the Tempe Cells were evaluated for control of pH and metals by the various amendments. The leached and amended tailings or wastes were also analyzed. Results revealed that the amendments that most significantly reduced acidity were also most effective in limiting the concentration of soluble heavy metals. Combinations of $CaCO_3$, $Ca(OH)_2$, phosphogypsum, Fe_2SO_4, $CaCl_2$, and triple superphosphate performed best and were subsequently tested in greenhouse plant growth trials.

An equation for the calculation of the quantity of lime was developed:

$$t\ CaCO_3/1000\ t\ Soil = 1.25\ (A + B)$$

where t = tons
A = Potential Acidity
B = Active Acidity
Potential Acidity = 31.25 (% HNO_3 S + Residual S) + 23.44 (% HCl S)
where S = sulfur content extracted from or remaining in the sample
Active Acidity = SMP Buffer Test

The use of calcium carbonate alone did not raise the pH fast enough or high enough to permit seeding within a reasonable amount of time. We found that portioning the liming rate into 60% calcium carbonate and 40% calcium oxide rapidly raised the pH and reduced metal toxicity. All amendments were composed of these two calcium sources in these proportions.

Plant species used in the greenhouse trials were selected on the basis of their ability to tolerate acidic soil conditions, nutrient deficiencies and the climatic conditions at the Silver Bow Creek site (Reclamation Research Unit et al. 1989). In addition, some species were selected for site specific characteristics such as elevated salinity or textural extremes. Finally, all species were screened for commercial availability. Seventeen species and two cultivars of two species were grown in amended tailings in the greenhouse.

At the termination of the greenhouse growth period, the plants were evaluated for vigor, height, color, root growth, and production (Reclamation Research Unit et al. 1989). Plant tissues were also analyzed for metal levels. Livestock and wildlife toxicities from elevated metal levels were thought to present a concern if the area is revegetated. In the plant tissues analyzed, concentrations of boron, calcium, and lead were comparable to tissue levels in nonpolluted areas of Montana. Cadmium, copper, manganese, and zinc were elevated compared to background levels in Montana but these concentrations would not be toxic to any grazers and they were not phytotoxic. Aluminum and arsenic levels were higher than most background values in the literature, but they would not be toxic to

animals. The higher aluminum values were high enough to indicate some plant growth inhibition. Metal loading in and on plants grown in the field were much higher. These data are discussed later in this report.

PHASE II: PILOT-SCALE TREATABILITY STUDIES:
FIELD DEMONSTRATION INVESTIGATIONS

Activities involved in what is referred to as Phase II of STARS included the implementation of the best amendment options and plant species selections obtained in Phase I into a statistically sound field demonstration. Of the six groups of wastes located along Silver Bow Creek during Phase I, five were selected for further study. The chemical constituents of these materials were known to be quite variable even within an area of relatively uniform deposition. To reduce this variability in the demonstration, it was necessary to find homogeneous areas for the field plots. A sampling grid was established over each site. At each grid point, pH and electrical conductivity (EC) were measured in saturation pastes and total metal levels measured by x-ray fluorescence spectroscopy (XRF). The measured parameters were contour-mapped using geostatistical kriging methods and graphic software. The field plots were placed in the most homogeneous locations identified by the kriged maps. Other factors such as the thickness of the waste materials and the depth to groundwater were also important in selecting plot locations.

After location of the field demonstration sites but before treatments were actually applied, soil (waste) samples were collected from each experimental plot to:

- measure chemical parameters required to determine amendment rates;
- measure plant nutrient status to determine fertilizer rates; and
- measure total metal levels to verify XRF data.

Amendments incorporated into the various waste materials in the field phase of the STARS study included combinations of hydrated lime ($Ca(OH)_2$), agricultural lime ($CaCO_3$), triple superphosphate, and iron sulfite. There were five primary soil treatments replicated four times at each STARS site. These included:

- control, no amendment;
- agricultural tillage (moldboard plow, discing) incorporation (15 cm depth);
- mechanical incorporation (122 cm depth) by deep plow;
- Lime slurry pressure injection throughout the 122 cm profile; and
- a 0 to 45 cm thick coversoil wedge applied over tailings amended to a depth of 15 cm.

A dormant fall seeding was planned for 1988. However, analyses of materials from plots after treatment revealed that the pH ranged from 8.5 to 12 in most plots. One month after amendment application, some plots still revealed pH values up to 12. Seeding was, therefore, postponed until April 1989.

Seed mixtures for each site varied widely. As in the greenhouse study, plant species selection was based upon the characteristics of each individual site, acid and drought tolerance, greenhouse performance, and land use. Unfortunately, most acid tolerant plant species require the equivalent of over 50 cm of precipitation per year. The study area receives approximately 35 cm. Therefore, plant species were carefully selected that met the requirements of the sites and were as acid tolerant as possible. Two seed mixtures were constructed for each site (Schafer and Associates et al. 1989).

To permit water movement studies during the monitoring phase of the STARS project, selected sites were instrumented with suction lysimeters, a neutron access tube, and a piezometer. All sites

were fertilized with nitrogen, phosphorus, potassium, and boron. Rates were based upon nutrient analyses. Unfortunately, there were indications from the greenhouse study that the analyses of nitrogen, phosphorus, and potassium in these severely impacted may not be good indicators of actual field conditions. Plants showed signs of nitrogen and phosphorus deficiency despite attempts to bring these nutrients to adequate levels in the growth tubes. We found similar problems in the field demonstration.

PHASE III: MONITORING AND EVALUATING FIELD PLOTS

The success of the field treatments was determined by evaluating plant species performance, the quality and quantity of water leaching through the amended materials, and the quality of surface water runoff. The plant parameters were monitored in 1989, 1990 and 1991. Suction lysimeters were monitored in 1991 and 1992. Surface runoff and root penetration into the wastes were monitored in 1992. Vegetation evaluation parameters included plant cover, production, root penetration, and elemental levels. Leachate quality and surface runoff were evaluated in terms of pH, EC, and the concentration of heavy metals.

Plant Performance

Five unique plant rootzone materials were selected for field growth trials. Four different soil treatments and a control were implemented at each of these sites. It is known that caps or cover soils will permit rapid revegetation and stabilization of waste materials. This treatment was evaluated at the study sites to establish a minimum depth necessary for the cover material and to provide a comparison for the other treatments.

At one study site, all plants seeded directly into the amended wastes died by the third growing season. This site was considered the most difficult of the revegetation study sites. Pre-treatment metal levels in the waste material were very high (1,750 mg As/kg soil, 11,000 mg Cu/kg soil, and 22,000 mg Zn/kg soil), salinity was elevated at 6.7 dS/m, and the site exhibited poor drainage. This resulted in vegetation being submerged on several occasions during the study. Waste material at this site will be excavated and transported to a storage area because of unacceptable risk to humans living in close proximity. Response, in terms of vegetation performance at the other four sites, was variable but generally indicated that the toxic properties of the soils had been ameliorated sufficiently to permit plant growth and soil stabilization. Results are summarized for two of the sites in this paper. These two are Site 7, which has soil, and Site 21 with a silty clay soil. Information gathered at the other two sites is comparable to that itemized for Sites 7 and 21 in this report.

Site 7

Root zone materials at Site 7 were high in metals and very sandy (Reclamation Research Unit et al. 1989). Despite these plant growth limitations the Deep Plow (DP), the Agricultural (A) treatment, and the Coversoil Wedge (CW) produced significantly greater total plant cover than the Control plots (Table 2). At this particular site the DP treatment produced almost as much plant cover as the CW.

Production data (Table 3) revealed numerous significant differences among the various treatments in grass and total production. The most numerous differences were between the CW's and the Controls or between the DP treatment and the Control. On the basis of plant cover and production data at this site, the DP treatment must be designated as the most desirable soil incorporation method.

Importance Values (IV's) were calculated (relative % cover + relative % production) for the most common seeded species (Table 4). These values provide a relative estimate of how well a species performed in this soil under the treatment and amendment conditions of this study. The Control plots generally were devoid of vegetation and data from them reinforces the plant inhibitory information previously inferred from cover and production values, therefore, IV's were not calculated for these treatments. The IV's from the other treatments indicate that two of the wildryes and one of the wheatgrasses performed well in this sandy soil. Observations revealed that these grasses were established and reproducing within three growing seasons. Mammoth wildrye (*Elymus giganteus*) established well and was spreading by rhizomes (Mean IV = 112.3). Altai wildrye (*E. angustus*) was also growing very aggressively (Mean IV = 92.7). Thickspike wheatgrass (*Agropyron dasystachyum*) was spreading vegetatively on the plots (Mean IV = 35.8). Basin wildrye (*Elymus cinereus*) performed better on the coversoiled plots (IV = 53.7) than on the other treatments (Mean IV = 20.7). Legumes did not perform well on this site (IV = 0.0 to 22.5), but they can be expected to increase in cover and production throughout the next few growing seasons. The fescues (*Festuca ovina* and *Festuca longifolia*) will probably decrease in cover as the taller wildryes shade them out of the community.

On this type of material and this method of soil acid neutralization mammoth wildrye, altai wildrye, and thickspike wheatgrass constitute an aggressive seed mix. However, a legume should be added to the vegetation on the site to lend a nitrogen fixing component and diversity to the new plant community.

Metal levels in or on two of the dominant grasses at this site are shown in Table 5. For comparison, metal analyses of related wheatgrasses (*Agropyron smithii*) collected in southeastern Montana are presented in this Table. Although elevated, the metal levels in or on the vegetation from this site will not pose a threat to grazing wildlife or the occasional livestock that might pass through the area.

Table 2. Mean vegetation cover (% areal cover) at Site 7, Summer 1991.

Treatment	Seed Mix 1			Seed Mix 2			Mean of Total Cover
	Grasses	Forbs	Total	Grasses	Forbs	Total	
Agricultural	22.2 A*	5.9 A	28.1 AB	35.4 A	6.0 A	41.4 BC	34.8 B
Control	0.5 A	0.0 A	0.5 A	0.5 A	<0.1 A	0.5 A	0.5 A
Deep Plow	41.9 A	3.5 A	45.4 B	47.9 A	4.3 A	52.2 C	48.8 CB
Slurry Injection	28.3 A	2.8 A	31.1 AB	20.2 A	<0.1 A	20.2 AB	25.7 AB
Coversoil Wedge							
38 cm	33.4 A	30.4 A	62.6 CB	41.1 A	29.5 A	70.6 C	66.6 CD
15 cm	49.5 A	44.3 A	93.0 C	36.5 A	32.9 A	69.4 C	81.2 D

* Multiple mean comparison based on LSD at significance level of 0.10. Mean followed by same letter in columns are not different.

Table 3. Mean vegetation production (kg/ha*) at Site 7, Summer 1991.

Treatment	Seed Mix 1			Seed Mix 2			Mean of Total Production
	Grasses	Forbs	Total	Grasses	Forbs	Total	
Agricultural	528 AB**	30.2 A	558 AB	829 BC	29.0 A	858 AB	708 AB
Control	31 A	0.0 A	31 A	8.5 A	0.5 A	8.9 A	19.6 A
Deep Plow	1530 B	18.0 A	1548 B	1211 C	47.3 A	1258 BC	1403 BC
Slurry Injection	531 AB	4.1 A	535 AB	171 AB	0.5 A	172 A	353 A
Coversoil Wedge	1242 AB	498 B	1740 B	792 BC	1159 B	1950 C	1845 C

* To convert to lbs/a multiply by 0.89.

** Multiple mean comparison based on LSD at significance level of 0.10. Mean followed by same letter in columns are not different.

Table 4. Importance values for the dominant seeded species on Site 7, Summer 1991.

Species (Seed Mix 1)	Relative % Cover	Relative % Production	Importance Value*	Species (Seed Mix 2)	Relative % Cover	Relative % Production	Importance Value*
Agricultural				Agricultural			
Ag da	18.7	23.7	42.4	Ag da	9.5	9.7	19.2
El an	29.9	38.0	67.9	El ci	16.3	22.8	39.1
Fe ov	10.3	11.0	21.3	El gi	58.5	48.8	107.3
Po co	1.6	4.4	6.0	Fe lo	1.1	2.6	3.7
Legumes	17.1	1.4	18.5	Legumes	11.7	1.5	13.2
Deep Plow				Deep Plow			
Ag da	13.8	19.5	33.3	Ag da	11.6	23.8	35.4
El an	59.5	74.7	134.2	El ci	3.7	12.4	16.1
Fe ov	10.5	1.8	12.3	El gi	62.7	57.5	120.2
Po co	0.0	0.0	0.0	Fe lo	13.7	2.1	15.8
Legumes	7.6	1.2	8.8	Legumes	0.0	3.6	3.6
Slurry Injection				Slurry Injection			
Ag da	32.2	31.7	63.9	Ag da	13.9	10.3	24.2
El an	48.3	59.1	107.4	El ci	0.3	6.5	6.8
Fe ov	0.0	0.0	0.0	El gi	85.4	82.8	168.2
Po co	0.0	1.1	1.1	Fe lo	0.0	0.0	0.0
Legumes	7.7	0.2	7.9	Legumes	0.0	0.0	0.0
Coversoil Wedge				Coversoil Wedge			
Ag da	14.8	27.7	42.5	Ag da	12.1	13.2	25.3
El an	25.8	35.7	61.5	El ci	28.3	24.8	53.7
Fe ov	11.7	7.5	19.1	El gi	3.2	0.1	3.3
Po co	0.0	0.0	0.0	Fe lo	6.9	2.4	9.3
Legumes	23.0	3.8	26.8	Legumes	15.9	6.6	22.5

* Importance Value = Relative % Cover + Relative % Production.

Table 5. Comparison of mean elemental levels (mg/kg) in dominant vegetation collected (July 1991) from Site 7.

Element	Treatment: Agricultural Tillage	Control	Deep Plow	Slurry Injection	Topsoil	SE Montana Western Wheatgrass** (*Agropyron Smithii*)
	Thickspike wheatgrass (*Agropyron dasystachyum*)					
Aluminum	90.2 B*	119.0 CB	86.1 B	165.0 C	33.4 A	5 to 50
Arsenic	3.1 A	2.2 A	2.1 A	2.5 A	3.9 A	<1 to 2
Cadmium	0.27 B	0.41 B	0.29 B	0.23 AB	0.12 A	0.01 to 0.1
Copper	14.9 B	26.6 C	15.7 CB	21.7 CB	6.4 A	2.5 to 4.5
Lead	12.3 A	3.90 A	8.8 A	6.4 A	20.9 A	1.0 to 2.0
Manganese	78.1 A	58.4 A	53.3 A	76.4 A	59.4 A	25 to 65
Zinc	69.7 A	72.0 A	56.4 A	47.1 A	35.6 A	12 to 21
	Altai wildrye (*Elymus angustus*)					
Aluminum	66.5 AB	***	62.9 AB	134.0 B	36.4 A	
Arsenic	7.1 A		3.3 A	7.6 A	1.8 A	
Cadmium	0.39 A		0.36 A	0.26 A	0.09 A	
Copper	13.4 A		12.2 A	15.0 A	6.0 A	
Lead	14.8 A		3.3 A	6.7 A	1.2 A	
Manganese	75.1 A		53.0 A	60.8 A	71.0 A	
Zinc	63.8 A		57.2 A	44.4 A	42.4 A	

* Multiple mean comparison based on LSD at significance level of 0.05. Means followed by same letter in rows are not different.

** Al and As estimated from the literature, Cd, Cu, Pb, Mn, and Zn from Munshower et al. 1987).

*** Species did not grow on control plot.

Two soil pits were opened at Site 7 to examine root penetration and distribution. All soil pits were placed in DP treatments because previous excavations had provided evidence that penetration was deepest in this treatment. In both pits, roots penetrated to 25 cm. There did not appear to be any impediment to deeper penetration and the plant roots may still be developing. It is doubtful that the roots have reached their point of maximum penetration.

Site 21

Table 6 summarizes plant cover at Site 21. Both grass and total cover data revealed significant differences among the treatments. Cover in these two categories was higher on all treatments than on the Control plots. As at Site 7, the average total cover on the A and DP treatments were similar.

Table 6. Mean vegetation cover (% areal cover) at Site 21, Summer 1991.

Treatment	Seed Mix 1			Seed Mix 2			Mean of Total Cover
	Grasses	Forbs	Total	Grasses	Forbs	Total	
Agricultural	56.8 B*	0.5 A	57.3 CB	53.5 CB	0.0 A	53.5 CB	55.4 CB
Control	0.0 A	0.0 A	0.0 A	0.0 A	0.0 A	0.0 A	0.0 A
Deep Plow	55.0 B	7.3 A	62.3 CB	59.7 C	2.0 A	61.7 C	62.0 C
Slurry Injection	47.7 B	2.4 A	50.1 B	38.4 B	0.6 A	39.0 B	44.6 B
Coversoil Wedge							
38 cm	65.3 CB	4.4 A	69.7 CD	63.4 C	0.3 A	63.6 C	66.7 CD
15 cm	81.6 C	3.9 A	85.5 D	67.8 C	2.4 A	70.1 C	77.8 D

* Multiple mean comparison based on LSD at significance level of 0.10. Mean followed by same letter in columns are not different.

The production data (Table 7) also revealed differences between the Control plots and the treatments, but there were almost no differences between the treatments and the CW. As at Site 7, production and cover on the DP plots appeared to be very successful and at this site production on the DP plots were comparable to the CW.

Importance Values suggest that the crested wheatgrasses performed very well on the CW's but not on the other treatments (Table 8). On Seed Mix 1 plots, Tall wheatgrass (Agropyron elongatum) dominated the landscape. Intermediate wheatgrass (Agropyron intermedium) revealed an identical pattern on Seed Mix 2 plots. Both of these wheatgrasses shared dominance with the crested wheatgrass species (Agropyron cristatum and Agropyron desertorum) on the CW's.

Metal levels in or on the plant tissues from these plots (Table 9) were generally ten times those found in similar vegetation at Site 7. The differences between metal levels in the vegetation growing on the CW's at the two sites were much lower, however. Lower metal levels in this vegetation may be due to lower metal levels in the root zone or reduced dust accumulation on the elevated wedges. The possibility of trace element imbalances in livestock and wildlife forages must be considered. Copper and zinc levels in or on vegetation are high enough to produce problems to animals grazing these sites throughout the year. Cadmium levels are elevated above levels recommended for livestock consumption (NRC 1980).

Root penetration at Site 21 was quite variable. In two soil pits opened in one plot amended by slurry injection, roots penetrated to 12 cm and 20 cm. In a replicate of the slurry injection treatment, root penetration was to 17.5 cm. In a third replication of this treatment, roots had penetrated to 27.5 cm. This latter example was noted as the best revegetated slurry injection plot. In the only A plot examined, roots stopped growing vertically and branched laterally at 17.5 cm. This was the limit of lime incorporation on this plot. In a DP treatment, roots had penetrated to 38 cm. Even at this depth there was no indication that the roots were at their limit of penetration.

The increase in production (and cover) of the crested wheatgrasses on the CW's indicates that these species will do well when a coversoil is available, but are not capable of successfully competing on finer textured, acid producing wastes even if amended. On this type of materials, Tall wheatgrass, Intermediate wheatgrass, and Russian wildrye (Elymus junceus) appear to be most successful. Their use with a legume must be recommended on this type of amended waste.

Water Quality

One of the major impacts of mining related wastes is upon water. Both ground and surface water are affected by these materials with detrimental effects upon water for

Table 7. Mean vegetation production (kg/ha*) at Site 21, Summer 1991.

Treatment	Seed Mix 1			Seed Mix 2			Mean of Total Production
	Grasses	Forbs	Total	Grasses	Forbs	Total	
Agricultural	1883 B**	2.6 A	1885 B	2065 B	3.1 A	2068 B	1977 CB
Control	0 A	0.0 A	0 A	0 A	0.0 A	0 A	0 A
Deep Plow	2805 B	126 B	2932 B	2354 B	17.4 A	2371 B	2651 C
Slurry Injection	2128 B	21.3 A	2149 B	1299 AB	1.8 A	1301 AB	1725 B
Coversoil Wedge	2274 B	27.0 A	2301 B	2006 B	6.7 A	2017 B	2159 CB

* To convert to lbs/a multiply by 0.89.

** Multiple mean comparison based on LSD at significance level of 0.10. Mean followed by same letter in columns are not different.

Table 8. Importance values for the dominant seeded species on Site 21, Summer 1991.

Species (Seed Mix 1)	Relative % Cover	Relative % Production	Importance Value*	Species (Seed Mix 2)	Relative % Cover	Relative % Production	Importance Value*
Agricultural				Agricultural			
Ag cr	0.0	0.0	0.0	Ag de	1.6	1.0	7.6
Ag el	98.3	98.5	196.8	Ag in	74.2	63.2	137.4
Ag ri	0.0	0.4	0.4	Ag tri	3.6	0.0	3.6
Legumes	0.0	0.0	0.0	El ju	16.9	35.3	52.2
				Legumes	0.0	0.0	0.0
Deep Plow				Deep Plow			
Ag cr	3.8	3.0	6.8	Ag de	3.9	3.6	7.5
Ag el	80.7	89.6	170.3	Ag in	64.0	67.7	131.7
Ag ri	0.0	0.3	0.3	Ag tri	0.7	3.0	3.7
Legumes	0.7	0.0	0.7	El ju	25.1	19.7	44.8
				Legumes	0.0	0.0	0.0
Slurry Injection				Slurry Injection			
Ag cr	0.0	0.7	0.7	Ag de	7.2	1.8	9.0
Ag el	94.3	93.2	187.5	Ag in	45.4	61.5	106.9
Ag ri	0.1	0.7	0.8	Ag tri	0.0	0.0	0.0
Legumes	0.0	0.0	0.0	El ju	44.8	36.6	81.4
				Legumes	0.0	0.0	0.0
Coversoil Wedge				Coversoil Wedge			
Ag cr	45.8	48.9	94.7	Ag de	46.3	46.2	92.5
Ag el	45.2	47.2	92.4	Ag in	30.9	38.6	69.5
Ag ri	3.7	2.7	6.4	Ag tri	10.1	12.1	22.2
Legumes	0.0	0.0	0.0	El ju	10.0	0.0	10.0
				Legumes	0.0	0.0	0.0

* Importance Value = Relative % Cover + Relative % Production.

Table 9. Comparison of mean elemental levels (mg/kg) in dominant vegetation collected (July 1991) from Site 21.

Element	Treatment: Agricultural Tillage	Control	Deep Plow	Slurry Injection	Topsoil	SE Montana Western Wheatgrass** (*Agropyron Smithii*)
			Tall wheatgrass (*Agropyron elongatum*)			
Aluminum	316.0 AB*	**	384.0 B	302.0 AB	74.0 A	5 to 50
Arsenic	26.1 AB		33.2 B	21.9 AB	4.3 A	<1 to 2
Cadmium	2.0 B		1.7 AB	1.5 AB	0.61 A	0.01 to 0.1
Copper	278.0 B		247.0 AB	216.0 AB	63.9 A	2.5 to 4.5
Lead	30.5 AB		60.6 B	25.1 AB	5.4 A	1.0 to 2.0
Manganese	917.0 B		672.0 AB	512.0 A	320.0 A	25 to 65
Zinc	750.0 B		541.0 AB	393.0 A	249.0 A	12 to 21
			Intermediate wheatgrass (*Agropyron intermedium*)			
Aluminum	311.0 B	***	266.0 B	286.0 B	48.4 A	
Arsenic	21.7 B		22.3 B	26.6 B	3.1 A	
Cadmium	1.9 B		1.6 AB	2.1 B	0.57 A	
Copper	228.0 B		196.0 AB	268.0 B	59.5 A	
Lead	28.3 B		38.2 B	29.7 B	3.5 A	
Manganese	790.0 B		622.0 B	677.0 B	243.0 A	
Zinc	589.0 B		491.0 AB	575.0 B	195.0 A	

* Multiple mean comparison based on LSD at significance level of 0.05. Means followed by same letter in rows are not different.

** Al and As estimated from the literature, Cd, Cu, Pb, Mn, and Zn from Munshower et al. 1987).

*** Species did not grow on control plot.

human consumption, agricultural uses, and fishery resources. Toxic elemental concentrations in these two water sources were measured to clarify the changes produced by the amendments and treatments used in this study.

Pore Water Quality

The impact of amendment and treatment upon soil pore water chemistry was measured in water pulled from suction lysimeters. Pore water quality at Site 7 appears to indicate that there was an effect of the addition of lime amendment to the soil at this site (Table 10). Most of the metals in water pulled from the treated plots show a decrease in soluble concentrations and calcium an increase in concentration compared to the levels reported in the Control plot.

Data from Site 21 do not show these differences. Control plot data are very limited because the soil zones at 40 and 150 cm were too dry to provide a pore water sample with these lysimeters. Furthermore, concentrations of metals in pore waters from the various treatments at this site (Table 11) were higher than the values recorded at Site 7.

Runoff Water

The study area has a history of fish kills resulting from the erosion of metals, salts, and acid into the receiving river after precipitation events of sufficient magnitude to wash mining wastes into the river. It has been assumed that amendment of the materials and vegetation growing on them will increase infiltration, leach salts deeper in the profile and reduce erosion of surface salts. During the third growing season simulated rainfall was applied to the plots to evaluate this phenomenon. Precipitation rates were 5 cm/hr for two hours. Runoff was collected, measured, and elemental levels in the water determined.

The coarse textured material at Site 7 had relatively little runoff, especially after amendment application and plant establishment (Table 12). The DP and Ag treatments yielded insufficient runoff for chemical analyses for the elements in question. Runoff at Site 21 was adequate from all treatments for the analytical determination of the elemental levels. Results of the analyses support the hypothesis that amendment application will reduce pollutant concentrations in the water. Table 13 shows the remarkable decrease in heavy metal levels in runoff from all treatments at Site 21.

CONCLUSIONS

Lime amendment of acid generating mining wastes has been shown to reduce the phytotoxicity of the materials but incorporation of the amendment into the wastes has been and continues to be a major problem. This study evaluated amendment and incorporation of wastes into several unique types of mining wastes. Amendments consisted of calcium carbonate and calcium hydroxide but the amount of the amendment added to each study site varied with the properties of the individual sites. Incorporation techniques included slurry pressure injection, agricultural plowing, deep plowing, and covering the waste with a soil cap. Several parameters were measured to determine the effectiveness of each incorporation technique.

While capping any waste with soil is a preferred rehabilitation technique, expense and lack of suitable materials often prevents the use of this technique. Of the alternative incorporation techniques the DP appears to produce the most satisfactory response. Plant cover and production were higher and runoff was reduced and improved by this technique. Nontoxic plant rootzones were also deepest under this method of amendment incorporation.

Table 10. Pore water chemistry from Site 7, 1992 (µg/l)

Treatment Sample Depth (cm)	Elemental Concentration (µg/L)											
	Al Zn	As(V)	Cd	Ca	Cu	Fe	Pb	Mg	Mn	K	Na	
Control												
40	Dry	--	--	--	--	--	--	--	--	--	--	--
90	4110	16	166	18,300	37,000	178	125	3,730	32,200	2,300	68,700	49,700
150	184	12	54	139,000	2,340	25	5	21,300	26,700	7,820	157,000	19,100
Slurry Injection												
40	Dry	--	--	--	--	--	--	--	--	--	--	--
90	61	7	211	69,500	17,500	13*	4	7,230	41,600	3,110	59,900	61,400
150	83	41	2*	153,000	36	31,000	1*	10,600	79,400	7,270	25,300	1,370
Deep Plow												
40	59	176	10	17,500	102	14	1*	2,820	1,870	3,330	284,000	2,070
90	162	206	16	116,000	60	36	4*	14,000	82	7,110	439,000	1,400
150	61	17	2*	143,000	10	29	1*	21,000	2	7,000	122,000	500

* Below detection limit.

Table 11. Pore water chemistry from Site 21, 1992 (µg/l)

Treatment Sample Depth (cm)	Elemental Concentration (µg/L)											
	Al Zn	As(V)	Cd	Ca	Cu	Fe	Pb	Mg	Mn	K	Na
Control											
40	Dry	--	--	--	--	--	--	--	--	--	--
90	68	u*	5	517,000	239	75	u	135,000	180	25,000	179,000	4,130
150	Dry	--	--	--	--	--	--	--	--	--	--	--
Slurry Injection												
40	Dry	--	--	--	--	--	--	--	--	--	--	--
90	111	604	3	287,000	97	81	1	170,000	770	11,800	559,000	373
150	57	460	2	36,400	45	16	u	14,800	92	1,880	414,000	23
Deep Plow												
40	358	67	105	289,000	204	35	6	138,000	365,000	5,830	449,000	65,600
90	86	13	134	429,000	389	58	u	215,000	86,000	9,820	206,000	40,400
150	43	u	3	126,000	57	25	u	27,400	65	5,410	76,000	388
Agricultural												
40	117	3	198	411,000	1030	36	23	259,000	146,000	15,300	212,000	54,900
90	45	171	3	302,000	67	44	u	52,600	1,280	6,012	119,000	918

* Below detection limit.

Table 12. Rainfall simulation studies, 1992, dissolved metals in µg/l.

Site	Treatment	As	Cu	Fe	Mg	Mn	Zn	Runoff (ml)
7	Control	12	14	124	167	4	8	1,200
	Injection	28	13	48	2,170	3	4	7,900
	Deep Plow	No Runoff						0.0
	Agricultural	No Runoff						0.0

Table 13. Rainfall simulation studies, 1992, dissolved metals (µg/l)

Site	Treatment	As	Cu	Fe	Mg	Mn	Zn	Runoff (ml)
21	Control	51	245,000	212	26,500	86,400	66,400	20,100
	Injection	91	44	30	25,100	360	22	16,300
	Deep Plow	47	390	18	7,400	6,900	2,700	7,000
	Agricultural	40	120	56	17,100	3,800	330	2,800

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NONPOINT SOURCES AND WATER QUALITY

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INTRODUCTION

In the twenty years since passage of the Clean Water Act in the United States, much progress has been made in decreasing pollution by sewage and industrial wastes of the nation's rivers, lakes, and coastal waters. Water pollution problems from point sources, such as municipal and industrial outfalls and other sources that are identified as coming from a clearly defined location, still exist and work is continuing in this area. However, it is important to concentrate more efforts on reducing and controlling nonpoint source pollution. Nonpoint source (NPS) pollution comes from diffuse sources and enters surface and ground waters in many ways, often in surges or storm events and often in large quantities. NPS is the largest remaining category of contamination threatening water quality in the United States. Effectively dealing with NPS pollution is a much more difficult task than correcting point source problems and requires different approaches.

Fisheries, wildlife, and recreation are the uses most affected by NPS pollution. Severe damage has been caused to aquatic communities nationwide, and the aesthetic values of many of the nation's recreational waters have been destroyed. Current estimates assign 45% of impaired estuarine areas, 76% of impaired lake areas, and 65% of impaired river miles to NPS causes.

The United States has lost billions of dollars of benefits from aquatic resources since the NPS problem was documented almost 20 years ago. Sediment causes decreased light transmission through water, which results in decreased plant reproduction, interference with feeding and mating patterns of aquatic animals, decreased viability of aquatic life, decreased recreational and commercial values, and increased drinking water costs. Increased siltation also destroys critical habitat for certain sport fishes and prematurely fills storage capacity of reservoirs. Nutrients promote premature aging of lakes and estuaries. Pesticides and herbicides hinder photosynthesis in aquatic plants, affect aquatic reproduction, increase the susceptibility of biota to stress, accumulate in the tissues of fish and other aquatic organisms, and are hazardous to both human health and wildlife through drinking water and the food chain. NPS pollution has resulted in beach closures, fishing bans, decreased property value, erosion, loss of aesthetic appeal of lakes and streams, and contamination of drinking water supplies.

SOURCES OF NPS POLLUTION

NPS pollution is contributed by agriculture, urban runoff, atmospheric deposition, highway drainage, hydromodification (stream channelization, flood prevention, lake drainage), silviculture, livestock grazing, construction practices, land disposal activities, septic systems, landfills/spills, and mining. Some NPS problems are generated from more than one kind of source and only recently have been recognized as requiring multimedia control programs. For example, nitrogen has diverse sources, plays an essential role in biogeochemical cycling, and can create ecological and human health problems. Pesticides and pesticide transformation products represent a major NPS concern that has potential environmental and health effects, and that can adversely impact both groundwater and surface water resources.

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AGRICULTURE

Agriculture is the largest NPS, affecting 50-70% of impaired surface waters. Siltation of surface waters is a major pollution problem arising from eroded croplands and overgrazed pastures and rangeland. Fertilizers and manures are major pollutants causing impacts in surface waters. Nitrates and pesticides are increasingly found in groundwater, arising from their use on crops and from livestock wastes. Animal waste from confined livestock facilities contains, in addition to nutrients and oxygen-demanding organic matter, bacteria that can cause drinking water contamination, fish kills, and shellfish bed closures. It also contains phosphorus that accelerates eutrophication of freshwater lakes. Agricultural activities often dramatically alter the landscape, increasing the vulnerability of sensitive ecological habitats including wetlands and riparian zones.

URBAN RUNOFF

Urban runoff NPS problems arise from streets and parking lots, and from industrial sites. Such runoff carries salts and oily residues from road surfaces and may also carry nutrients and toxic organic chemicals and metals.

ATMOSPHERIC DEPOSITION

Short- and long-range transport in the atmosphere and subsequent deposition on land and surface waters of a variety of pollutants, notably ammonia, nitrate, heavy metals, phosphate, and pesticides, are significant sources of NPS pollution in surface waters, both fresh and marine.

HIGHWAY DRAINAGE

Runoff from highway surfaces results in salts and oils being transported as pollutants to waters. Nutrients and combustion product organics may also be in highway runoff. Pollutants from highway construction and maintenance may find their way into surface waters.

HYDROMODIFICATION

Habitat modification, polluting chemicals, and increased sedimentation adversely affect water quality; these problems result from construction and other engineering projects such as dam building and stream channelization.

SILVICULTURE

Forestry operations, such as road construction and timber cutting, result in erosion, causing siltation problems in streams and lakes. Debris and pollutants from logging roads and habitat modification from logging cause NPS problems in surface waters and for forest wildlife.

CONSTRUCTION PRACTICES

Development activities such as clearing land, building new structures, and modifying existing structures produce sediment and toxic substances that can result in adverse effects in receiving surface waters.

LAND DISPOSAL ACTIVITIES

Toxic pollutants from hazardous and nonhazardous waste disposal sites can contaminate ground and surface waters. Sewage sludge spread on land and leakage from household and commercial septic systems can also adversely affect both surface and ground water systems.

SPILLS

Inadvertent spills during transport of chemicals or chemical wastes may cause pollution of surface waters with hazardous chemicals, sometimes resulting in kills of aquatic plants and fishes.

MINING

Runoff from mining activities can result in sedimentation problems and the addition of surfactants, acids, metals, and toxic organics to surface waters. NPS problems can also arise from abandoned mines, waste piles, and oil and gas wells.

REGULATORY MANDATE

Legislative mandates for the U.S. Environmental Protection Agency (EPA) to address NPS pollution are primarily the Clean Water Act of 1972, as amended in 1987, and the Coastal Zone Management Act (reauthorization amendments of 1990).

The Clean Water Act as amended establishes a national program to control nonpoint sources of water pollution. Two major new requirements were established: (1) reports from the 50 state governments describing water impairment due to nonpoint sources, the types of sources causing the problems, and state and local control programs; and (2) state programs for controlling nonpoint source pollution including methods and a time frame for remedying problems. The U.S. Environmental Protection Agency began a three-year grants program in 1990 through which EPA is awarding $140 million to states to support approved state nonpoint source programs.

The Coastal Zone Management Act Reauthorization Amendments of 1990 require that states develop a Coastal Nonpoint Source Control Program. The Act establishes a "technology-based" approach to NPS management with the associated presumption that best management practices will meet water quality goals.

Multimedia issues will require coordination across legislative mandates; for example, "trading" permits for nitrogen may be issued across the Clean Air Act and Clean Water Act. Solutions to NPS pollution problems will also require close cooperation among many local, state, and federal (U.S. EPA, U.S. Department of Agriculture, U.S. Geological Survey, U.S. Fish and Wildlife Service, Federal Highway Administration) agencies.

In the United States much of the regulatory activity directed toward controlling NPS pollution is voluntary. That is, landowners and operators like farmers, ranchers, and foresters, are encouraged to adopt Best Management Practices (BMPs) that help control NPS pollution while sustaining production and profitability. Federal and state government institutions provide technical and financial assistance in this activity.

RESEARCH APPROACH

The logical assessment, management, and restoration unit for research addressing NPS problems is the watershed, the surrounding land and associated land uses that contribute to the aquatic system. Problems are identified in a watershed context; long-term solutions require watershed or geographic area management plans that address the multiple sources of, and solutions to, the NPS problem.

The U.S. Environmental Protection Agency's research strategy is to develop the data, assessment tools, and technical outreach to answer four key questions: (1) What are the specific causes of the observed problems within impacted watersheds? (2) How can major nonpoint sources be reduced by prevention, restoration, and watershed management? (3) How do we determine the effectiveness of prevention, restoration, and management efforts? (4) What policies and mix of technologies are most effective in solving problems at different geographic scales?

The focus is on watershed scale processes and responses that will enable an integrated, multimedia approach to problem identification, human health and ecological assessment, pollutant runoff and leaching, control, landscape and stream system restoration, and pollution prevention. The research embodies geographic targeting of watersheds and problem areas within watersheds, integration of the effects of all stresses (physical, chemical, and biological), incorporation of designated use considerations, and trading of "pollution rights" between point and nonpoint sources within watersheds.

EPA's current NPS research plan has five major scientific components:

(1) Watershed Assessment Methods. Methods are needed that integrate geographic information systems, remote sensing, modeling, monitoring, and diagnostic bioassessments to characterize the sources, stressors, controls, and management methods needed to improve, protect, or restore watershed ecology and water resources. Currently, such methods do not exist. Assessments are typically limited in scope, address single media impacts, apply to limited scales, and often lack a comprehensive basis for action. Priority is now being given to developing a systems analysis capability for geographic targeting, policy analysis, watershed management planning, and relating source controls to impacts.

(2) Watershed Management and Control Technology. Best management practices applied to individual sources and watershed practices applied to mitigate multiple sources are the currently mandated approaches for NPS management within developed watersheds. Data documenting their effectiveness are limited, cause-and-effect relationships are uncertain, and field programs that measure the cumulative impacts of watershed level controls are urgently needed.

(3) Watershed Restoration. Substantial public investments are proposed for restoration of severely damaged watersheds. NPS management compatible with restoring the ecological integrity of watersheds is a major design and implementation challenge for such projects. Priority is being given to developing data on the costs and effectiveness of various NPS management approaches for watersheds under restoration.

(4) Pollution Prevention. Largely undeveloped but developing watersheds should be managed where possible to sustain natural conditions. Innovative approaches that limit pollutant inputs to watersheds and replace existing NPS-generating practices with sustainable development concepts are emerging and must be verified, documented, and promoted. Priority is being given to research on integrated farm management systems such as farming by soils; integrated pest management; chemical

and waste input reductions (variable application technologies); innovative forestry practices; new mining methods; and sustainable development in urban, suburban, and rural environments.

(5) Multimedia Assessment for Nitrogen Management. Gaps in knowledge of biogeochemical cycling processes are being filled by conducting process studies of forest response to wet/dry fall nitrogen loads; by developing better understanding of nitrogen movement and utilization in agricultural, urban, and suburban settings; and by developing methods for quantifying the effects of anthropogenic nitrogen inputs to estuarine systems in terms of measurable changes in estuarine community structure and function.

The research begun in 1992 emphasizes a concerted, systematic approach to solving the NPS pollution problem, beginning with the primary source of many of these problems--agriculture--and also focusing on the most pervasive pollutants--nitrogen and pesticides.

THE MASTER RESEARCH PROJECT

In 1992 the U.S. EPA, U.S. Department of Agriculture, and U.S. Geological Survey initiated a major research project to begin to implement the long-term NPS research strategy. This project is a five-year, $35 million study on the environmental effectiveness of midwestern agricultural NPS controls. The EPA component of the tri-agency project is called MASTER (Midwest Agrichemical Surface/Subsurface Transport and Effects Research).

Through the research being conducted in the MASTER project, EPA is working to understand the ecological impacts of agrichemicals and to separate out and distinguish these impacts from those of other types of agricultural practices such as habitat and hydrologic modification. At the Walnut Creek Watershed field site in the state of Iowa (Figure 1), MASTER studies are underway to determine the effects of agrichemicals on aquatic ecosystems' structure and function (Figure 2), to quantify the subsurface assimilative capacity for agrichemicals, and to quantify interactions of groundwater and surface water in this watershed. The effectiveness and longevity of alternative agricultural management practices are being projected and monitored to enable the prevention of ecological degradation and derive potential ecosystem restoration methodologies. Diagnostic and predictive ecological and hydrological modeling tools are being developed (Figure 3) to assess and minimize the ecological risk from agricultural management practices in this watershed. This involves linking existing fate and transport models and ecosystem effects models to predict chemical movement and resulting ecological impacts at different spatial and temporal scales as a function of control/restoration strategy.

The research project is still in its early stages. However, an example of the kind of information being obtained is shown in Figure 4. The current distribution of stream atrazine concentrations for the western corn belt plains can be used to determine the proportion of streams in this region with atrazine concentrations that exceed the proposed atrazine water quality criterion of 3 micrograms/liter. If alternative agricultural practices were implemented on the highly vulnerable watersheds within this region, it might be possible to reduce the proportion of streams exceeding the criterion from 70% to 30%. This reduction would reduce the risk to stream resources.

By the end of 1992 a preliminary assessment of the ecological effects of agricultural management practices, including ecological restoration techniques, will be completed for the Walnut Creek Watershed (15 square miles). By the end of 1996 the MASTER project will provide the tools, criteria, guidelines, and scientific information for the development of regional and national agricultural

policies and regulations that will improve the quality of the nation's water resources while maintaining economic sustainability of the nation's agricultural industry.

FEDERAL, STATE, AND LOCAL COOPERATION IS NEEDED

U.S. EPA and other federal agencies can provide scientific information through research, technical expertise, education, and some funding to help solve water quality problems arising from NPS pollution. But state and local governments must address NPS pollution through careful land-use and development planning that protects aquatic resources and aquatic and terrestrial habitats. Farmers and foresters must employ production practices that protect nearby streams and groundwater. Individual citizens must help by such measures as taking used oil to collection centers, applying lawn and garden chemicals carefully, and disposing of chemicals and debris in a safe manner. Only if all sectors of the populace do their part can the major problem of nonpoint source pollution be effectively solved.

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ECOLOGICAL ENGINEERING SYSTEM FOR THE CONVERSION OF MUNICIPAL WASTEWATER INTO RESOURCES IN ZHENJIANG

Yu-hui Zhuo[1,2] Xu Shen[1] and Xiao-liu Geng[1]

ABSTRACT

Xhenjiang (E 119°58', N 32°19') is a mid-sized industrial and tourism city with a population of 350,000, located in the south of Jiangsu Province and closely situated to the Yangtze River. With the rapid development of industry and increase in population in the past two decades, the problem of water pollution has become very serious, resulting in industrial water shortages and restraints on further economic development. To solve these problems, a research program (198601991) was undertaken cooperatively by the Environmental Science Department of Nanjing University and the local Environmental Protection Authority. After technical and cost-efficiency evaluation of a number of wastewater treatment plans, ecological engineering for the conversion of municipal wastewater into resources (oxidation ponds + land treatment systems) was selected as a cost-effective and environmentally sound strategy. This plan was accepted by the local government and listed on the World Bank's loan program for environmental protection in the southern part of Jiangsu Province. In June of 1992, the design plan successfully passed the comprehensive assessment of the World Bank. The final wastewater treatment capacity of the project will be 300,000 tons per day and the total investment will be 160,000,000 RMB (including a $20 millon US loan from the World bank). The construction period will cover 5 years (1993-1997). The two key parts of the project are the three municipal sewage intercepting systems which will be built along the main water bodies in the urban district (Harbour, Old Grand Canal and Yueliang River) and the ecological engineering systems which will be constructed on approximately 13 square kilometers in a suburban region 2 kilometers away from the urban district of Zhenjiang. The ecological engineering system will be comprised of oxidation ponds, land treatment systems, and several types of ecological farms. The treatment process involves collection of all the municipal wastewater by the three sewage intercepting systems, which will then be pumped into the ecological engineering system to be treated until discharge standards are met. Any wastewater that can be diverted or converted to resources (crops, fisheries, poultry and wood) will be. Treated water will eventually by discharged into the main stream of the Yangtze River. This paper will describe the project in detail and analyze its feasibility. It is expected that this project will solve the dual problems of water pollution and industrial water shortage in Zhenjiang and that the city will benefit socially, economically, and environmentally. And of equal importance, it will become a demonstration project and a major field experimental base for studying and developing low-cost ecotechnology suitable for the treatment and utilization of wastewater in cities and towns of southern China.

ENVIRONMENTAL SURVEY

GEOGRAPHICAL LOCATION

Zhenjiang city (E 119°58', N 32°19') is located on the southern shore of the Yangtze river in the center of the Jiangsu province. The cities of Nanjing and Shanghai lie 70 km upstream and 503 km downstream from Zhenjiang, respectively.

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CLIMATE

Zhenjiang experiences a northern, subtropical monsoonal climate that is typically warm and humid. The annual mean air temperature is 15.4°C and the frost-free period averages 238 days out of the year. During the summer, the prevailing wind direction is from the southeast with an annual mean wind speed of 3.6 m/s and an annual maximum of 16 m/s. The average annual rainfall is 1060 mm with 45% of the total rainfall occurring during the summer.

SOIL

The predominant soil type of the delta area near the Yangtze river is a fine, silty, sandy soil over clay, while in the floodland formed near the Yangtze are sand clays, slime sand clays, and silty fine sandy soil.

VEGETATION

Mixed broad-leaf forests of both evergreen and deciduous tree species comprise the dominant vegetative cover. On undeveloped river banks (such as the Zhenrun and Jiaobei shoals) there are helophytes such as reeds, medical zizanta caducifloria, acorus calamus, and sparganium stoloniferum.

LAND AREA AND POPULATION

The urban district of Zhenjiang covers 26 km^2, with a 1990 population estimated at 350 000. Therefore the population density is 13562 people per square kilometer.

ECONOMY

Zhenjiang is a mid-sized industrial city with a diversified economic structure, of which electronics, machinery, textiles, paper, construction materials, and chemical production are the predominant industries. The gross industrial production of the urban district of Zhenjiang in 1988 was 3.4 billion RMB. Zhenjiang is also an important port city and a major, international tourist destination, plays a vital role in the economy of the city.

AQUATIC ENVIRONMENTAL QUALITY ASSESSMENT

AQUATIC ENVIRONMENTAL BACKGROUND

Within the urban district of Zhenjiang, the primary water bodies are the Yangtze river, the Great (Old) Grand Canal, the Harbour, and the Yueliang river (Fig. 1).

INVESTIGATION OF WATER POLLUTIONS SOURCES

In 1990, the total wastewater discharge from the city reached 200 000 m^3 per day, of which most was untreated (Table 1). Over the years, this practice has resulted in serious water pollution problems in all of the main water bodies in Zhenjiang.

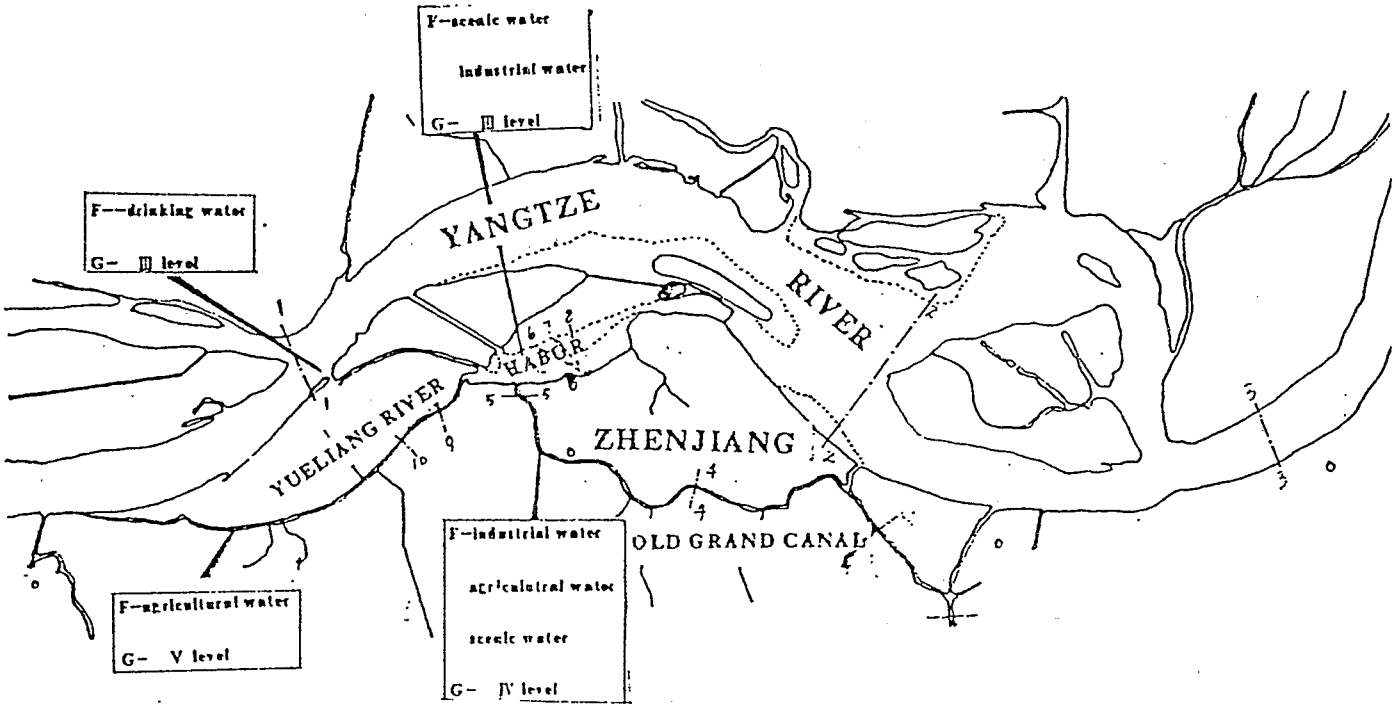
COMPREHENSIVE ASSESSMENT OF WATER QUALITY

Evaluation of water quality was made using the P.R. China national surface water quality standard (GB3838-88) and 8 water quality indices (chemical oxygen demand (COD), biological

oxygen demand (BOD5), ammonia nitrogen, volatile phenol, total As, Pb and Cr6+), selected from water quality data gathered from 1985 to 1989 (Table 2). The results indicate that the water quality in the Yangtze river is at an acceptable level while the other water bodies within the region are polluted to varying degrees. The most serious indicators are COD, BOD5, ammonia nitrogen, and volatile phenol.

ECONOMIC ANALYSIS OF WATER POLLUTION

Water pollution has not only restrained further development of the local economy, but has also threatened the beauty of the region and therefore the associated tourism. The highest density of factories in the urban district is found along the harbour and the Old Grand Canal. Of these, the harbour has become the most important industrial water source. However, deterioration of the Harbor water has resulted in unacceptable water quality for industrial use, and the consequent water shortage has both decreased industry production (for example, the Dadon Paper Mill showed a loss in profit of 5 million RMB in 1989) and increased factory demand upon the municipal tap water supply. In 1989, the total municipal water supply was 150 000 m^3 per day of which the industrial demand accounted for 80 000 m^3. The demand upon the municipal water supply by further industrial development in the area combined with the decreasing water quality in the Harbour will likely lead to a shortage of drinking water in the city. The negative impact of water pollution has already been felt by the tourism industry of the Zhenjiang area, and if no steps are taken to improve the water quality, the long term viability of the tourist trade may be seriously jeopardized.



Note: F, G respectively stand for function of water body and water quality goal

Figure 1. Map of functional regionalization of main water bodies in Zhenjiang.

Table 1. Characteristics of wastewater discharged from the urban district of Zhenjiang.

Receiving Water	Wastewater Discharge	SS	COD	Phenol	Cyanide	Oil	As	Pb	Ammonia
Harbour	97,800	56.77	75.57	0.08	0.06	1.12			1.31
Old Grand Canal	84,600		29.26	0.01	0.01		0.03	0.01	
Yuenliang River	20,300		8.17	0.05		0.05			

Table 2. Comprehensive assessment of water quality in Zhenjiang.

Cross-section		Yangtze River			Old Grand Canal		Harbour			Yueliang River	
		1	2	3	4	5	6	7	8	9	10
Dry Period	1985	+	+	+	-B,C,N,A	-B,C,A,N	-B,C,A,N	-B,C,A,N	-B,A,N		
	1986	+	-A	+	-B,C,N,A	-B,C,A,N	-B,C,A,C	-B,C,A,N	-B,C,A,N		
	1987	+	-A	+	-A,N	-A	-B,A,N	-B,C,A,N	-B,A,N		
	1988	+	-A	+	+	-B,A,N	-B,A,N	-B,C,A,N	-B,A,N	-B,N	-B,N
	1989	+	+	+	-A	-B,A,N	-B,C,A,N	-B,C,A,N	-B,A,N	-B,C,N	-B,N
Normal Period	1985	+	+	+	-A	+	-B,C,A,N	-B,C,A,N	-N		
	1986	+	+	+	-A	-A,N	-B,A,N	-B,C,A,N	-B,A,N		
	1987	+	+	+	+	+	-B,A,N	-B,A,N	-B,A,N		
	1988	+	-A	+	+	+	-B,A,N	-B,A,N	-B,A,N	-N	-N
	1989	+	+	+	-A,N	-A,N	-B,N	-B,A,N	-B,A,N	+	+
Wet Period	1985	+	+	+	+	+	+	-B,A,N	-A,N		
	1986	+	+	+	+	+	+	-N	-N		
	1987	+	+	+	+	+	-N	-B,A,N	-B,A,N		
	1988	+	+	+	+	-A	-A	-B,A,N	-A,N	+	+
	1989	+	+	+	+	-A,N	-N	-N	+	+	+

PRIMARY FEASIBILITY ANALYSIS OF INTEGRATED WATER POLLUTION TREATMENT IN ZHENJIANG

FUNCTIONAL REGIONALIZATION OF MAIN WATER BODIES

Classification by water use of the main water bodies in the Zhenjiang region is illustrated in Figure 3-1.

WATER QUALITY GOALS

The short-term water quality goals for the main water bodies within the Zhenjiang region, are for the harbour and those portions of the Yangtze river and Great Grand Canal within the Zhenjiang district to reach the 2nd, 3rd, and 4th levels of the national surface water quality standards (GB3838-88) by the year 2000.

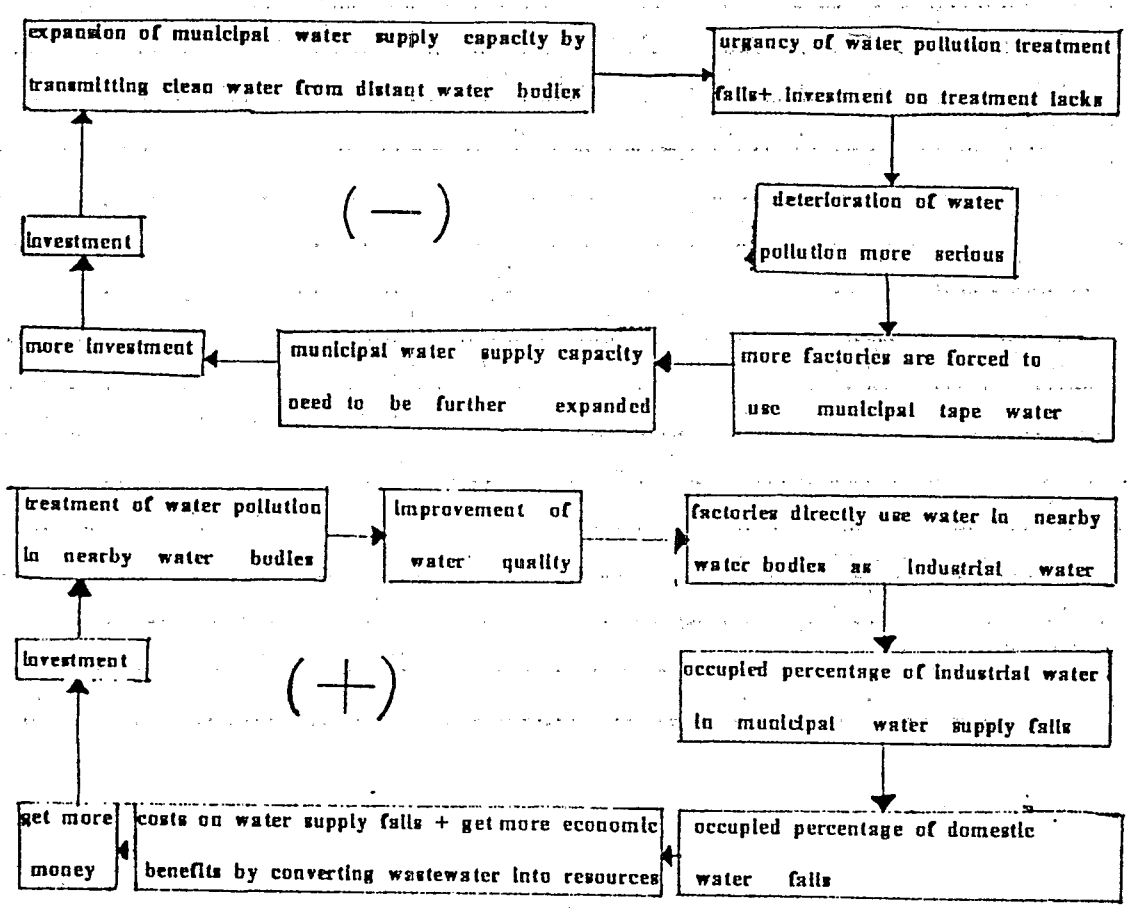


Figure 2.

ANALYSIS OF THE PROJECT DESIGN SCHEME

Reliance of Zhenjiang's industrial core upon the harbour as a primary water source means that the harbour is strongly linked to the economic health of the Zhenjiang region. In recent years, continued deterioration of the harbour water has created a shortage of water for industrial needs. There are two possible solutions to address the shortage of water. One is to expand the municipal water supply capacity by diverting water to the system from the Yangtze river. The other solution is to treat industrial waste water and allow the water quality of the Harbour to recover to a level where it would again be suitable for industrial use. The two paths lead to very different situations, and the resulting event flow diagrams are shown in Figure 2.

The flow diagrams indicate that treatment of the waste water will ultimately provide the better solution, both economically and environmentally. This has in fact been established in other urban areas of southern China, such as Shanghai, where the total costs of municipal water supply expansion have generally exceeded that of methods undertaken to improve the water quality.
Just to the north of Zhenjiang, 2 km from the urban core, are two large deltas referred to as the Zhenrun and Jiaobei Shoals. These deltas occupy a flat area of 13 km², the soil is soft floodland sediment, and they are currently undeveloped. The deltas may be a possible location for the establishment of low-cost oxidation ponds and land treatment systems to treat the industrial pollution from Zhenjiang. Part of the pollution problem in the Harbour is a lack of adequate water mixing or exchange from the Harbour back out to the Yangtze river. By pumping the sewage to an area closer to the Yangtze river and treating it before discharge, water pollution should be effectively controlled.

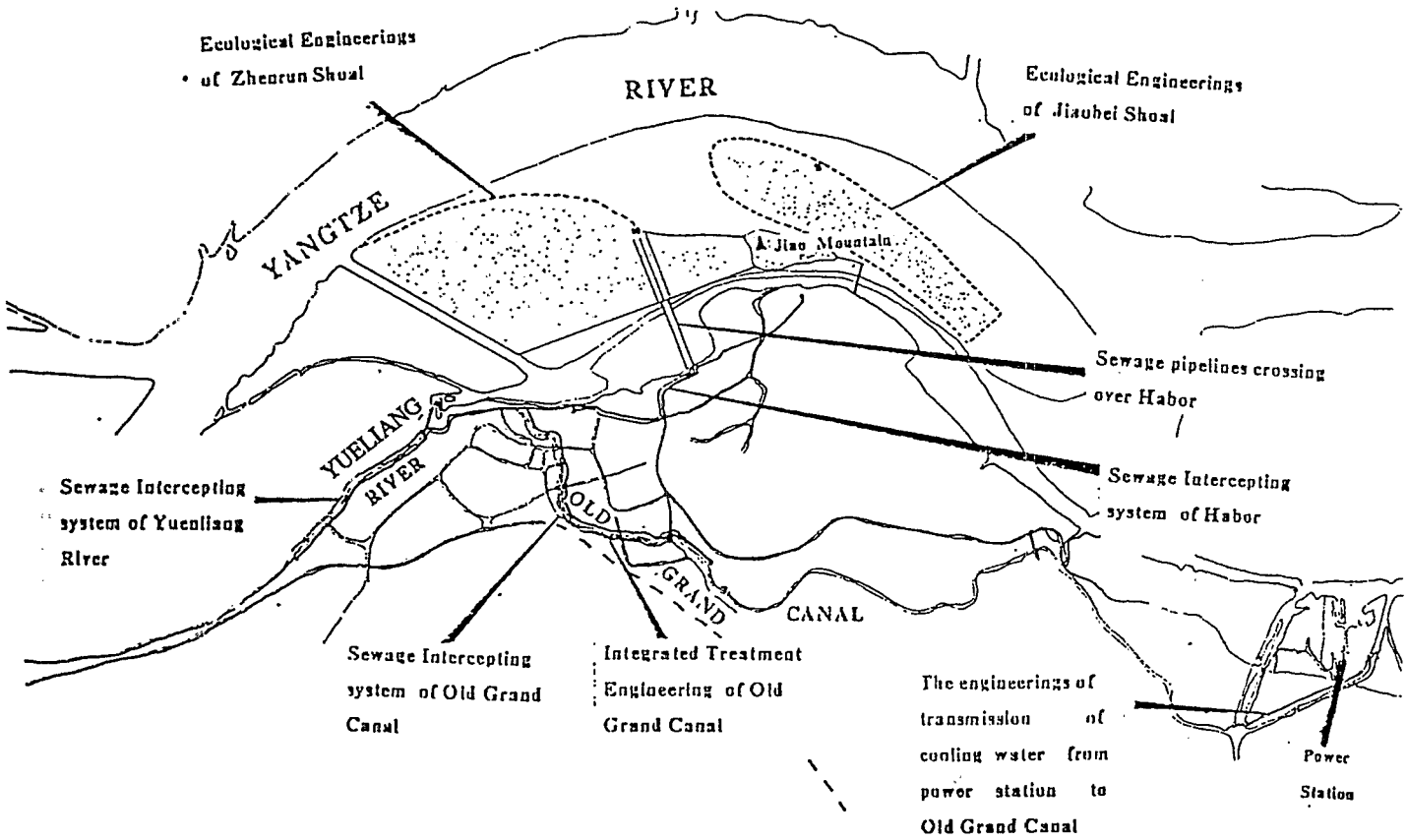


Figure 3. Integrated treatment project of water pollution in Zhenjiang.

OUTLINE OF PRIMARY DESIGN SCHEME

Three municipal sewage intercepting systems will be constructed along each of the three main water bodies in Zhenjiang; the Harbour, the Old Grand Canal, and the Yueliang river, all of which currently collect municipal waste water (Fig. 3). The Jiangbin pumping station will direct sewage onto the Zhenrun and Jiaobei Shoals through pipelines crossing over the harbour. Thirteen km^2 of the deltas will be developed into waste water treatment facilities where the waste water will be treated and diverted as a supply for other industries (such as agriculture, fisheries, poultry, and forestry), before being discharged into the Yangtze river on the condition that water quality standards are met.

MUNICIPAL WASTE WATER TREATMENT PLAN

OUTLINE

The water pollution treatment plan for Zhenjiang is comprised of two components. The primary system is the treatment facilities for general treatment of city waste water as a means to improve water quality and provide a water source for industries. An auxiliary system will be provided to improve the water quality of the Old Grand Canal as a means of enhancing the tourism potential of the area. Only the primary treatment plan is discussed below. Table 3 lists the major components included in the design of the water treatment facilities. The complete project has been divided into three stages of construction as outlined in Table 4.

FIRST STAGE OF DEVELOPMENT

The treatment facility is being designed to handle a daily flow of 124 500 m^3 of waste water, of which the industrial contribution is 91 000 m^3 and the domestic portion 33 500 m^3. The primary goals of the first stage of development are to establish the sewage interception system in the harbour, and intermediate-testing experimental oxidation ponds on the Zhenrun Shoal.

(1) Sewage Interception System of the Harbour.

An overview of the sewage interception system for first stage development is illustrated in Fig. 4. The total land area to be serviced is 449.25 hectares with a population of 221 800. Systematic analysis suggested that the interception ratio of the system would be optimal at 3. The 1990 estimation of the total cost of this stage was 46 722 900 RMB.

(2) Intermediate-Testing Experimental Oxidation Ponds.

As previously discussed, the Zhenrun Shoal is located to the north of Zhenjiang city between the Harbour and the Yangtze river. This delta is composed of floodland sediment and covers an area of more than 2 000 hectares. The main soil types on the delta are sand clay and slime sand clay. The soil texture is very soft and the land is flat, making it readily suitable as a site for the design, construction, and maintenance of biological oxidation ponds and other treatment facilities.

In June of 1992, the North China Civil Engineering Design Institute made a primary design of the experimental oxidation ponds on Zhenrun Shoal based on the report "Primary design scheme of first-stage project of ecological engineerings for the conversion of wastewater into resources in Zhenjiang", and the results of an environmental impact assessment of the area. The location chosen occupies a 10 hectare area in the northeastern corner of the Zhenrun Shoal (Fig. 3).

Once in operation, the facility is designed to treat 5 000 m^3 of waste water per day. The predicted intake water levels of BOD5 at 30 mg/l and SS at 60 mg/l will be treated to give output levels of BOD5 at 200-250 mg/l and SS at 200-300 mg/l. A flow chart of the treatment processing steps, a table outlining some of projected operating data, and a schematic of the layout of the collecting ponds are all given in Figure 5. The total investment into the project was estimated in 1992 as 3 470 000 RMB.

Table 3. The major components of the principal sewage treatment facilities planned for Zhenjiang city.

Sewage Intercepting Engineerings (SIE)	Engineering of Sewage Pipeline Crossing Over Harbour	Sewage Treatment and Utilization Engineerings
• SIE in south bank of Harbour • SIE in both banks of Old Grand Canal • SIE in both banks of Yueliang River • Engineerings of remaking municipal water discharge system	• Depositing reservoir • Jiangbin Pumping Station • Sewage mainlines crossing over Harbour	• Depositing tanks • North and south man-made banks of Zhenrun Shoal • Intermediate-testing experimental oxidation ponds • Pumping stations of Zhenran Shoal and Jiaobei Shoal • Ecological farms of Zhenrun Shoal and Jiaobei Shoal • Scientific Experimental Base for ecological engineerings

Table 4. The three stages of construction of the principal sewage treatment facilities for Zhenjiang city.

Stage	Duration	Engineerings	Expected Benefits
First Stage	1993-1995	• SIE of Harbour • Transmission engineerings of acidic wastewater • Jiangbin Pumping station • Main Pipeline over Harbour • Zhenrun Shoal Pumping station • Experimental Oxidation ponds	• Obvious improvement of water quality in Harbour • Pollution load reduction: 50% • Industrial water shortage of factories along Harbour can be almost solved
Second Stage	1994-1996	• Immediate testing base of ecological engineerings • Ecological farms on Zhenrun Shoal • Expansionj Engineering of Zhenrun Shoal Pumping station • SIE of Old Grand Canal	• Water quality of Harbour reaches 3rd level • Pollution load reduction: 80% • Economic benefits: 500,000RMB in 1996
Third Stage	1995-1997	• SIE of Yueliang River • Construction of ecological farms completed • developing Jiaobei shoaly land • Engineerings of transmitting water from Power Station to Old Grand Canal	• Water quality of Yueliang River reaches 5th standard • Pollution load reduction: 99% • Economic benefits: 1 million RMB per year

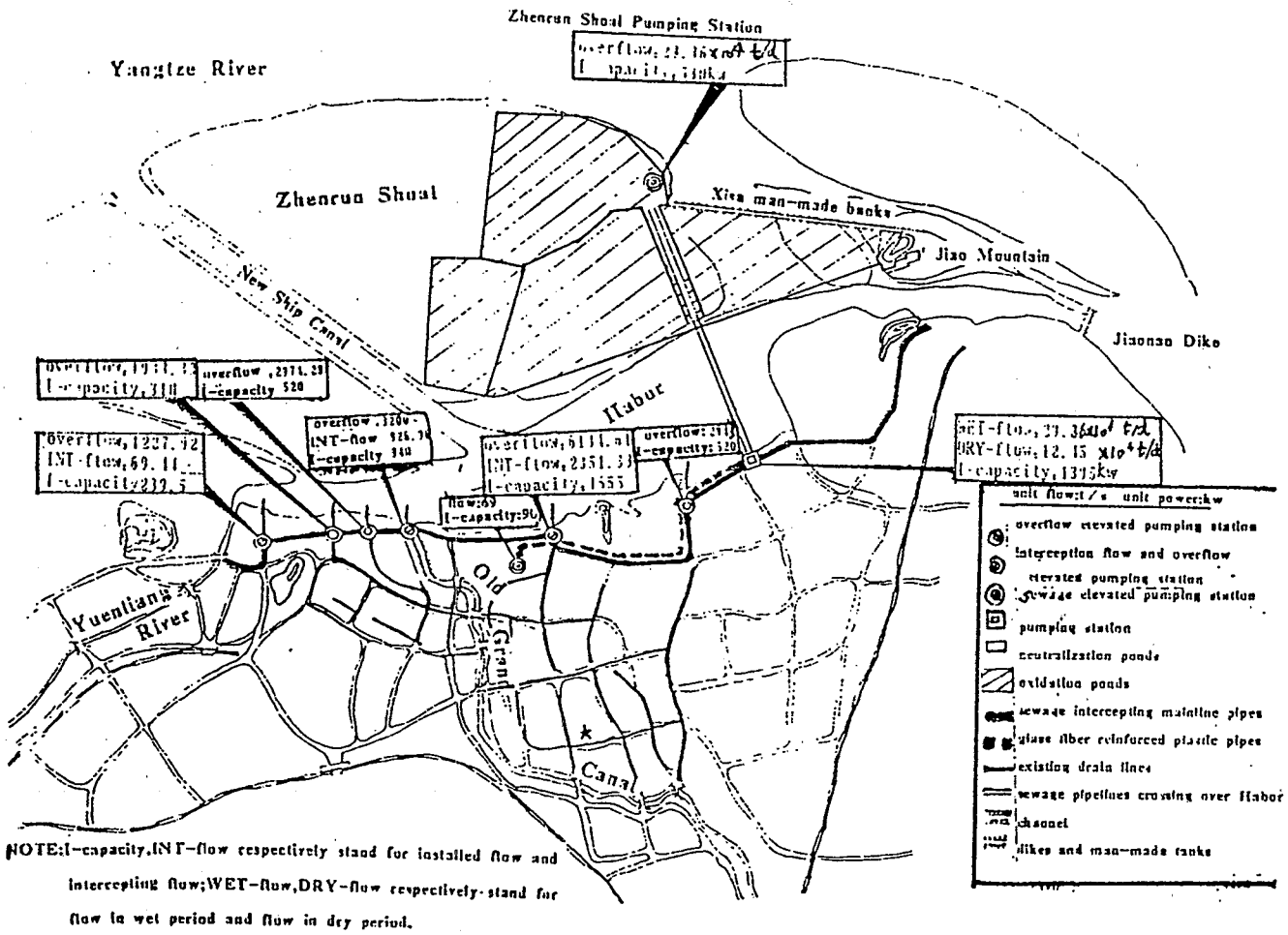
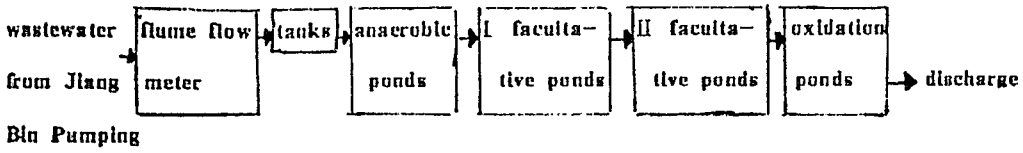


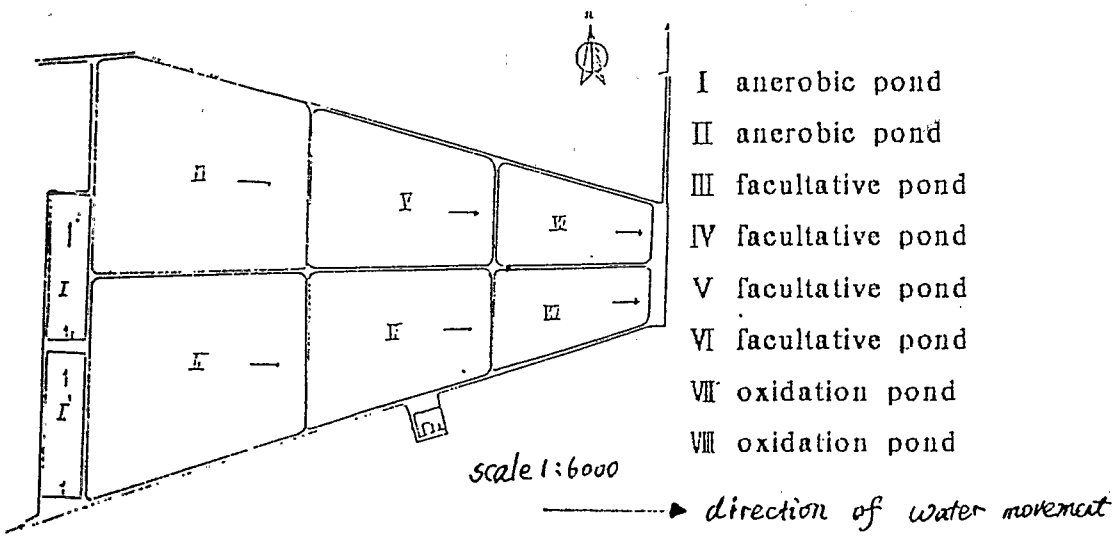
Figure 4. First-stage project of Ecological Engineerings for the conversion of wastewater into resources.



* technical parameters *

type	BOD_5				effective water depth (m)	effective area (m^2)	effective volume (m^3)	retention time (d)	reaction rate constant K (d^{-1})
	inlet water (mg / L)	outlet water (mg / L)	removal rate(%)	areal load(kg / $10^4 m^2 \cdot d$)					
anaerobic ponds	200 ~250	120	40		4.5	6902.0	16353.0	3.3	0.20
facultative ponds	120	60	50	72.140	2.5	83183.7	129975.3	26	---
oxidation ponds	60	30	50	176.8	2.0	16967.5	21278.1	4.3	0.23

* layout of the project *



* Total investment *

Total investment on this project is 3,470,000 RMB according to prices in

Figure 5.

ENVIRONMENTAL IMPACT ASSESSMENTFOR THE ZHENJIANG HARBOUR

The Environmental Science Department of the Nanjing University in China and the Athens National Environmental Research Laboratory in the U.S.A. worked jointly on a research program in which the DYNHYD4 hydrodynamic and the WASP4 entrophication models were used to develop a water quality model of the Zhenjiang harbour. The results indicate that completion of the first-stage of the project will permit the water quality of the harbour to reach or even surpass the 3rd level of the national surface water quality standard (GB3838-88). The model also predicts that the water quality will reach the desired standard within the first month of operation of the first-stage facilities.

ENVIRONMENTAL IMPACT ASSESSMENT FOR THE YANGTZE RIVER

Fully treated water from the oxidation ponds and other land treatment systems will be discharged into the Yangtze river at a zone near some man-made banks known as the Xisa banks. The distance from the discharge site to Dantu city, the nearest developed center downstream, is 9.8 km. It is assumed that the high volume of the Yangtze will dilute and further biodegrade the treated waste water, which will further reduce any negative impact on downstream water bodies.

ENVIRONMENTAL IMPACT ASSESSMENT FOR THE RESIDENTIAL SCENIC AREAS OF ZHENJIANG

The oxidation ponds on the Zhenrun Shoal will be 2 200 m from Jiao Mountain, an important natural resource, and 2 550 m from the Jiangbi residential area. During most of the year, the prevailing wind is from the southeast and such nearby scenic and residential areas are upwind from the water treatment site. During the winter, however, the prevailing wind is from the northwest and could carry odours from the oxidation ponds to these areas. An attempt is being made to reduce this effect by adjusting the layout and design of the oxidation ponds, establishing green belts, or using other technological methods.

TREATMENT OF TOXIC WASTES

In order to ensure that the treatment facility is able to maintain an acceptable quality of discharged treated water, it will be necessary to minimize the toxic pollutants entering the system. To do this requires integrated utilization of new management methods and technologies. This is viewed as one of the major difficulties facing the successful operation of the project. Ultimately, the project will include measures to ensure that the discharge of toxic wastes into the sewage collection system is only done when the waste meets a certain standard. This may require pre-treatment facilities in some factories. Part of the long-term environmental management of the city must include conditions that will prevent the establishment of new factories that produce unacceptable toxic waste, and measures that can force the closure of those factories unable to meet the minimum standard requested.

LATER STAGE DEVELOPMENT PROSPECTS

The oxidation pond treatment system on the Zehrun Shoal is intended to serve only as a transitional method of water treatment. The ultimate goal of the project is to establish a large-scale facility that will not only be able to treat waste water, but will also be efficient enough to allow for the recovery and reuse of treated water. For this later stage of development, the Zhenrun and Jiaobei Shoals are able to offer several site advantages, including:

(1) Ample space for expansion - 13 km^2, equal to half of the existing urban district.
(2) Fertile topsoil - subclay/sand, rich in organic materials and suitable for vegetation.
(3) A warm and damp climate - average annual air temperature of 15.4°C with a 238 day frost-free period.
(4) Close proximity to the industrialized area - permits inexpensive transport of sewage.
(5) Flat topography.
(6) Close proximity to the Yangtze river - allows water from the Yangtze to be used to adjust waste water treatment and discharge concentrations.

The Environmental Science department of Nanjiang University will construction an experimental field station on the Zhenrun Shoal. In addition, a major national research laboratory entitled "Water Pollution Control and Conversion of Wastewater into Resources" has been recently established within the Environmental Science department. This new laboratory will allow for the study and development of models relating to waste water treatment. These developments together with technological assistance from the World Bank, should ensure that the final stages of the project will be realized.

SUMMARY

The outlined plan was developed with preliminary scientific investigation, and consideration of the available technology and the rich land resources available in Zhenjiang. The economic, environmental, and social benefits derived from the project for Zhenjiang include:

(1) The majority of domestic and industrial waste water of the urban district of Zhenjiang will be treated and reused, effectively controlling the water pollution problem.

(2) The factories near the harbour and adjacent rivers will once again be able to use those water bodies for source water, solving the industrial water supply problems. The costs associated with municipal water supply expansion can thus be avoided.

(3) The Zhenrun and Jiaobei Shoals will be developed for light industry such as rice fields and fisheries.

Finally, and importantly, the facility will serve not only as a demonstration project, but also as a major field experimental base for the study and development of low-cost waste water treatment and post-treatment water uses for other regions of southern China.

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WATER QUALITY MODELING OF TOXIC ORGANIC CHEMICALS IN THE YANGTZE RIVER AND ITS APPLICATIONS

by

Ouyong Xu, Guangyao Sheng and Haixan Zou[1]

ABSTRACT

The maximum permissive loads of five organic pollutants (phenol, nitrobenzene, chlorobenzene, bis-2-chloroethyl ether, and di-n-butyl phthalate), were calculated in sewage effluence being discharged into the Yangtze River at a site up-stream from a newly constructed water pumping station. A two-dimensional, non-steady state, differential equation was used to simulate chemical flow patterns within the river in order to account for width and tidal variations. The first-order attenuation coefficient (K) in the differential equation represents the sum of the pseudo first-order rate constants of environmental processes such as volatilization, direct photolysis, hydrolysis, oxidation, and microbial transformation. Rate constants were computed using the Exposure Analysis Modeling System (EXAMS) developed by Burns et al. (1982), and also from corresponding parameters measured in the field. The results indicate that attenuation of the organic compounds is significant compared with the transport process provided that the coefficient of attenuation exceeds 10-2 /hr.

INTRODUCTION

The site of sewage discharge from Changzhou city into the Yangtze River is located approximately 8.5 km up-stream of the Ligang Water Works pumping station (currently under construction). The water source of the new pumping station will be the Yangtze River. The close proximity of the sewage discharge to the pumping station raises questions about the impact of organic pollutants from sewage effluents upon the water quality at the Ligang pumping station, and suggests a need for assessment of the maximum permissible load of these organic pollutants. The maximum permissive loads of five organic pollutants (phenol, nitrobenzene, chlorobenzene, bis-2-chloroethyl ether, and di-n-butyl phthalate) were calculated using a two-dimensional non-steady state mass transfer equation and the mixed coefficients for the Changzhou section of the Yangtze River (Institute of Environmental Water Conservancy, He Hai University, unpublished), and the Exposure Analysis Modeling System (EXAMS) (Burns et al. 1982). The effects of changes in the magnitude of the first-order attenuation coefficients of organic compounds upon their maximum concentration levels within the source water at Ligang are discussed.

MODEL AND PARAMETERS

The section of the Yangtze River under study, from the sewage discharge site at the mouth of the Zaojiang stream to the pumping station at Ligang, is wide and subject to tidal bores occurring twice daily. The direction of water flow changes during the dry season, but during the rainy season, these tidal effects are reduced and only flow velocity is affected. Toxic organic compounds discharged into the Yangtze River are both diluted and attenuated, resulting in a cline in the

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concentration of pollutants over the study area. The distribution of these pollutants can be simulated using a two-dimensional dynamic equation that includes the effects of flood and ebb tides:

$$\frac{\delta (HC)}{\delta t} + \frac{\delta (UHC)}{\delta x} + \frac{\delta (VHC)}{\delta y} = \frac{\delta (HDx\ \delta C)}{\delta x} + \frac{\delta (HDy\ \delta C)}{\delta y} + H(S-KC)$$

where:
t = time
H = depth (m)
x and y = plane coordinates where x is positive in the direction of water flow, and y is perpendicular to x and positive from right to left
U, V = average water velocities (m3/sec) in X and Y planes respectively; their magnitude and direction are variable with the tide.
C = concentrations of toxic organic compounds (ppb)
Dx, Dy = mixing coefficients in the X and Y planes (m2/sec)
S = load of toxic organic compounds (g/m3.sec)
K = first order attenuation coefficient of the toxic organic compounds (hr-1)

The equation shown was solved to provide a numerical solution by splitting the equation using the control volume method and then solving by the implicit difference approach. A rectangular grid was imposed over the study area of the river using 200 m divisions in the Y-axis, while the X-axis was divided unequally into 200, 400 and 600 m divisions. A total time period of 8 days divided into 30 minute time steps was used in the computation. The mixed coefficients (diffusion and dispersion) in the X and Y planes were calculated by the following formulas which were the results of field study:

$Dx = 6.00\ HU^*$
$Dy = 0.52\ HU^*$, where U^* is the shear velocity.

EXAMS (Burns et al., 1982a, b) is an essential model for studying the transport and dispersion of organic compounds in an aquatic environment. Using the physical and chemical properties of an organic compound combined with some aquatic environment parameters the first-order attenuation coefficient, K, can be computed for use in the two-dimensional dynamic equation given above. Theoretical and applied studies using EXAMS have been reported in the literature (Wolf et al., 1980; Zepp, 1980; Honeycutt and Ballantine, 1983). In a previous study, the transport and dispersion of nitrobenzene, bis-2-chloroethyl ether, quinoline, and phenol were examined in the Changzhou section of the Great Canal, which is connected to the Yangtze River and within 20 km of the current study area. Using the data collected from the Great Canal on nitrobenzene and bis-2-chloroethyl ether, we were able to verify the reliability of EXAMS as well as the precision of the parameters selected (Xu and Sheng, 1987). Therefore, it seems reasonable to expect that the application of EXAMS to the current study section of the Yangtze River will provide reliable, reproducible results. The first-order attenuation coefficient, K, and the rate constant were estimated from environmental parameters such as temperature, bacterial population, etc.

The important kinetic processes to be considered when evaluating the behavior of organic pollutants in a water body are hydraulic transport, volatilization, direct photolysis, hydrolysis, oxidation, and microbial transformation. The main function of EXAMS is to calculate the pseudo first-order rate constants of these processes. The first-order attenuation coefficient in the differential equation described above is calculated as the sum of the pseudo first-order rate constants of volatilization (KV), direct photolysis (KP), chemical oxidation (KO), hydrolysis (KH), and microbial transformation (KB). The physico-chemical properties (from Mabey et al. 1981) and rate constants of

the five organic compounds examined are shown in Table 1. The effect of season on the measured or estimated environmental parameters for the section of the Yangtze river under study are given in Table 2. The pseudo first-order rate constants (K) calculated by EXAMS of the five organic compounds examined are listed by season in Table 3. The calculated values of K show that phenol had the fastest rate of degradation while di-n-butyl phtalate had the slowest.

Following completion of a new sewage pipe line, the load of organic pollutants discharged into the Yangtze will increase. A review of the literature indicates that bacteria counts can be expected to increase up to 20 times their current population at the discharge site and directly downstream. The increased pollutant load requires modification of the bacterial degradation rate constants (KB) and the corresponding first-order attenuation coefficients (K) for the compounds examined (Table 4).

Table 1. Physico-chemical properties and process rate constants of selected organic compounds.

Compounds	Bis-2-chloro ethyl ether	Nitro-benzene	Phenol	Chloro-benzene	Di-n-butyl phthalate
mol.wt.	143	123.11	94.11	112.56	278.3
solubility (ppm)	1.02×10^{4}	1.9×10^{3}	9.3×10^{3}	4.88×10^{2}	13
sediment/water distribution coef. (mg/kg)/(mg/l)	13.9	36	14.2	330	1.7×10^{5}
Herry constant (atm·m^3/mol)	1.3×10^{-5}	1.31×10^{-5}	4.54×10^{-7}	3.58×10^{-3}	4.50×10^{-6}
vapour pressure (Torr)	0.71	0.15	0.341	11.7	1.6×10^{-4}
acidic hydrolysis rate constant (/h/M)	0.0000	0.0000	0.0000	0.0000	7.92×10^{-3}
neutral hydrolysis rate constant (/h)	4×10^{-6}	0.0000	0.0000	0.0000	0.0000
alkaline hydrolysis rate constant (/h/M)	0.0000	0.0000	0.0000	0.0000	79.2
oxidation rate constant (/h/M)	24.0	0.0000	1.0×10^{-7}	0.0000	1.4
microbial transformation rate constant (mg/cell/h)	3×10^{-9}	3×10^{-9}	3×10^{-6}	3×10^{-9}	3×10^{-8}

Table 2. Some environmental parameters of the Yangtze River study site in three different seasons.

	Dry	Intermittent	Rainy
wind velocity near surface (m/s)	3.5	3.5	3.5
water temperature (°C)	15	20	25
oxidant concentration (M)	1 x 10-9	1 x 10-9	1 x 10-9
bacterial cell population (cells/ml)	200	2000	4000
aeration coefficient (cm/h)	3.80	3.80	3.80
suspended solids (mg/L)	100	300	600
pH	8.0	8.0	8.0

latitude 31.77'; average cloud cover (%) 69.0

Table 3. Pseudo first-order rate constants (hr-1) and attenuation coefficients of selected organic compounds.

Compounds	K_p	K_H	K_o	K_s	K_V	K
			dry season			
chlorobenzene	0	0	0	5.87×10^{-7}	8.79×10^{-4}	8.80×10^{-4}
nitrobenzene	0	0	0	6.00×10^{-7}	3.79×10^{-4}	3.80×10^{-4}
bix-2-chloro ethyl ether	0	3.99×10^{-6}	0	5.99×10^{-7}	3.51×10^{-4}	3.56×10^{-4}
phenol	0	0	0	5.99×10^{-4}	2.55×10^{-5}	6.25×10^{-4}
di-n-butyl phthalate	0	4.4×10^{-6}	0	3.3×10^{-7}	6.77×10^{-6}	1.15×10^{-5}
			medium period			
chlorobenzene	0	0	0	5.46×10^{-6}	7.34×10^{-4}	7.40×10^{-4}
nitrobenzene	0	0	0	5.94×10^{-6}	3.54×10^{-4}	3.60×10^{-4}
bix-2-chloro ethyl ether	0	3.98×10^{-6}	0	5.98×10^{-6}	3.29×10^{-4}	3.39×10^{-4}
phenol	0	0	0	5.97×10^{-3}	2.50×10^{-5}	6.00×10^{-3}
di-n-butyl phthalate	0	1.52×10^{-6}	0	1.15×10^{-6}	2.25×10^{-6}	4.93×10^{-6}
			raining season			
chlorobenzene	0	0	0	1.00×10^{-5}	5.99×10^{-4}	6.09×10^{-4}
nitrobenzene	0	0	0	1.17×10^{-5}	3.28×10^{-4}	3.40×10^{-4}
bix-2-chloro ethyl ether	0	3.97×10^{-6}	0	1.19×10^{-5}	3.07×10^{-4}	3.23×10^{-4}
phenol	0	0	0	1.19×10^{-2}	2.44×10^{-5}	1.19×10^{-2}
di-n-butyl phthalate	0	7.69×10^{-7}	0	1.17×10^{-6}	1.09×10^{-6}	3.03×10^{-6}

Table 4. Modified microbial degradation rate constants and first-order attenuation coefficients of five organic toxins.

Rate Constants	K_s			K		
	dry season	intermittent season	rainy season	dry season	intermittent season	rainy season
chlorobenzene	1.17×10^{-5}	1.09×10^{-4}	2.00×10^{-4}	8.91×10^{-4}	8.43×10^{-4}	7.99×10^{-4}
nitrobenzene	1.20×10^{-5}	1.19×10^{-4}	2.34×10^{-4}	3.91×10^{-4}	4.73×10^{-4}	5.62×10^{-4}
bix-2-chloro-ethyl ether	1.20×10^{-5}	1.20×10^{-4}	2.38×10^{-4}	3.67×10^{-4}	4.53×10^{-4}	5.49×10^{-4}
phenol	1.20×10^{-2}	0.119	0.238	1.20×10^{-2}	0.119	0.238
di-n-butyl phthalate	6.66×10^{-6}	2.30×10^{-5}	2.34×10^{-5}	1.78×10^{-5}	2.68×10^{-5}	2.53×10^{-5}

Table 5. Maximum permissive concentrations of selected compounds in source water (ppb).

compounds		chloro-benzene	nitro-benzene	bis-2-chloro ethyl ether	phenol	di-n-butyl phthalate
max. permissive dissolved conc.		20	20	0.3	1.0	200
total max. permissive conc.	dry season	20.007	30.001	0.300	1.000	234
	intermittent season	20.020	30.003	0.300	1.000	302
	rainy season	20.040	30.006	0.300	1.000	404

RESULTS AND DISCUSSION

Before the permissive loads of the organic toxins selected can be calculated, several conditions need to be defined.

(1) Flow rates of 8000, 50000, and 30000 m3/sec were used to represent river flow during the dry, rainy, and intermediate seasons, respectively. These flow rates were obtained from data from the Institute of Environmental Conservancy, He Hai University.

(2) The submerged discharge pipe (200 m in length) at the mouth of the Zaojiang stream is to be installed at a depth of 26 m below the surface of the river.

(3) The organic compounds studied are assumed to remain bound to suspended solids and not to redissolve, and therefore will be removed in the water treatment processes at the Ligang Water

Works pump station. Therefore, the maximum permissive concentrations of chemicals in drinking water (Sitting, 1985; Verschueren, 1983) may be treated as the maximum permissive dissolved concentrations in source water at the intake of the pump station (as shown in Table 5). Based on estimates of the seasonal concentrations of suspended solids, the formula for the derivation of the total maximum permissive concentration of an organic compound at the Ligang station is as follows;

$$Cd = \frac{1}{1 + Kp[p]} C$$

where

Cd = the maximum permissive dissolved concentration (ppb)
C = the total maximum permissive concentration (ppb)
[p] = the concentration of suspended solids (ppm)
Kp = the sediment/water partition coefficient of an organic compound.

(4) Only the organic toxicants released from the mouth of the Zaojiang stream into the Yangtze river are considered in this study, and other sources of pollutants further upstream are ignored.

As previously indicated, the change in the direction of the flow of the Yangtze River from tidal effects results in an accumulation of pollutants and greatly complicates the concentration cline of the pollutants. At the end of ebb tide, the river flow velocity approaches a minimum and a cloud of highly concentrated sewage forms about the discharge site. During flood tide, the cloud of concentrated sewage along with sewage from further downstream is carried upstream above the site of discharge. At the cessation of flood tide, the river flow velocity again approaches a minimum and a cloud of concentrated sewage reforms. When ebb tide begins again, sewage being discharged combines with sewage being carried back downstream to form yet another type of concentration pattern. Tidal changes cause a dynamic pollutant concentration distribution that results in variability of the pollutant concentration at the pump station in Ligang. In order to protect the quality of drinking water the maximum concentration of a given organic pollutant at Ligang should not exceed the maximum permissive concentration calculated for that pollutant.

The maximum permissive pollutant concentrations were calculated for the three different river flow rates corresponding to the three different seasons identified. The maximum permissive concentrations were also calculated for the years 2000, 2010, and 2020 during which the sewage discharge volumes are projected to be 320, 500, and 700 thousand tons per day. The results indicate that the maximum permissive loads are lowest during the dry season due to the minimal flow at this time. Therefore, during this season the discharge of organic toxins into the river must be closely monitored.

In order to compare the relative importance of transportation and degradation of organic compounds, the maximum permissive loads of "conservative" organic toxins can be calculated if it is assumed that the first-order attenuation coefficient of each organic compound is equal to zero. Comparison of the ratios of permissive loads of "degrading" organic compounds to "conservative" compounds indicates that only the ratios for phenol are quite high (1.26, 2.52, and 3.04) and hence, only for phenol is the degradation process significant compared to that of transport. Low ratios for the other four compounds suggest that the degradation process is of little importance and can be ignored. Therefore, these compounds should be treated as "conservative" ones.

As previously discussed , tidal effects can cause the concentrations of organic compounds at the pump station in Ligang to vary considerably. The ratios of minimum to maximum concentrations

for each of the organic pollutants examined is given in Table 6. Further calculations indicate that during the dry season (8000 m3/sec flow rate) the maximum concentration of organic pollutants at the Ligang pump station occurs 1.5 hr after the beginning of the highest water velocity. During the intermediate season (flow rate of 30000 m3/sec), the highest pollution concentration at the Ligang pump station occurs 1 hr before the beginning of the highest water velocity, while during the rainy season (50000 m3/sec flow rate) the highest concentration is reached 2.5 hr before high water velocity. In the event that the concentration of organic pollutants at these times exceeds the maximum permissive concentration, the pumping station should be stopped for 1-2 hr to avoid contamination of the water supply.

When the first-order attenuation coefficient of an organic pollutant becomes large enough, the attenuation ratio (the ratio of concentration of a degrading compound to a conservative compound) becomes larger than unity. A sample set of attenuation ratios derived with a first-order attenuation coefficient (K) of 0.1 hr-1 is given in Table 7 for different flow rates and contaminant loads. The results indicate that attenuation ratios are related to hydraulic factors and are independent of the organic pollutant load. At a given flow rate and pollutant load, the attenuation ratio and pollutant concentration at the pump station varies with the first-order attenuation coefficient. This is illustrated in Fig. 1 using a flow rate of 8000 m3/sec and a pollutant load of 200 g/sec. In this study, when the first order attenuation coefficient, K, exceeds 0.01, the attenuation process becomes significant compared with the transport process. When K is smaller than 0.01, the attenuation process can be ignored and the pollutant can be treated as a conservative compound. The first-order attenuation coefficient is a critical value and should be used to decide if attenuation is important in the computation of models for river studies.

Table 6. Variations in the concentration of organic toxicants in the river water at the Ligang Water Works pumping station.

Compound	Flow (m3/sec)	Ratio of minimum to maximum concentration
phenol		0.631
chlorobenzene		0.637
nitrobenzene	8000	0.637
bis-2-chloroethyl ether		0.637
di-n-tutyl phthalate		0.637
phenol		0.379
chlorobenzene		0.508
nitrobenzene	30000	0.508
bis-2-chloroethyl ether		0.508
di-n-tutyl phthalate		0.508
phenol		0.302
chlorobenzene		0.409
nitrobenzene	50000	0.409
bis-2-chloroethyl ether		0.409
di-n-tutyl phthalate		0.409

Table 7. Relationship between attenuation ratio, flow, and load.

Flow (m3/sec)	Load (g/sec)	Concentration of organic compound	Concentration of conservative compound	Attenuation ratio
8000	5.0	0.171	0.772	4.51
	80.0	2.743	12.348	4.50
30000	5.0	0.146	0.302	2.22
	80.0	2.179	4.830	2.22
50000	5.0	0.108	0.178	1.65
	80.0	1.720	2.855	1.66

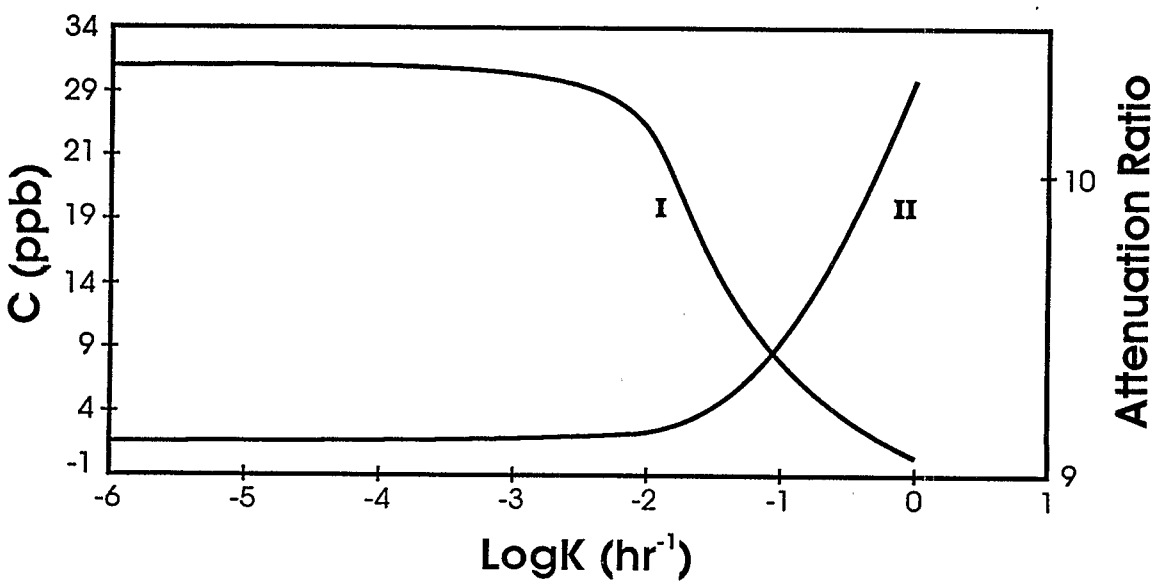

Figure 1. The concentration (I) of organic toxicant and attenuation ratio (II) vs attenuation coefficient K.

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A PLAN OF REGIONAL WATER POLLUTION CONTROL IN RURAL AREAS OF CHINA

Yongchun Zhang[1]

ABSTRACT

A plan for controlling regional water pollution caused by town and village industries (TVI's) in rural areas of China where stream networks intersect, was developed using the county of Wuxi as an example. A number of techniques and methods were used in the formation of the pollution control plan, including analysis of stream network structure, examination of stream flow and relative hydraulics, analysis of the relationship between waste discharge and water quality, and field and laboratory experiments for the derivation of appropriate model parameters. This led to formation of a mathematical model for assessing stream network water quality and utilization of the model to calculate the total permissible levels of TVI waste discharge in the region. The maximum allowable amount of pollutant discharge from each town or plant was determined using an optimized systematic analysis method based on a stratified structure.

INTRODUCTION

In the past decade, the development of town and village industries (TVI's) in China has increased dramatically, especially in coastal regions and the downstream regions of large rivers such as the Yangtze, Yellow, and Pearl rivers. The expansion of TVI's in some areas has been so rapid that industrial output has increased 88% per year. Outside of the more developed centers, TVI's tend to be scattered and small in scale, with limited funding, few trained technicians, and only crude, inefficient equipment. The limited supply of electric power is scarcely enough for the TVI's, which means that little, if any, power is diverted to waste treatment. Despite the low concentration of TVI's in rural areas, the relative output of pollutants is high. The environmental monitoring capacity in rural areas is very limited, and as a result, the discharge of untreated waste water into the water systems surrounding rural TVI's has led to serious water pollution problems.

Located south of the Yangtze river at the Taihu catchment beside Taihu Lake, Wuxi county is a typical district marked by a network of intersecting streams. Within the county's 1235 km^2 area there are more than 3000 streams of varying size and approximately 1 million people. Increasing industrial output within Wuxi county has been an governmental priority that has resulted in the establishment of 6000 TVI's within the county. Correspondingly, water pollution has increased to the point where the waste discharge has reached 15 million tons annually. This has led to serious problems that include a shortage of drinking water, the destruction of fish breeding habitat, a shortage of clean water for agriculture (and vegetable irrigation in particular), an increase in the number of water pollution-linked accidents, and a lowering of the water table. Wuxi county was therefore selected as an excellent case study site.

The aims of this study were to provide a water pollution control plan for the Wuxi county government, and to develop a systematic approach that could be used to form regional water pollution controls in other areas of rural China. The focus of the plan was to provide feasible and realistic

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guidelines to limit waste discharge. By scientifically determining the maximum allowable levels of waste discharge that still protected water quality, environmental standards could be met, TVI's could operate and discharge pollutants at this maximum level, and financial investment in waste treatment would be minimized.

METHODS

The pollution load of any given water body is the result of discharge from sources directly along that water body, both within the county and further upstream, and also, due to the high level of stream integration, from neighbouring water bodies. As a result, all pollution sources communicating with the water body, even at a distance, must be controlled.

The following factors were considered while producing this water pollution control plan:

(a) User demands
- geographical distribution of users
- water quality desired by users
- water quantity desired by users
- future users and their demands regarding water quality and quantity
- variation of future water demands (quality and quantity)
- basic and maximum demand levels (quality and quantity) at different periods of time

(b) Pollution sources
- geographical distribution of main sources of pollution
- main categories of pollution
- amounts of pollution from various sources
- capacity of pollution control and waste water treatment
- probable future status of pollution sources
- probable amount and categories of pollution in the future
- probable control levels and treatment capacity

(c) Water resources
- structure of the channels in a stream network
- hydrological and aquatic conditions in streams
- present water quality
- probable unfavourable hydrological conditions in the future
- probable water quality under the most unfavourable hydrological and aquatic conditions
- required treatment of waste influxes to meet the desires of the users under different hydrological and aquatic conditions
- sewage treatment needed to meet the basic requirements of users under the most unfavourable hydrological and aquatic conditions

Ideally, the appropriate mathematical approach to evaluate the stream network pollution load would be to take every stream within the network and every TVI pollution source, and treat it as one big system using the method of systematic optimum planning. However, the complexity of the necessary calculations and the power of the computer that would be needed precludes use of such an approach in a rural situation. The method used, therefore, was a simplification of the above approach and involved the following changes:

(a) The complicated stream network was simplified by considering only the principle streams within it. While the smaller streams were not considered directly, their role in pollutant transport was taken into account.

(b) Each town with a relatively high concentration of TVI's was considered to represent the principle pollution source for the immediate surrounding area. It was assumed that waste water from different industries in the district was concentrated in the town.

(c) Since 90% of the total pollutant load could be attributed to 30% of all the TVI's, and since 36% of these TVIs discharged the most pollutants, only those industries were considered. In Wuxi county, and within the area of the Taihu catchment in general, the main pollution discharging industries are textile, chemical, construction materials, pulp and paper, beverage, metal processing, non-metal processing, food processing, and machinery industries. Therefore, only these plants were selected for pollution control, and so the complicated system was generalized into a relatively simple one.

(d) Streams were classified into stratification categories according to their relative importance, position, and function within the stream network.

(e) Calculation of the allowable TVI waste discharge amount into each stream depended upon the order of stream stratification. The towns and associated nodes where streams intersected were considered as pollution control sections. In order to optimally assess the allowable waste discharge from each pollution source so that the permissible water pollution load was maximized, a mathematical model of water quality and systematic methods were used.

The objective function (Z) of the systematic analysis was expressed as:

$$Z = \sum_{i=1}^{n} Lei * Qei \rightarrow Max$$

where:

Lei = the concentration of a pollutant type discharged at different pollution sources (mg/l)
Qei = the flow of waste water discharged from different pollution sources (l/min).

The limiting condition which ensured the water quality of each segment of the stream met the goal of water resource protection was given by:

$$A_1^{-1} * B_1 * Lei + A_1^{-1} * g \leftarrow Le_2$$

where:

Le_2 = the vector of water quality standards of each stream segment (mg/l)
A_1, B_1 = the coefficient matrix related to the waste water discharge and the discharge of water flow in streams.

$$
\begin{pmatrix}
1 & 0 & \cdots & 0 & 0 \\
a1 & 1 & \cdots & 0 & 0 \\
 & & \ddots & & \\
 & & & \ddots & \\
 & & & & \ddots \\
0 & 0 & \cdots & an & 1
\end{pmatrix} = A1
$$

$$
\begin{pmatrix}
b1 & 0 & \cdots & 0 & 0 \\
0 & b2 & \cdots & 0 & 0 \\
 & & \ddots & & \\
 & & & \ddots & \\
 & & & & \ddots \\
0 & 0 & \cdots & 0 & bn
\end{pmatrix} = B1
$$

$$g = (g1, 0 \ldots\ldots 0)T$$

$$g1 = a_1 * L2$$

$$a_1 = \frac{\alpha i(a1i - Q3i)}{Q1i - Q3i + Q2i}$$

$$bi = \frac{Qei}{Q1i - Q3i + Q2i}$$

$$\alpha i = e^{\beta x}$$

$$\beta = \frac{U}{2Ex}(1 - \sqrt{1 + 4Ex(K1 + K3)/U2})$$

After running the systematic analysis model, the optimal waste discharge amounts for each pollution control spot was calculated. For streams on the lower level of stratification, these calculated discharge amounts for the corresponding nodes were treated as the limiting factors. By repeating the above calculations, the total limiting amount of waste discharge for the given area, and the maximum allowable pollutant discharge amount for each pollution source was calculated by systematic optimization.

DISCUSSION OF TECHNIQUES

In order to conduct the optimal systematic analysis of water pollution control within a stream network, some preliminary calculations must be made.

WATER QUALITY MODEL

When modelling stream networks, the assessment of water quality includes models that consider hydraulics, water quality, and conditions linking the nodes of a stream network. The hydraulics model is composed of the so-called Saint-Venant equations, while the water quality model used the convection-diffusion equation. The model form was altered for different pollution and water current conditions. Using the biochemical oxygen demand (BOD) and dissolved oxygen (DO) models, the equations are as follows:

$$\frac{\alpha H}{\alpha t} + \frac{1}{B}\frac{\alpha Q}{\alpha x} = 0i$$

$$\frac{\alpha U}{\alpha t} + U\frac{\alpha U}{\alpha x} + g\frac{\alpha H}{\alpha x} + g\frac{U:U:}{c^2 d} = 0$$

$$\frac{\alpha(A.L)}{\alpha t} + \frac{\alpha(AUL)}{\alpha x} = \frac{\alpha}{\alpha x}\left(A.Ex.\frac{\alpha L)}{\alpha x}\right. - \sum_{io=1}^{ro} Kio.A.L. + s \qquad i = i1$$

$$\frac{\alpha(A.D)}{\alpha t} + \frac{\alpha(AUD)}{\alpha x} = \frac{\alpha}{\alpha x}\left(A.Ex.\frac{\alpha D)}{\alpha x}\right. + K_2.A.(Ds-D) - \sum_{io=1}^{Po} Kio\ A.L. + S1$$

$$\sum_{i1=1}^{P1} Qj1,il = 0$$

$$Hj1,1 = Hj1,2 = \ldots = Hj1,il = \ldots = Hj1,p1$$

$$\frac{\alpha L}{\alpha t} . Vj1 = \Delta Fj1 \qquad i = i1$$

$$\frac{\alpha D}{\alpha t} \cdot vj1 = \Delta Dj1 \qquad /$$

Marginal condition, i = f
H (i,j) = h1 (f,j)
L (i,j) = l1 (f,j)
D (i,j) = d1 (f,j)

Initial condition, j = 0
H (i,0) = h (i)
U (i,0) = u (i)
L (i,0) = l (i)
D (i,0) = do (i)

Where,
Eq. ( ) = so-called continuity equation
Eq. ( ) = momentum equation
Eq. ( ) = equation of pollution conservation
Eq. ( ) = equation of DO conservation
B = average width of cross sections, for shallow and wide channels. It approximately equals the width of the water surface (m).
A = area of the cross sectional water flow (m^2)
Q = water discharge (m^3/s)
c = coefficient expressing the roughness of the stream bed (Chezy coefficient)
d = hydraulic radius, for wide and shallow rivers, it usually approximates the average depth of the water in the channel
Kio = coefficients defining the decay rate of pollutants; for organic pollutants, these coefficients define the oxidation rate, (K1), settling/resuspension rate, (K3), etc.
s = other sources or sinks, including branch influxes, etc.
Pp = amount of coefficients
io = ordinal number
i1 = ordinal number of cross sections located in the areas of stream network nodes
j1 = ordinal numbers of the nodes of a stream network
Vj1 = water volume at node j1 of the stream network
ΔFj1 = net flux of pollutants at node j1
h1(f,j) = water level at marginal sections
l1(f,j) = concentration of pollutants in marginal sections
h(i) = original water level of a section
u(i) = original flow velocity in a section
l(i) = original concentration of pollutants in a section
f = ordinal number of marginal sections
i = ordinal number of segments of the stream network
j = ordinal number of time steps
m = total number of time steps
n = total number of segments of the stream network
d1(f,j) = concentration of dissolved oxygen in marginal sections
do(i) = original concentration of DO of a section

Dji = net flux of dissolved oxygen at node j1
s1 = other sources or sinks of dissolved oxygen
K2 = coefficient defining the rate of re-aeration of water in the stream network

When the marginal and original conditions are known, the model equations can be solved using the implicit difference method.

PARAMETER ESTIMATION

In order to be able to mathematically assess water quality variation in a stream network, the model parameters needed to be estimated from data collected in the field. The parameters which typically need to be estimated in a water quality model the longitudinal dispersion coefficients, the coefficient of river bed roughness, the pollution degradation rate, the net rate of air/water oxygen movement, and the rate of pollutant settling/resuspension.

(a) Dispersion Coefficient

Several representative stream segments of the stream network were selected for a dye tracing experiment. Dye was mixed homogenously in the river at one site, and then cross-sectional samples were taken at two or three downstream sites. The longitudinal coefficient was estimated with the following formula:

$$Ex = \frac{U}{2} * \frac{\sigma t_2 - \sigma t_1}{t1 - t2}$$

where;

σt_1, σt_2 are the standard deviation of the dye concentration distribution over time
t_1, t_2 represent the time of the dye cloud passing through the sampling sites

(b) Degradation Rate

Water samples were collected from different segments of the stream network. Water samples were maintained in the laboratory at a steady temperature and the BOD was analyzed daily for a 20 day period. The degradation rate of organic pollutants was estimated using the minimum square regression method:

$$Y = a_3 f_1 + b_3 f_2$$

$$fl = 1 - e^{-k't}$$

$$f_2 = t * e^{-k't}$$

$$a_3 = \frac{\Sigma f_2^2 \Sigma f_1 y - \Sigma f_1 f_2 \Sigma f_2 y}{\Sigma f_1^2 \Sigma f_2^2 - (\Sigma f_1 f_2)^2}$$

$$b_3 = \frac{\Sigma f_2 Y \Sigma f_1^2 - \Sigma f_1 f_2 \Sigma f_1 Y}{\Sigma f_1^2 \Sigma f_2^2 - (\Sigma f_1 f_2)^2}$$

(c) Multiple Parameters Estimation

Using a systematic analysis method, several parameters could be estimated simultaneously. The procedures was as follows:

Water quality testing was performed at both up and downstream locations of different stream segments within the network. Optionally, several groups of parameters could be taken from the respective valid range. The mathematical model was applied to each group of parameters separately, and following comparison of the mathematically derived results with the field observations, the data set that showed the largest deviation was discarded. A new group of parameters was formed and the process repeated until finally a group of the most ideal parameters was estimated.

The following model equations were used to estimate multiple parameters simultaneously. The objective funciton provides minimum deviation and is given by:

$Z - :: Lc - Lm ::^2 \rightarrow \min$

where: Lc, Lm are the calculated and monitored results, respectively.

The following limiting conditions were used to ensure the parameters and the calcualted results were within the valid range.

$ai \leftarrow Ki \leftarrow bi$

$Ai \leftarrow Li \leftarrow Bi$

where: ai, bi are the valid parameters ranges, and Ai, Bi represent the valid range of pollutant concentration.

The parameters of the mathematical model of water quality estimation based upon the information collected from the stream network in Wuxi county are given in Table 1.

Table 1. Model parameters estimated from field data.

Parameter	Unit	Symbol	Value
1. dispersion	m/s	Ex	33.35
2. degradation	l/d	K1	0.26
3. settlement	1/d	K3	0.51
4. re-aeration	1/d	K2	0.75

SUMMARY

A regional water pollution control plan for Wuxi county was derived for the town and village industries and the associated rural stream network. The pollutant limits for 36 towns within the county were assessed. Economically and technically, the plan is usable. By using the mathematical model of water quality, it is possible to protect water quality. If th eplan is followed in Wuxi county, the water quality should recover to a level suitable for fisheries and agriculture, and some streams should be suitable as a source of drinking water.

(a) A water pollution control plan is important to initiate in areas of stream networks and wehre TVI's are densely distributed. The crux of regional pollution control is to make clear the relationship between the pollutants discharged in different areas, by different mechanisms and in varying amounts, with the changes in water quality at different locations. The mathematical model of water quality and the systematic analysis method are powerful tools in achieving an udnerstanding of this relationship.

(b) The relatively complex association of parameters within the model can be simplified for practical use. The systematic optimum plan in the stratified structure is one such mehtod of simplification.

(c) Data collected in the field is important to maintain th eaccuracy of the model. Reliable and consistent field experimentation and sampling is therefore an important component of the overall control plan.

(d) The water current proved to be the determining factor in assessing the concentration and distribution of pollutants within the stream network. Changing flow characteristics, therefore, are a potentially important mechanism towards improving water quality. Further research in this area is needed.

ADSORPTION OF NEUTRAL AND IONIC ORGANIC POLLUTANTS ON SOILS AND SEDIMENTS

John C. Westall[1]

INTRODUCTION

Sorption is one of the most fundamental processes in controlling the transport, fate, and environmental effect of organic pollutants in aquatic environments. Sorption processes directly control the distribution of these compounds, and thereby indirectly affect transport, bioavailability, and degradability. Whereas the sorption of neutral organic compounds has been studied extensively and is understood comparatively well for an environmental process, the sorption of ionic organic compounds has not been researched nearly as thoroughly. In this paper we review the data and models for sorption of neutral compounds and summarize some of our recent work with ionic and ionizable compounds. The literature cited simply provides a key to more detailed descriptions of the data presented; no attempt has been made to provide a comprehensive review of the literature.

CLASSES OF ORGANIC COMPOUNDS

For this discussion, all organic compounds will be assigned into three classes: neutral, ionogenic, and ionic, as shown in Figure 1. The neutral compounds, as exemplified by the chlorobenzenes and methylbenzenes, generally interact weakly with water and sorb strongly onto environmental organic matter. The predominant driving force for sorption is "the hydrophobic effect", which arises from relatively weak interactions between the solute and the solvent, the solute and the sorbent, and the solvent with itself. The extent of sorption is relatively independent of solution chemistry (e.g., pH and major ion composition). Due to the weak interaction of these compounds with both the solvent and the sorbent, sorption is relatively easy to describe for a wide range of compounds and a wide range of sorbents with relatively simple models.

The ionic surfactants, as exemplified by linear alkylbenzene sulfonate (LAS) and dodecylpyridinium (DP) possess both a hydrophobic group and an ionic group. The hydrophobic group is expected to undergo the same weak, nonspecific interactions as observed for the neutral organic compounds. However, the ionic groups interact much more strongly with both the solvent and the sorbent; the negatively charged LAS is repelled by the predominantly negatively charged environmental surfaces, while the positively charged DP is attracted. The question with respect to the sorption of these compounds is the extent to which both the hydrophobic and the ionic terms contribute to sorption -- does one predominate, or are they roughly equivalent and additive.

The ionogenic compounds, as exemplified by the weak acid chlorophenols, and the weak base methylanilines, exhibit both neutral and ionic forms in solution, depending on the pH. Like the ionic compounds, both hydrophobic and electrostatic interactions are anticipated for the ionic forms, while for the neutral form, the ionic interaction is absent. However, characterization of the sorption of ionogenic compounds as a function of pH is generally difficult; since many environmental surfaces have pH dependent charge, it is difficult to separate the effect of pH on the solute from the effect of pH on the sorbent. In contrast, the charge of the ionic compounds is fixed and not pH dependent.

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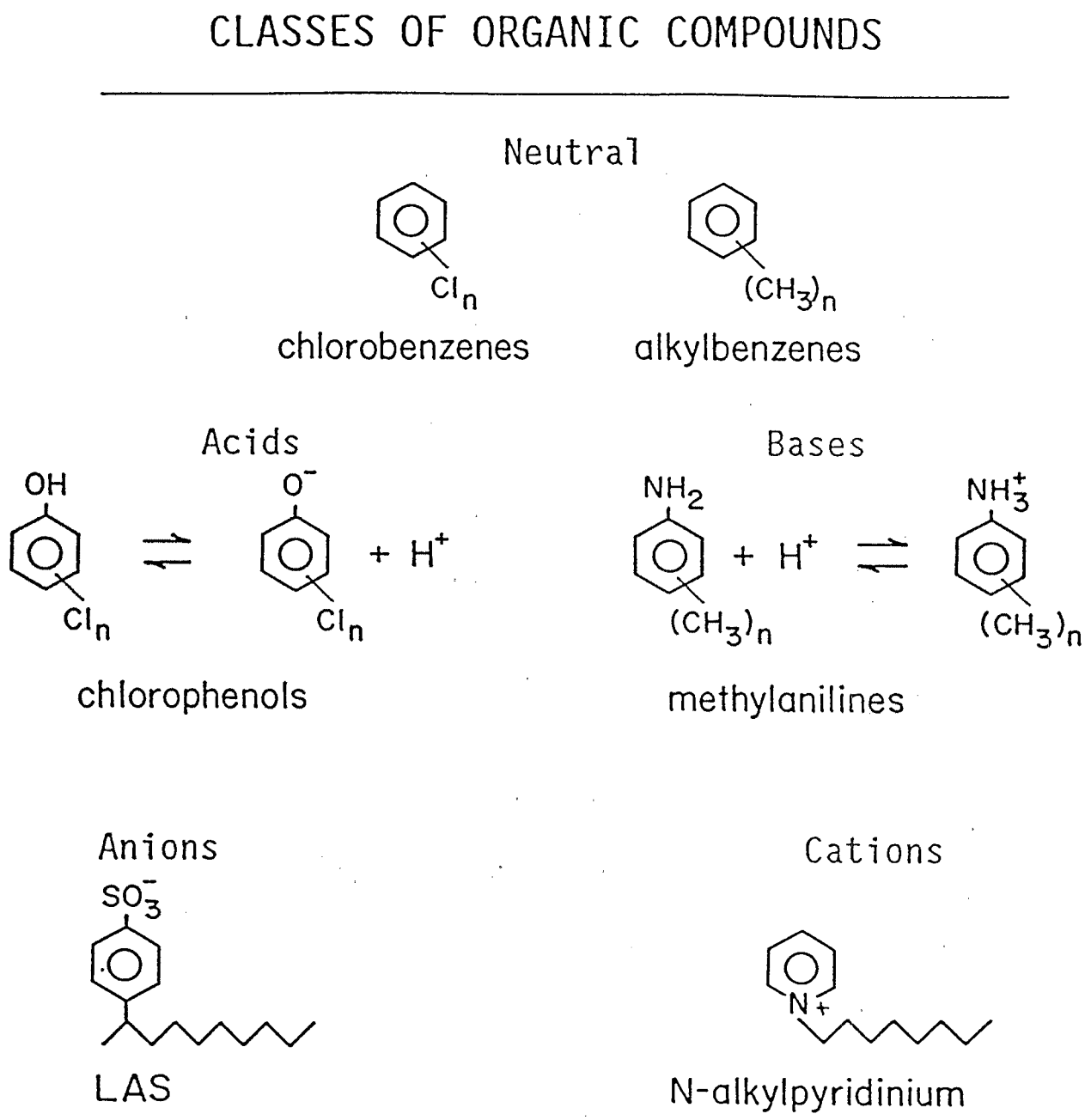


Figure 1. Classes of organic compounds.

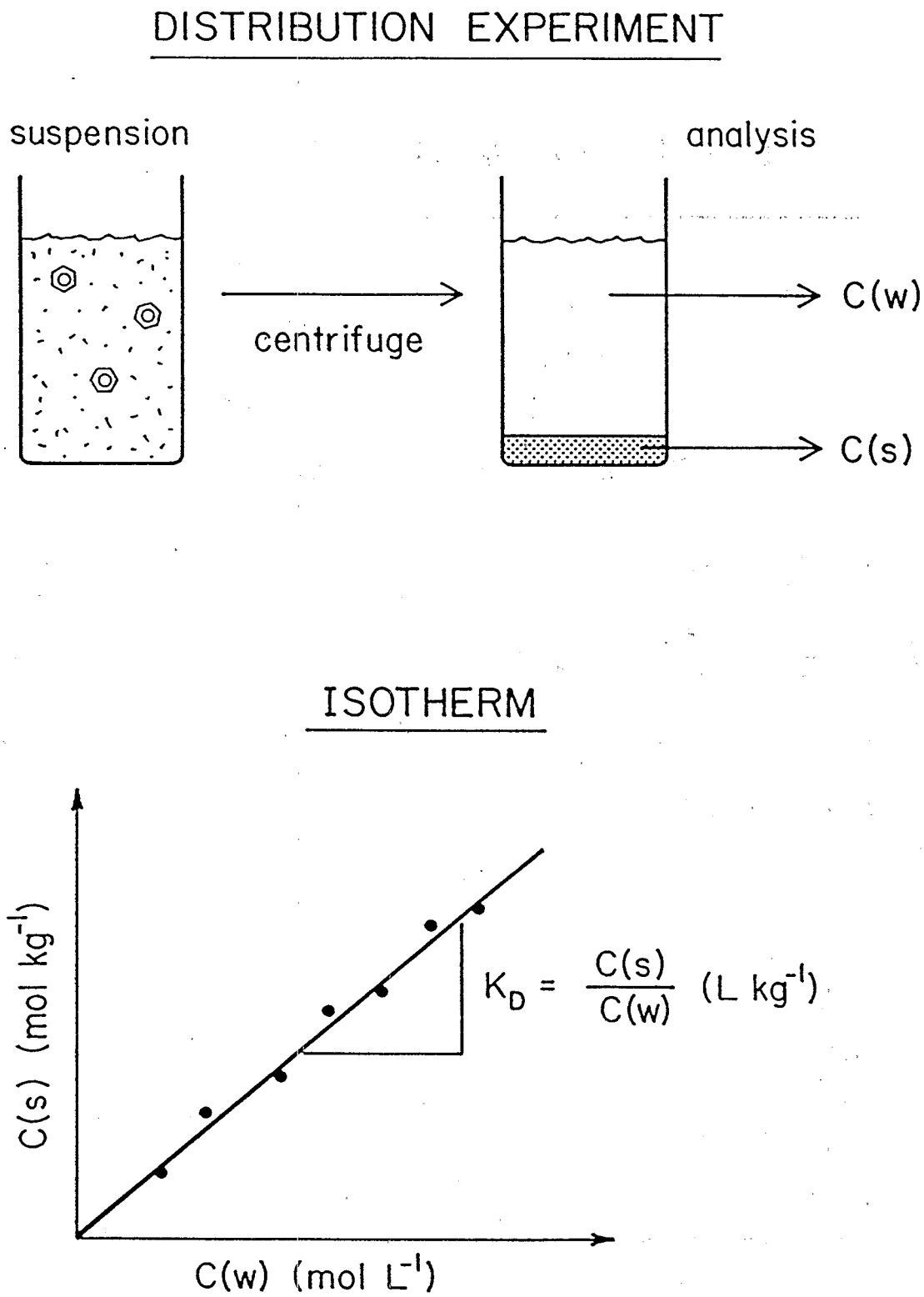


Figure 2. Experimental procedure.

The remainder of this discussion will be focused on the compounds in Figure 1, grouped according to charge: (i) neutral, (ii) anions and anionogenic, (iii) cations and cationogenic.

EXPERIMENTAL METHODS

Essentially all of the data presented in this study were obtained in simple batch experiments (1, 2), as shown in Figure 2. A suspension containing the solvent, the sorbent, and the solute were mixed until apparent equilibrium distribution was reached, the phases were separated by centrifugation, and the concentration of the target organic compound was determined both in the solution and on the sorbent. The data are generally plotted as isotherms: C(s), the amount on the sorbent per mass of sorbent (mol kg^{-1}) vs C(w), the equilibrium concentration in solution (mol L^{-1}). For linear isotherms, the slope is the distribution constant, K_d (L kg^{-1}):

$$K_d = \frac{C(s)}{C(w)} \tag{1}$$

For the neutral compounds, linear isotherms are the rule, while for ionic compounds, nonlinear isotherms are the rule. In the case of nonlinear sorption, the distribution is represented as the concentration-dependent distribution ratio, D:

$$D = \frac{C(s)}{C(w)} \tag{2}$$

DISTRIBUTION OF NEUTRAL ORGANIC COMPOUNDS (3,4)

It has been shown that the solution-sorbent distribution of the neutral (particularly nonhydrogenbonding) organic compounds can be explained very well based on a property of the compound and a property of the sorbent. The property of the compound is the octanol-water partition constant, and the property of the sorbent is the organic carbon content of the sorbent, f_{oc}, the fraction organic carbon:

$$\log K_d = a \log K_{ow} + \log f_{oc} + b \tag{3}$$

The correlation between log K_d and log f_{oc} is illustrated in Figure 3 for the compound 1,4-dichlorobenzene and a collection of sorbents including lake sediments, river sediments, aquifer materials, and activated sludge. The correlation between log K_d and log K_{ow} is illustrated in Figure 4 for the methyl- and chloro- benzenes for sorption on an aquifer material with 0.25 % organic carbon.

The correlation expressed by Equation 3 is among the most widely used and widely useful in the field of environmental organic chemistry. The reason for the success of this simple correlation is the weak, nonspecific interaction of the neutral organic compounds with the solvent and the sorbent. As the specificity of the interaction of the solute with the solvent and the sorbent increases (e.g., hydrogen bond formation, strong dipole interactions), the success of the simple model decreases.

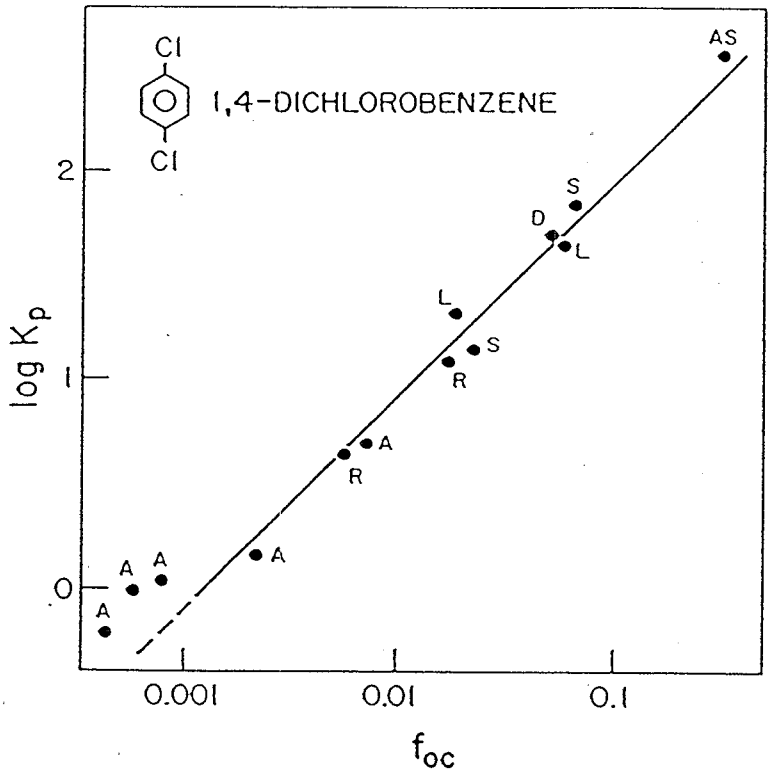


Figure 3. K_d vs property of sorbent, f_{oc}.

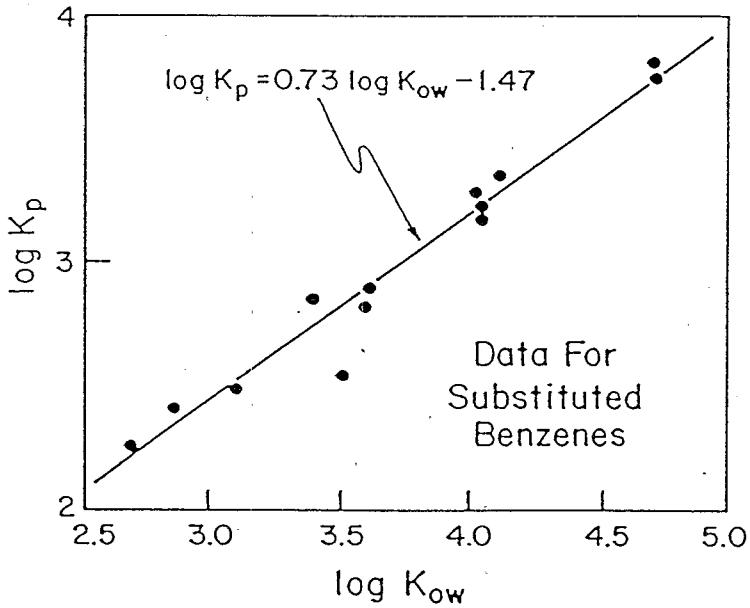


Figure 4. K_d vs property of sorbent, K_{ow}.

ORGANIC ANIONS AND ORGANIC ACIDS

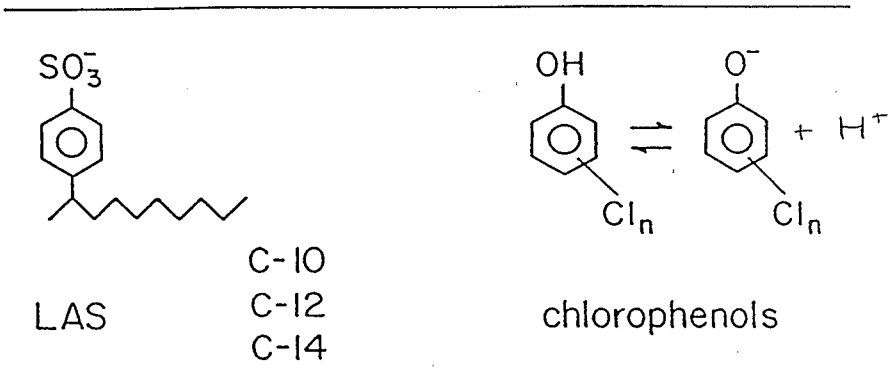
ORGANIC ANIONS (5)

The organic anions that are considered are homologs of 4-alkybenzenesulfonates containing 10, 12, and 14 carbons in the alkyl chain, referred to as C-10, C-12, and C-14 LAS (4-(1-methylnonyl)benzenesulfonate, 4-(1-methylundecyl)benzenesulfonate, and 4-(1-methyltridecyl)benzenesulfonate, Figure 5). The questions posed about the sorption of these compounds are, (i) the linearity of the isotherm, (ii) the effect of pH, and (iii) the effect of the number of $-CH_2-$ groups.

The isotherms for adsorption of LAS on sorbent EPA-12 in 0.01 M monovalent salt solution are shown in Figure 6 on a log-log plot. The isotherms are nearly linear: the slope of log-log plots for all three compounds is approximately 0.9. (The slope would be 1.00 for linear isotherms on a log-log plot.) Furthermore it is seen that the curves for the homologs are displaced along the x-axis approximately 0.4 log units per $-CH_2-$ group, as expected for the hydrophobic effect.

The effect of pH on the adsorption of LAS is shown in Figure 7. The change in distribution ratio with pH was d log Dc/d log $[H^+]$? 0.17. (These data were obtained from the very low concentration range of the isotherm in which the isotherm was effectively linear, yielding a constant value of $K_d = D$.) This effect of pH may be due to (i) the influence of H^+ as a surface potential determining ion or (ii) specific pH-dependent surface-complexation or ligand-exchange reactions (e.g., ligand exchange with -OH of =FeOH or =AlOH groups). Competition of SO_4^{2-} and HPO_4^{2-} with adsorption of LAS has been observed.

The correlation of distribution ratio with sorbent properties is shown in Figure 8. A weak correlation with organic carbon content is seen and attributed to the hydrophobic effect. The apparent correlation with clay content is attributed to the covariability of clay content with organic carbon content for these sorbents.



Linearity of isotherm?

Effect of pH?

Effect of n_{-CH2-}?

Figure 5. Organic anions and acids in this stuoy.

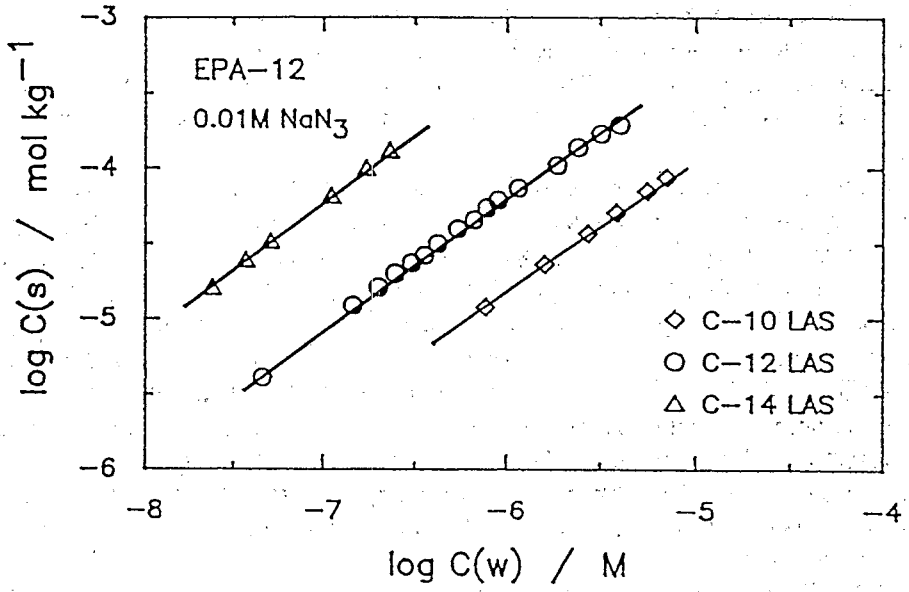


Figure 6. Isotherms of LAS.

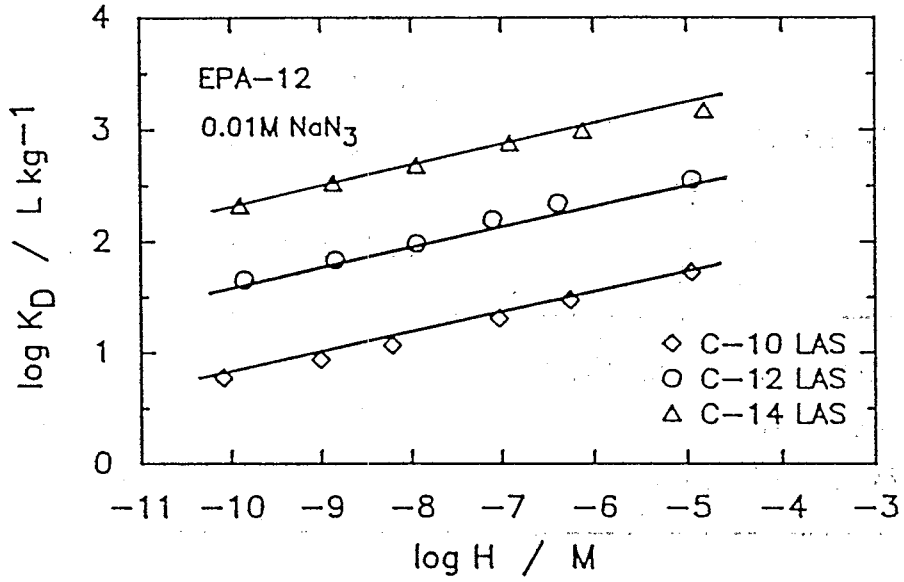


Figure 7. Effect of pH on sorption of LAS.

HYDROPHOBIC IONIZABLE ORGANIC COMPOUNDS (6-9)

Hydrophobic ionizable organic compounds exist in the aqueous phase as a neutral species and as an ionized species. The sorption of the neutral species can be characterized by the same paradigm as that used for neutral organic compounds, while adsorption of the ionic form requires consideration of the charge, as outlined above for sorption of LAS.

The organic acids with pH dependent charge are the chlorophenols, shown in Table I. These weak acids ionize according to the reaction:

$$AH = A^- + H^+ \qquad K_a \tag{3}$$

and the neutral species is distributed between octanol and water according to the reaction

$$AH = \overline{AH} \qquad K_{ow} \tag{4}$$

where the overbar represents a species in the nonaqueous phase. The pH-dependent concentration distribution ratio is formulated as

$$D = \frac{[\overline{A^-}] + [\overline{AH}]}{[A^-] + [AH]} \tag{5}$$

where the total concentration of the acid in both phases is taken into account.

Table I. Properties of chlorophenols.

Compound	log K_a	log K_{ow}
2-chlorophenol	-8.52	2.17
2,4-dichlorophenol	-7.85	2.75
2,4,6-trichlorophenol	-5.99	3.38
pentachlorophenol	-4.74	5.01

Reactions

$$AH = A^- + H^+ \qquad K_a$$

$$AH = \overline{AH} \qquad K_{ow}$$

Distribution ratio

$$D = \frac{[\overline{AH}] + [\overline{A^-}]}{[AH] + [A^-]}$$

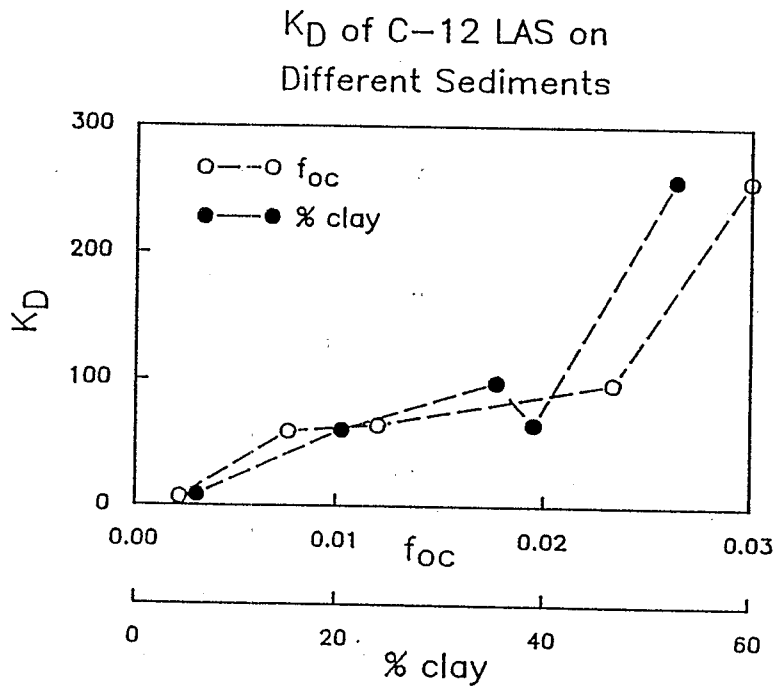


Figure 8. Effect of sorbent properties on sorption of LAS.

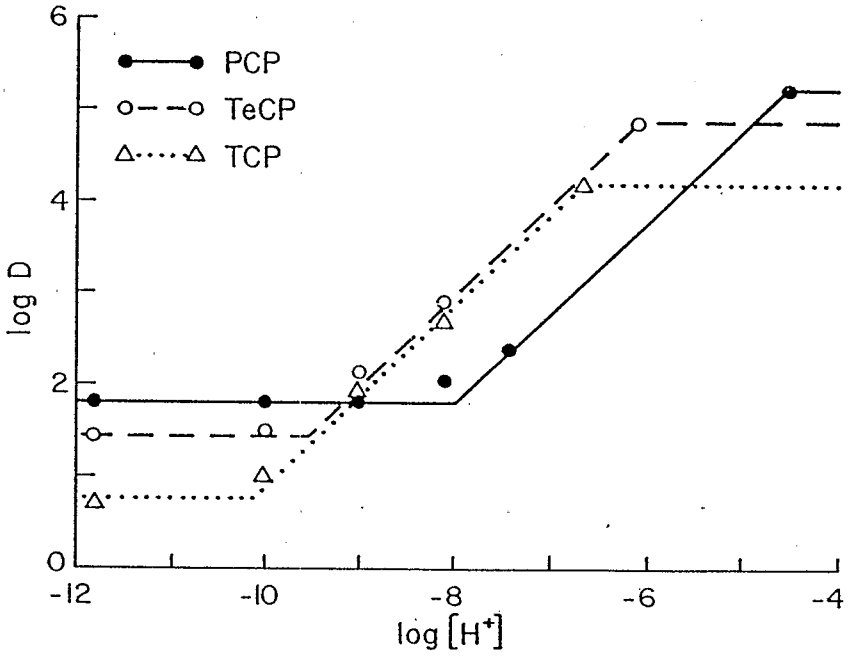


Figure 9. Effect of pH on octanol-water distribution of chlorphenols.

The distribution of the chlorophenols between octanol and water is shown as a function of pH in Figure 9. Three regions are seen in the figure. At high H^+ concentration, the neutral species is dominant in both phases, partitioning is independent of pH and given by Equation 4. At intermediate H^+ concentration, A- is the dominant species in the organic phase and AH is the dominant species in the organic phase; the distribution is pH dependent with a "knee" in the curve at pH = pK_a. At low H^+ concentration, the ionic species is the dominant form in both phases, and partitioning is again pH independent. Often the presence of the anionic species (A-) in the organic phase has been ignored, but as shown in Figure 9, it is significant.

The partitioning of pentachlorophenol with a variety of sorbents reported in the literature (9) is depicted in Figure 10; the pH dependence is similar to that observed in Figure 9 for water-octanol distribution, indicating that octanol is a good "model sorbent" for these compounds. However, note that the ionic species of the compound in both phases must be taken into account, that the relation of K_d to K_{ow} for the ionic species is not the same as it is for the neutral species, and that the K_{ow} and K_d for the ionic species are both dependent on the concentration and nature of salt in solution.

SUMMARY OF ORGANIC ANION ADSORPTION

The sorption isotherms of the organic anions were weakly nonlinear and pH dependent. The dependency of adsorption on hydrophobic chain length was about that expected from hydrophobic theory. The sorption of the neutral species of the organic base is favored over adsorption of the ionic species, as predicted for organic partitioning. Organic cations and organic bases.

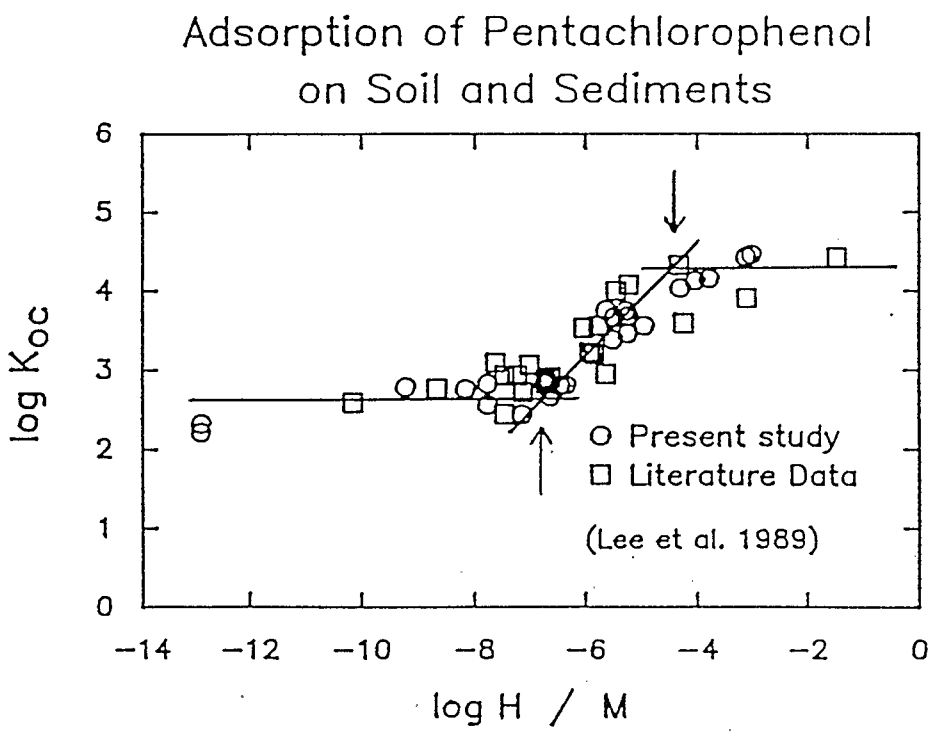


Figure 10. Effect of pH on soil-water and sediment-water distribution of pentachlorophenol (9).

ORGANIC CATIONS (5)

The organic cation used in this study was N-dodecylpyridinium (DP) (Figure 11). Adsorption of organic cations is predicted to be stronger than adsorption of organic anions of comparable hydrophobicity due to the electrostatic attraction of the cations to the negatively charged environmental sorbents.

The isotherms for adsorption of DP on three environmental sorbents and two specimen minerals is shown in Figure 12 on a log-log plot. Highly nonlinear isotherms are observed, with the log-log slope of approximately 0.6 in the lower concentration range. The aqueous concentrations cover seven orders of magnitude in one case. Although these isotherms show an almost step-function appearance when plotted on a linear scale (not shown), it is clear that the isotherm is continuous if a wide range of concentrations is considered.

The effect of pH is shown in Figure 13 and the isotherms obtained in different salt concentrations are shown in Figure 14. Adsorption is shown to be virtually independent of pH but relatively dependent on salt concentration. The independence of pH and dependence on salt concentration is consistent with a classical ion-exchange mechanism. Two features of the isotherms at different salt concentrations are noted: at low concentrations, the isotherms are shifted along the x-axis by about 0.8 log units; at higher concentrations, at about the exchange capacity of the sorbent, the isotherms cross over, with DP adsorption becoming favored at high salt concentrations. This behaviour is consistent with the reactions:

$$\overline{NaX} + R^+ = \overline{RX} + Na^+ \qquad (6)$$

and

$$\overline{Y} + R^+ + Cl^- = \overline{YRCl} \qquad (7)$$

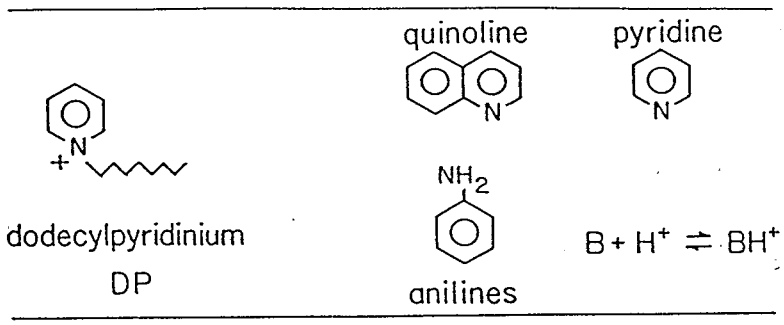
where:

$\overline{NaX}$ is the exchanger in the Na+ form, R+ is the DP cation, and

$\overline{Y}$ is a site for "hydrophobic" adsorption;

one might expect that Y is related to natural organic matter or even to $\overline{RX}$ groups.

ORGANIC CATIONS



Linearity of isotherm?

Effect of pH?

Effect of salt?

Figure 11. Organic cations used in this study.

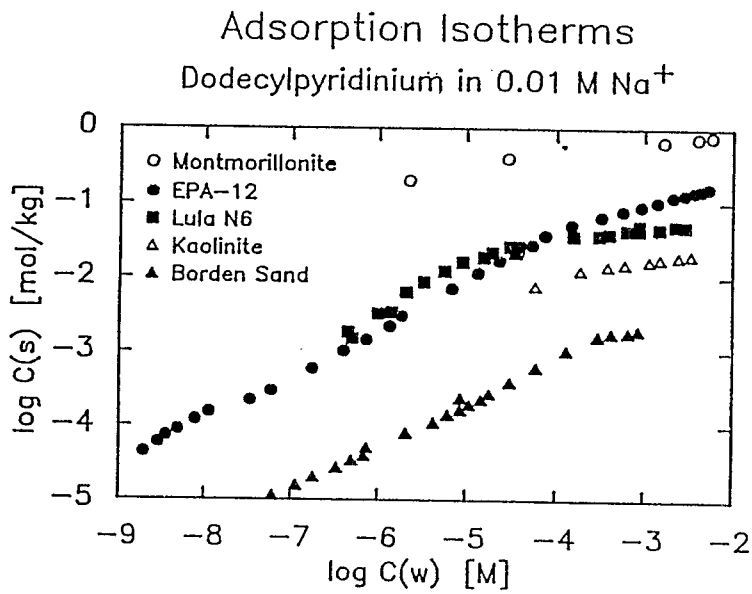


Figure 12. Isotherms of DP on different sorbents.

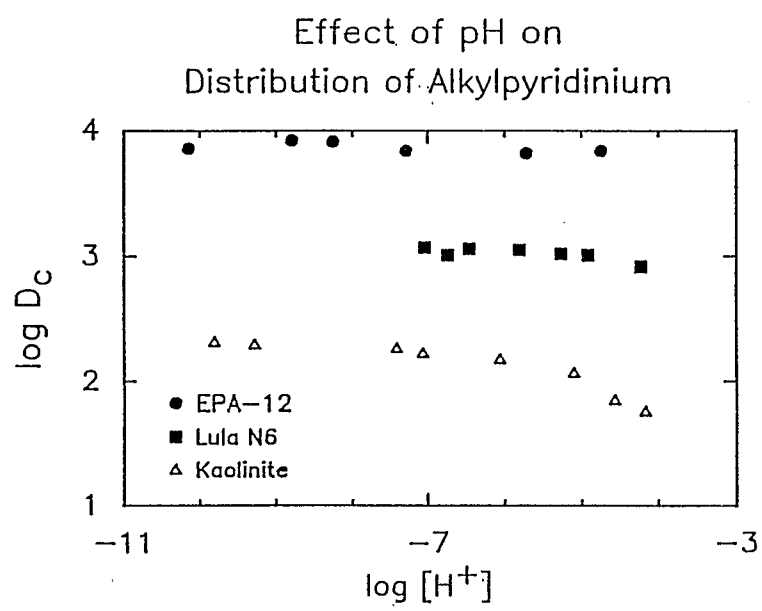


Figure 13. Effect of pH on distribution of DP.

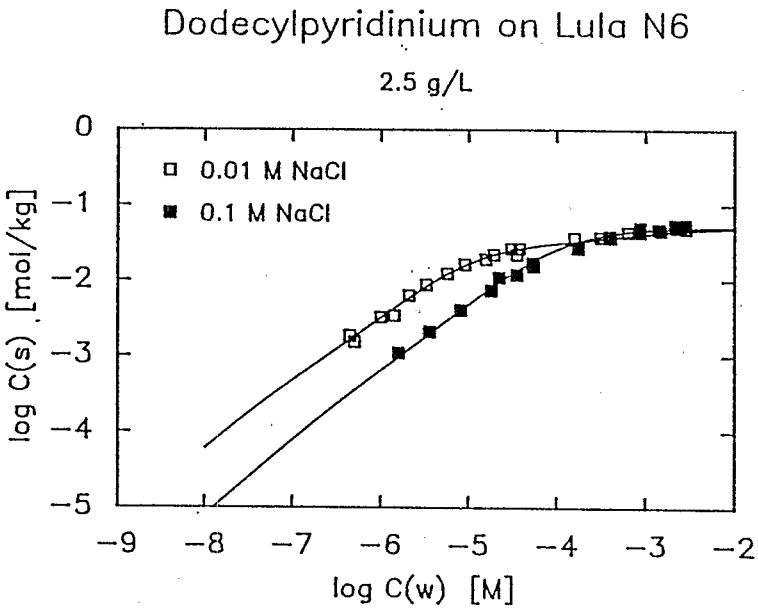


Figure 14. Effect of [NaCl] on adsorption isotherms of DP on aquifer material.

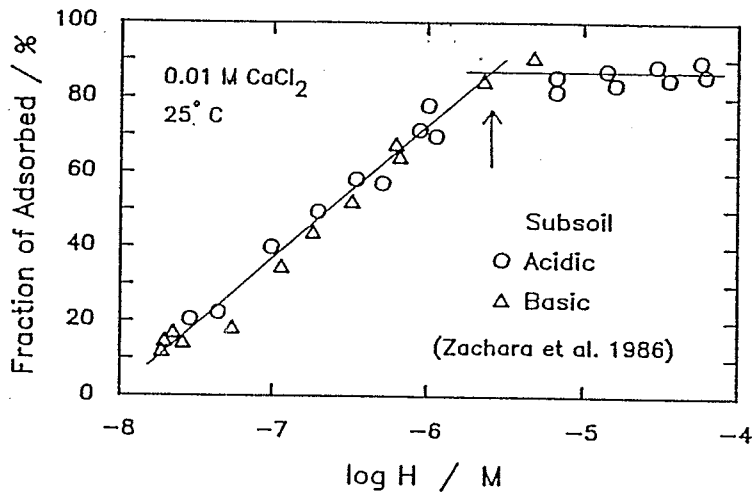


Figure 15. Effect of pH on sorption of the organic base, quinoline (10).

ORGANIC BASES (2,10)

The adsorption of the organic base quinoline on two subsoils as a function of pH (10) is depicted in Figure 15. A pH dependency similar to that seen in Figure 10 (pentachlorphenol) is seen, except that in this case, it is the ionic species that is bound more strongly than the neutral species. (Note also that Figure 15 is on a linear-log scale, and thus not quantitatively comparable to Figure 10.)

SUMMARY FOR ORGANIC CATION SORPTION

The isotherms of the organic cations are highly nonlinear, but not just step functions, although they may appear to be so if plotted on a linear scale. The adsorption of the cation is virtually independent of pH but dependent on salt concentration. The isotherms can be explained by a cation exchange reaction and a "hydrophobic" adsorption reaction.

CONCLUSION

These data and the accompanying explanations present a very simple picture of sorption of neutral and ionic organic compounds. As a first step in providing an overview, this approach is justifiable; however, the reader should be aware that even relations such as Equations 3, which I have cited as one of the most generally applicable in all of organic environmental chemistry, are still active areas of research, as details such as type of organic carbon, polarity of solute molecules etc. are further debated. Thus, this discussion should be considered a beginning, not an end.

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A STUDY OF HEAVY METAL SPECIATION MODELLING ON THE LE AN RIVER

Yuhuan Lin and Qi Li[1]

ABSTRACT

The toxicity of metal pollutants in aquatic systems varies from one metal species to another depending upon the water quality and the types and concentrations of heavy metals within the system. In this study, heavy metals within the water and sediment of a site along the Le An River, China, were examined and classified as dissolved or suspended. The suspended particulate metals were further divided into active and inert classifications. The active portion in water included a soluble part and a part comprised of elements in suspended particulate matter. The active portion in sediment included soluble, exchangeable, carbonate-bound, and ferric-manganese oxide- bound parts, while the inert portion included organic sulfide-bound and residual parts. A model was proposed to simulate the aquatic transport and transformation of dissolved and particulate heavy metal species. The model consisted of two components: the River model, and the MINTEQA2 Thermodynamic Equilibrium model (U.S.EPA, ERL, Athens, Georgia). The model was tested on field-collected water quality data from a section of the Le An river, and on hydrological and meteorological data from the main hydrological station along the Le An river (up-, mid- and downstream sites).

Using the River model, calculations of the concentration and speciation of copper in both water and sediment indicated that the impact of acidic mine drainage on the water quality of the Le An river was considerable. Near the discharge site, the concentration of copper in sediment reached levels of 3-5 g/kg, while the concentration of dissolved copper in the water at this site exceeded the national standard of 0.01 mg/l. The length of river polluted by the acidic mine drainage was about 50 km during the dry season, and up to 200 km (to Poyang Lake) during the flood season. The model-derived results for copper were in agreement with the values measured in the field for both water and sediment.

The species of heavy metals present within the water column of the river were calculated using the MINTEQA2 model, and recorded values of water quality and dissolved metal concentrations from the Le An river. The results indicated that large amounts of Al and Fe were introduced into the river from the mine drainage. These metals flocculated and formed a sediment on the river bottom during the dry season which was then carried downstream to the Le An river estuary during the flood season. A prediction of the effects of these metals on aquatic organisms was made by comparison of the actual metal concentrations with standard allowable levels.

INTRODUCTION

The study was conducted on a section of the Le An river system in the northeastern part of the Jiangxi province (Fig. 1). The Le An river forms from the drainage of a 9616 km2 water shed around the Huiyueshan mountain (lat. 29'11'') at an elevation of 860 m, and travels 279 km through Dexing and Leping Counties before reaching Poyang Lake (elevation 32 m) in Poyang County (lat. 29"2''). Within Dexing County, the Le An river is fed by two tributaries, the Dawu and Jishui rivers, which

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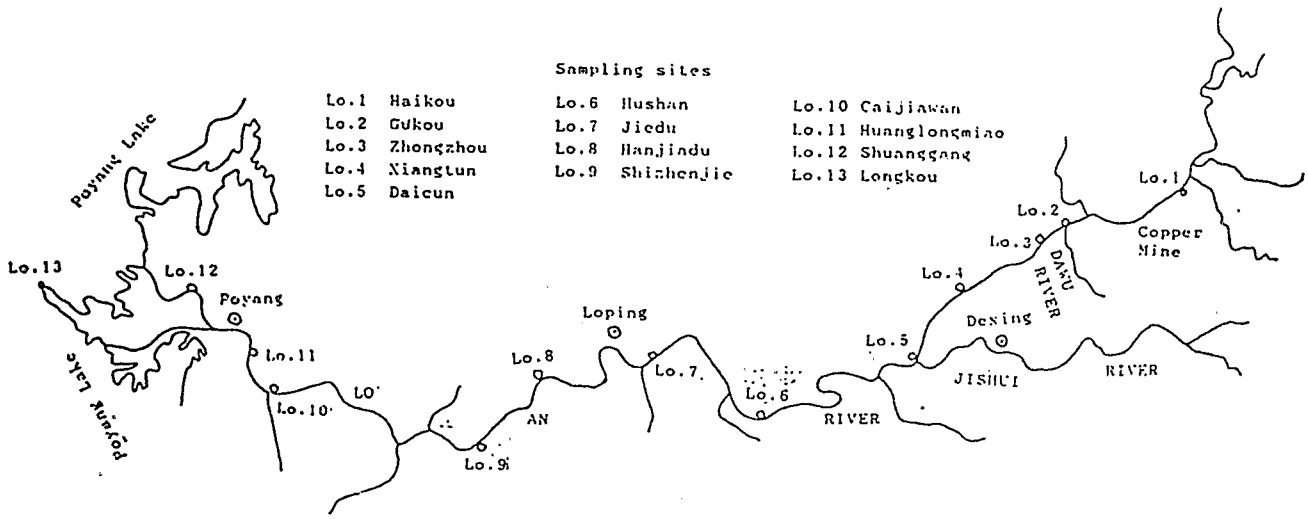


Figure 1. Sketch on Le An River, Poyang Lake area.

are surrounded by four different mining operations. The largest open-cast copper mine in China is located along the Dawu river, while the Fujiawu and Damaoshan copper mines and the Yinshan lead and zinc mine are found along the Jishui river. A large amount of acidic mine waste is discharged from these mines, which imposes a serious heavy metal pollution load (especially of copper) upon the Le An river system.

The toxicity of metal pollutants in aquatic systems varies from one metal species to another depending upon the water quality, and the types and concentrations of heavy metals within the system. In this paper, a model of heavy metal species in river systems is proposed. The model consists of two components:

(1) The River model, which treats the transportation of heavy metals within the river, including dissolved metals and suspended particles. Metals defined as suspended particles are further divided into active and inert parts.

(2) The geochemical, thermodynamic equilibrium model, MINTEQA2 (from U.S. EPA, Athens, Lab.), which can simulate metal species within an aquatic system.

RIVER MODEL FOR METAL SPECIATION

Suspended particulate metals can be classified as being active or inert. The active portion includes metal species that are water soluble, can exchange cations, or are either carbonate or ferric-manganese bound. These active portions are easily altered with changes in water quality, and so may take part in the movement and transport of elements in water.

The sulfide-bound and residual metals form the non-active or inert portion. It is necessary to simulate the movement and transport of the active and inert portions of metals in suspended sediment

separately. The grain size of metals transported in suspended sediment is generally less than 20 μm in diameter. A model describing the transport of dissolved and particulate metals is proposed below:

$$\frac{dC}{dt} = D\frac{\delta^2 C}{\delta x^2} + V\frac{\delta C}{\delta X} - Ks \cdot C + R \qquad (1)$$

where;
C is the metal concentration (active or inert portions) in suspended matter (mg/l)
V is the average flow velocity (m/s)
D is the dispersion coefficient of metal (m^2/s)
Ks is the sedimentation and resuspension coefficient of metal (l/s)
R is the point or nonpoint source load (mg/s).

The expression can also be used to calculate both the active and inert portions of metals in suspended sediment. In order to calculate the concentration of dissolved metal, it is necessary to define the partition coefficient representing the equilibrium relationship between the dissolved species in the water and the active portion of metals in suspended matter. The partition coefficient is expressed as follows;

$$Kp = Cse/Cdw \qquad (2)$$

where;
Kp is the partition coefficient of dissolved and suspended metals (l/kg)
Cse is the concentration of the active part of metal in suspended matter (mg/kg)
Cdw is the concentration of metal dissolved in the water (mg/l).

The total concentration of metal in the water is:

$$Ct = Cdw + Cac - Cin \qquad (3)$$

where;
Cac and Cin are the concentrations of the active and inert metal portions, respectively (mg/l)

The active metal concentration can be represented by:

$$Cac = Cse * SS * 10\text{-}6 \qquad (4)$$

where;
SS is the concentration of suspended matter (mg/l).

In the model, the balance of total suspended matter (SSt) in the water is:

$$SSt = SSi + SSp - SSout - SSd \qquad (5)$$

where;
SSi and SSp are the flux of suspended matter from the upstream and lateral boundaries, respectively (g)
SSout is the downstream output flux of suspended matter (g)
SSd is the flux of suspended matter as bottom sediment (g).

Finally, the total concentration of suspended matter is expressed as:

$$SS = SSt/Q \tag{6}$$

where;
Q is the volume of water (m3) within a river segment (defined below).

Expressions (1) to (6) can be used to represent the physical and chemical transport processes of metal within a river, including dispersion, precipitation, dissolution, adsorption, desorption, and sedimentation.

a) Segmentation of the River and Hydrological Data

In order to simulate the transport and transformation of metals within a river, it is necessary to divide the river into several segments based on hydrological data and river morphology. In this study, the section of the Le An River being examined was roughly divided into three sections; upstream, midstream, and downstream, corresponding to the locations of three hydrological stations (Table 1). The hydrological data used was collected from 1975 to 1986. In order to increase the accuracy of the simulation, the studied section of the Le An river (from the point of heavy metal discharge to a point 159 km downstream) was then subdivided into ten segments (Fig. 2). Water velocity, runoff, and runout from the boundary were estimated from the meteorological and hydrological data (Table 2). The results indicated that there was considerable variation between the upstream and downstream flow volumes and velocities. The seasonal runoff of the river also varied considerably, with flow rates of between ten to a few hundred cubic meters per second in the dry season, increasing to rates of several thousand cubic meters per second in the wet season.

Table 1. Characteristics of the three main sections of the Le An river.

Sections	Length (km)	Gradient (°/oo)	Width (m)	Depth (m)
Upstream	61.4	1.0	50-100	1-5
Midstream	83.6	0.34	100-150	2-8
Downstream	72.5	0.065	>200	5-15

Table 2. Flow velocity, runoff, and runout from the boundary in the normal season.

Segment	1	2	3	4	5	6	7	8	9	10	11
Q (m3/s)	116	129	134	149	184	199	204	219	224	224	
V (m/s)	0.92	0.80	0.75	0.69	0.42	0.38	0.32	0.28	0.27	0.26	0.20
Qb (m3/s)	10	3	5	15	35	15	5	15	15	20	

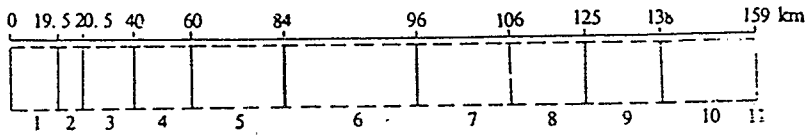


Figure 2. Scheme of segments on Le An River.

b) Calculation of Partition, Sedimentation, and Dispersion Coefficients

The partition coefficient for copper, Kp, was calculated for each river segment based upon the concentrations of dissolved metal in the water and of the active metal in suspended matter. The partition coefficient varied considerably between the first five segments (Table 3). The concentration of the active metal portion in segment 2 was so high that the dissolved concentration in the water may have reached levels up to several hundred parts per billion. Below segment 5, Kp stabilizes.

Table 3. Partition coefficient for copper, Kp, at different segments of the river.

Segment	1	2	3	4	5	6	7	8	9	10
Kp (l/kg)	5800	5099	1166	2825	500	500	500	500	500	500

The sedimentation coefficient, Kd, was calculated as:

$$Kd = dp/St \tag{7}$$

where,
dp is the average depth of water in each segment (m)
St is the sedimentation velocity of suspended particles (m/s).

The results are illustrated in Table 4.

Table 4. Sedimentation coefficient, Ks, at different segments of the river.

Segment	1	2	3	4	5	6	7	8	9	10
Ks (*10-3) (s-1)	0.92	1.98	0.51	0.16	0.16	0.15	0.05	0.05	0.05	0.05

The dispersion coefficient, D1, was calculated according to Fick's Law:

$$N = \frac{D_1 * A}{X} * \Delta C \qquad (8)$$

where;
D1 is the dispersion coefficient (m^2/s)
A is the cross-sectional area of the segment (m^2)
X is the distance between midpoints of adjacent segments (m)
ΔC is the differential concentration of segments (g/m3)
N is the mass flux of the heavy metal within the cross section (g/s). The value of N was assumed to be 100 m^2/s for calculation and had little effect upon the final results.

THE THERMODYNAMIC EQUILIBRIUM MODEL - MINTEQA2

The MINTEQ family of programs, of which the latest version is MINTEQA2, was developed by the USEPA. These programs employ the chemical equilibrium constant method to relate metal species within a chemical system and can calculate the equilibrium concentration of individual species in an aquatic environment.

The results from the river model for metal speciation were entered into the MINTEQA2 program and the species distribution of heavy metals throughout the water column were calculated for the individual river segments.

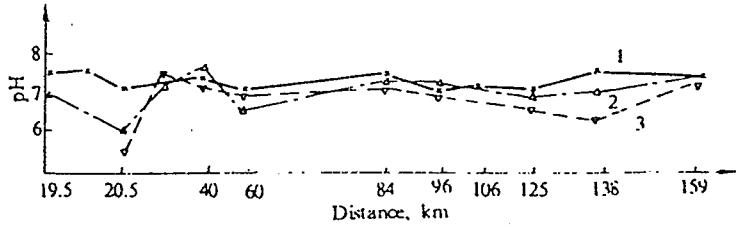
a) The Water Quality of the Le An River

Cation and anion concentrations and pH were measured from river samples from each segment during the field investigation portion of the study, and are shown with the results of the MINTEQA2 program in Table 5. The pH analysis indicated that pH tends to be higher during the dry season and lower during the wet season (Fig. 3), implying that the impact of acidic drainage on water quality was greater in the wet season. Dissolved oxygen and Eh (approximately 190 mv) were stable throughout the river.

Table 5. Water quality and ionic composition of the Le An river.

Lo.	pH	Ca	Mg	K	Na	Cu	Pb	Zn
1	6.56	7.068	1.54	1.73	1.84	0.003	0.021	0.031
2	4.79	20.59	11.07	1.05	2.55	1.94	0.021	0.053
3	6.46	13.76	2.48	1.51	2.11	0.10	0.001	0.010
4	7.17	9.33	2.33	0.87	2.10	0.18	0.001	0.013
5	7.20	5.82	1.43	0.93	1.61	0.07	0.001	0.021
7	7.25	9.05	2.18	1.09	1.81	0.04	0.01	0.057
	7.25	9.05	2.145	0.64	1.91	0.02	0.003	0.052
8	7.25	7.45	1.81	1.22	2.11	0.01	0.004	0.034
9	7.25	7.82	1.81	3.63	3.13	0.001	0.001	0.007
10	7.25	8.51	1.73	1.01	1.91	0.001	0.004	0.059
11	7.25	9.21	2.02	3.20	2.95	0.001	0.001	0.001
12	7.25	9.02	1.98	3.49	2.66	0.001	0.001	0.001
13	7.25	8.89	1.82	3.39	2.53	0.001	0.001	0.001

Lo.	Cd	Fe	Mn	Al	As	S	SO_7
1	0.00	0.24	0.030	0.098	-0	4.315	3.27
2	0.00	13.41	0.719	4.582	-0	58.82	152.08
3	0.00	3.20	0.112	0.299	-0.003	7.79	25.68
4	0.00	3.45	0.125	1.061	-0	7.82	28.35
5	0.00	1.10	0.078	0.462	-0	3.69	8.48
7	0.00	0.64	0.096	0.289	-0	4.18	12.62
	0.00	0.38	0.071	0.164	-0	3.54	9.62
8	0.00	0.39	0.070	0.186	-0	2.09	4.79
9	0.00	0.193	0.061	0.135	-0	3.08	4.80
10	0.00	0.36	0.054	0.225	-0	2.54	24.40
11	0.00	0.097	0.001	0.016	-0	4.62	6.60
12	0.00	0.035	0.001	0.010	0.0007	3.69	5.90
13	0.00	0.014	0.001	0.0023	0.0007	3.72	5.80



1. 1989, 4; 2. 1989, 12; 3. 1990, 4.

Figure 3. pH changes along Le An River.

DISCUSSION AND CONCLUSION

THE CONCENTRATION AND SPECIATION OF HEAVY METALS IN RIVER SEDIMENT AND WATER

In this study, copper was used as a representative heavy metal to assess the validity of the model in predicting heavy metal transport and deposition.

a) Total Sedimentary Copper Distribution

The calculated copper concentrations in sediment over the study area during three seasons (flood, dry, and normal) are depicted in Fig. 4. Copper concentrations reached highs of 3000-4000 mg/kg near the Dawu river mouth (location 2) which then decreased to 700-800 mg/kg at the downstream end of the study area (location 10). There was strong agreement between the calculated results (Fig. 4) and the field-collected data (Fig. 5) regarding the copper concentration distribution. The copper concentration in the segment near the point of pollutant discharge changed dramatically with the season and, according to the calculations, with different flow rates. The concentration of copper in the river segment near the source of discharge was typically higher while the transport distance of copper was shorter, in the normal season compared with the flood season. During the dry season the copper concentration in the segment near the Dawu river mouth reached a maximum but the transport distance was much shorter than in other seasons.

During the flood season, the concentration of copper along the river changed slowly, with the attenuation process dominating. Accordingly, the rate of sedimentation of pollutants along the river bottom diminished, and the distance that pollutants could be carried by the river exceeded 200 km (to Poyang Lake, location 13). The river velocity during the flood season also increased, and thus lateral transport of particles was greater than the sedimentation rate. During the normal and dry seasons, the results indicated that sedimentation was the principle transport process.

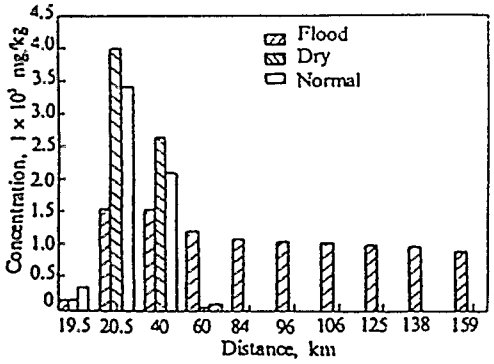


Figure 4. The calculated Cu concentration in sediments.

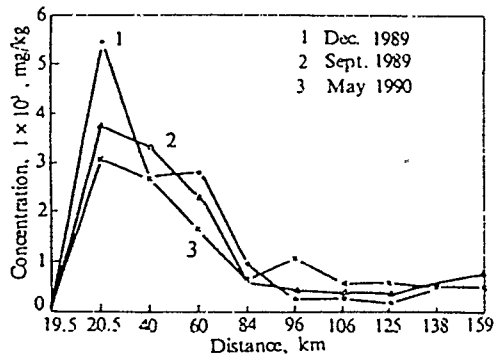


Figure 5. The concentrations of Cu in sediment.

b) Speciation of Copper in Sediment

The total, inert and active copper concentrations within sediment during the normal season are illustrated in Figure 6. The inert and active portions of the sediment-bound copper can be liberated into the water. Analysis of the speciation partition of the sediment-bound copper by sequential extraction indicated that 50-90% of the copper was inert, while the remainder, 10-50%, was active. This percentage calculated as inert was in agreement with the experimental results. Any difference

may be accounted for by loads coming from points other than the monitored discharge site, and temporal differences during estimation of the drainage composition.

The inert proportion of copper in sediment downstream was greater during the flood season (Fig. 7) than in the normal season (Fig. 6) because of the transport of suspended ore residue and tailings. During the normal season most of the copper particles were deposited in the river segment immediately below the discharge site. The proportion of active component at downstream sites was greater during the normal season than in the flood season.

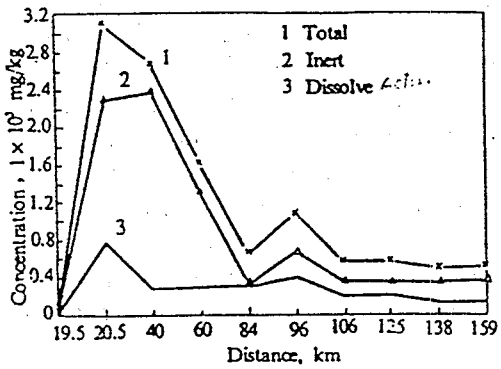


Figure 6. The speciation of Cu in sediments at normal season (Sept. 1989).

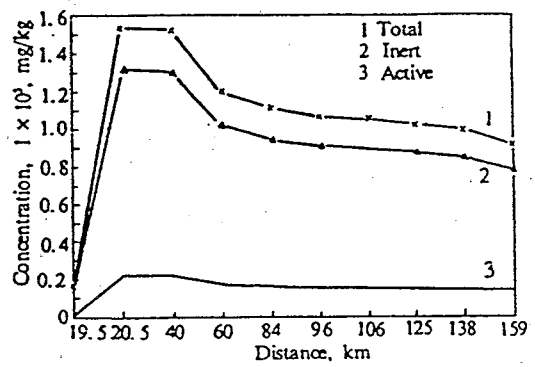


Figure 7. Speciation of Cu in sediments at flood season (May 1990)

c) Total Aquatic Cu Distribution

The calculated concentration distribution of copper in the river water (Fig. 8) was the reverse of what was in the sediment. During the dry season, the concentration in the river segment near the discharge site was less than in the dry season, and pollutants were generally carried no further than 50 km downstream. The concentration gradient along the river was steeper in the dry season.
The concentration of copper in the water at locations 2 and 3 varied seasonally from 4.5-25 ppm depending upon the initial load of pollutant. The concentration dropped to 0.5-0.05 ppm in river segments 50 km downstream, while at location 10 the level was 0.01-0.005 ppm.

A comparison of the calculated and field data indicated that the aquatic copper concentration was dependent upon the ratio of volumes of runout to drainage water. Higher flow rates and greater drainage led to higher levels of copper at downstream segments. Conversely, decreased flow rates and low drainage resulted in high copper concentrations only in the segment near the discharge site.

d) Aquatic Speciation of Cu

The total, dissolved, and particulate (inert and active portions) concentrations of copper were calculated from the model for the flood season (Fig. 8). Over 90% of the copper was found to exist in suspension, with 80% inert and less than 10% (53 to 100 ppb) dissolved. During the normal season, the concentration of the dissolved fraction can increase to 300 ppb (Fig. 9). The ratio of particulate to dissolved components remains constant (about 9:1) independent of the season.
The particulate component of the total aquatic Cu concentration estimated by the model was 10 to 20% higher than that found from the field data, while the value for the dissolved component was approximately the same. This difference may be the result of the model input assumption that 90% of the total concentration of copper in the drainage was suspended particulate copper.

THE CHEMICAL SPECIES OF HEAVY METALS THROUGHOUT THE WATER COLUMN

The thermodynamic equilibrium model (MINTEQA2) can be used to calculate the species of metals within a water column from water quality data and dissolved metal concentrations calculated from the river model. The results indicated that the species of metals found within the water column are dependent upon the pH, Eh and anion concentration.

The concentration of metals in the water at location 1 was low, with simple hydroxide compounds and Mn as the dominant metal species (Fig. 10). In contrast, the dominant metal species at the pollutant discharge site at the mouth of the Dawu river were very complex (Fig. 11). Copper was the main species found here, while ferric hydroxides, Fe(OH)2+ and Fe(OH)2+, and Al3+ were also predominant. The amount of free ferric iron and aluminum hydroxide was very low. The composition of metal species found in river segment 2 was similar to that found at the mouth of the Dawu river (Fig. 12).

There was a noticeable difference between the metal species composition of the pollutant plume found at location 2 and that found 10 km downstream at location 3 (Fig. 13). Large amounts of Al and Fe were initially found in a number of different forms, including Al(OH)3, Fe(OH)2+, Al(OH)2+, and Al(OH)4-, while copper was initially found as Cu(OH)2 or CuCO3. There was then a dramatic increase in the flocculation of Al and Fe species and precipitation from the water column into the sediment, so that the concentration of metals in the water rapidly decreased downstream. The aquatic metal species composition from location 3 to location 5 was similar.

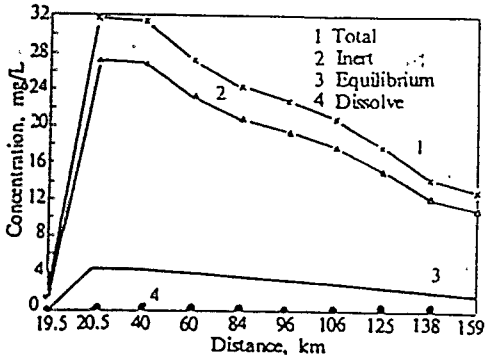


Figure 8. The total, dissolved Cu in water, active and inert species in suspension at flood season.

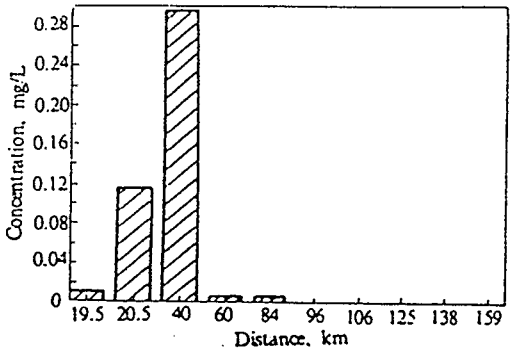


Figure 9. The dissolve Cu concentration in water at dry season.

Further downstream between locations 5 and 10, the concentration of metals was low (Fig. 14). Simple hydroxides predominated, and the water quality was essentially restored to normal levels.

BIOASSESSMENT OF METAL SPECIES

The toxicity of metal ions varies between species depending upon environmental conditions and species composition.

a) The formation of Al species in an aquatic environment is pH dependent, with free Al predominating at pH levels below 5.0. Free Al ions formed 52.8% of the total concentration at the

Dawu river mouth and 64.2% at location 2. The principle aluminum mineral formed at Gukou and at the Dawu river mouth may have been alunite ($3Al_2O_3*K_2O*4SO_3*6H_2O$). At location 3, the pH varied from 4.79 to 6.64 and $Al(OH)_3$ was the predominant species, forming 58.3% of the total concentration. The free Al ion concentration was very low from the middle river segments downstream to Poyang Lake. Dissolved Al concentrations above 1.5 mg/l can cause pronounced physiological stress on warm-water fish; 0.5 mg/l is the standard maximum concentration (Moore, 1984). The concentration of Al introduced into the Le An river through acidic mine drainage exceeds the national permissible level established for fish and other aquatic organisms, particularly during the flood season when pH is low. Locations 2 and 5 would be the most toxic with respect to Al, and form a serious environmental hazard. The impact of Al on the Le An river ecosystem needs to be closely monitored.

b) Copper is highly toxic to aquatic plants, invertebrates, and fish. The copper concentration at the mouth of the Dawu river and at location 2 reached 1.944 mg/l, which exceeds the established fisheries standard. The total downstream concentration almost exceeds the fisheries standard of 0.01 mg/l. Free Cu^{2+} is the dominant form below pH 5.0. Locations 2 (Gukou) and 3 (Zhongzhou) would be the most toxic.

c) Other elements: The concentrations of cadmium, zinc, and lead were very close to the established fisheries standards, particularly during the dry season. At location 3, free Cd was the predominate form of that element below pH 5.65 while $CdCO_3$ predominated above pH 7.25. The relatively high solubility of $ZnCO_3$ and $Zn(OH)_2$ resulted in high levels of dissolved Zn being found in the river.

There is no fisheries standard established for Fe in China, but the standard level in drinking water has been set at 0.3 mg/l. It is impossible to predict the impact of Fe upon the aquatic ecosystem. While the toxic effects of manganese on aquatic species are thought to be limited, the maximum allowable Mn concentration has been reported to be 0.77 mg/l for rainbow trout and 1.0 mg/l for drinking water. The large amounts of Fe and Mn found in the Le An river will cause a water treatment problem with regards to drinking purposes.

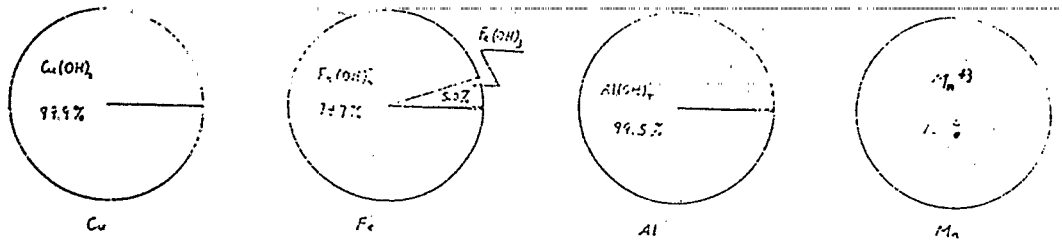


Figure 10. Speciations of metals at Location 1.

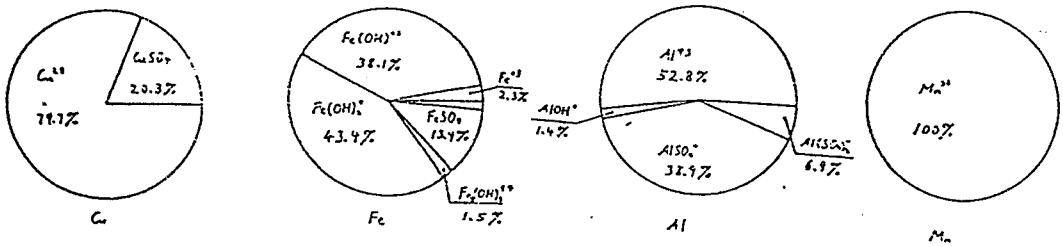


Figure 11. Speciations of metals at Dawu River mouth.

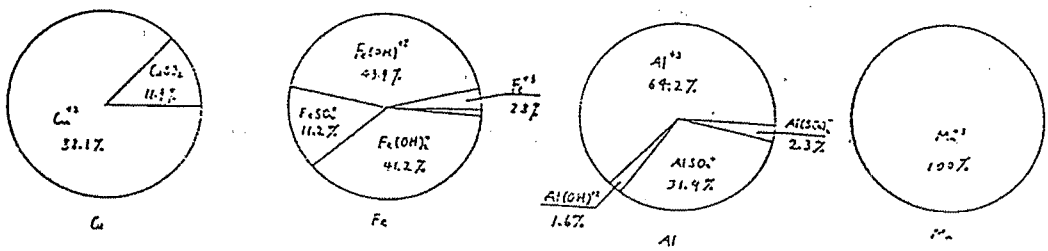


Figure 12. Speciations of metals at Location 2.

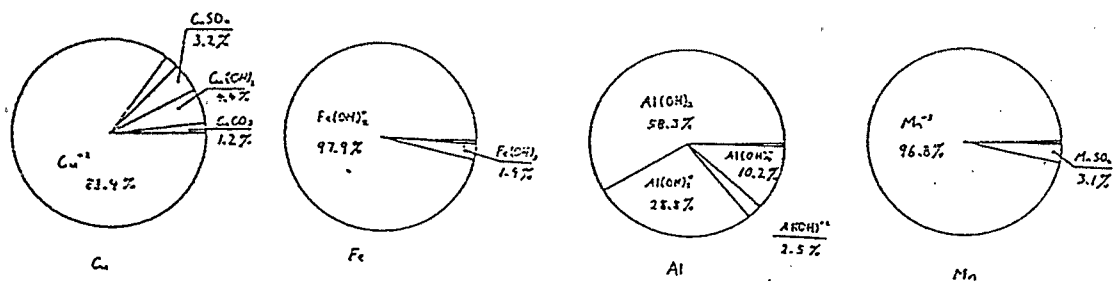


Figure 13. Speciations of metals at Location 3.

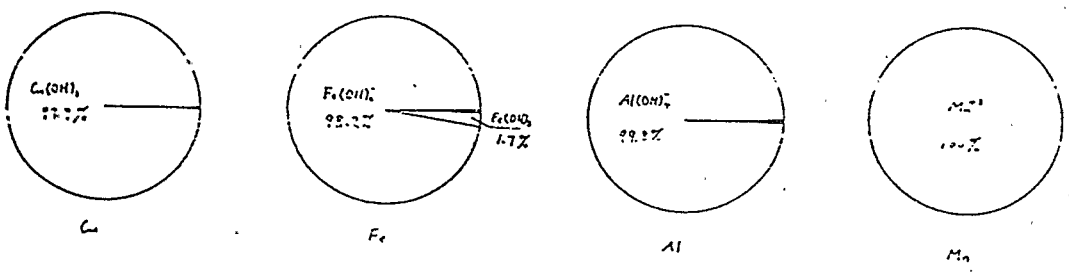


Figure 14. Speciations of metals at Poyang Lake.

SUMMARY

The ongoing drainage of acidic mining waste into the Le An river has significantly affected water pH and the concentrations of aluminum, iron, and copper. The River model and the MINTEQA2 model were used successfully to estimate heavy metal speciation over three seasons. The calculated data was found to agree well with the monitored data. Use of the River transport model and the Equilibrium model in combination allows for the description and prediction of water quality.

ACKNOWLEDGEMENTS

Many thanks to Dr. W. Salomon (Institute of Soil and Fertility RA, Haren, Holland) and Dr. R. Russo (Director, ERL, Athens, USEPA) for support of the river model and MINTEQA2 program.

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UNCERTAINTY ANALYSIS WITH CORRELATED INPUTS USING QUAL2E

Linfield C. Brown, Ph.D.[1]

INTRODUCTION

Quantitative uncertainty analysis is assuming increasing importance in surface water quality management. Decisions affecting the appropriate use of stream assimilative capacity, the permitting of waste water discharges, and the prediction of toxicant concentrations are increasingly subject to debate because of questions arising from the quality of the field data and the validity of model assumptions used to make forecasts. Conventional approaches to uncertainty analysis have generally assumed that all model inputs act independently. The significance of correlation among model inputs on errors in model predictions, though usually unknown, has always been presumed to be important. Only recently have investigators developed procedures for estimating the magnitude of its impact in model forecasting.

This study is a continuation of previous work (Brown, 1990), which reported on the effect of correlation among input parameters and variables on the output uncertainty of predicted dissolved oxygen (DO) concentrations for a single reach Streeter-Phelps model. The current study presents preliminary results from applying the input correlation computational strategy developed for the Streeter-Phelps model (analytical solution) to the overall uncertainty analysis framework of the multi-reach QUAL2E-UNCAS model (numerical solution). The QUAL2E-UNCAS model was selected for this application because it is a general purpose computer code, widely used by consultants and state regulatory agencies in waste load allocation and other water quality management activities. The QUAL2E-UNCAS model also has a substantial international user community.

PRIOR WORK

Burgess and Lettenmaier (1975) and Chadderton et al. (1982) performed first order error analysis on the DO deficit from Streeter-Phelps models, without including the effects of correlation among the input parameters. Song and Brown (1990) showed that the effect of correlation among input parameters in Streeter-Phelps type models could increase the variance of the predicted dissolved oxygen (DO) deficit by 20 to 40%, depending on the conditions of the simulations and the travel time at which the correlation effects are assessed. A monte carlo analysis showed that the effect of model non-linearities on the validity of the first order analysis was small compared to the correlation effects.

Brown (1990) demonstrated that the effect of correlation among input parameters and variables was also markedly dependent on the structure of the correlation matrix used in the uncertainty analysis. Working with hypothetical data sets for a single reach Streeter-Phelps model, he reported that input correlation could either increase or decrease the standard deviation of the simulated DO deficit, depending on whether the correlations represented model use in a calibration or a forecast mode. Typical forecast mode correlations tended to increase (25-50%), while calibration mode correlations tended to decrease (20-35%), the DO deficit standard deviations when compared to those obtained when assuming independence among input parameters and variables.

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The objective of the current study is to assess whether the effect of these two different correlation structures on uncertainty analysis would be manifested in a fashion similar to that reported by Brown (1990) when incorporated into a numerical water quality model applied to multiple reach stream network This assessment is made using field data from representative case studies.

QUAL2E-UNCAS

QUAL2E (Brown and Barnwell, 1985) is a comprehensive steady state water quality model that can simulate up to 15 different state variables, including: DO; carbonaceous biochemical oxygen demand (CBOD); temperature; algae (as chlorophyll-a); organic, ammonia, nitrite, and nitrate nitrogen; phosphorus; coliform bacteria; and conservative constituents. The model is applicable to dendritic streams that are well mixed, and can accommodate multiple waste discharges, withdrawals, tributary flows, and incremental inflow and outflow. QUAL2E-UNCAS is a recent enhancement to QUAL2E (Brown and Barnwell, 1987) which allows the modeler to perform uncertainty analysis on the steady state water quality simulations. Three uncertainty options are available: sensitivity analysis, first order error analysis, and monte carlo simulation.

One of the limitations of the original UNCAS framework in QUAL2E is that the uncertainty analysis methodologies assumed that each input variable or parameter used in the model acts independently of the others. This assumption was appropriate for obtaining a first approximation to the magnitude of the effects of input uncertainty on the precision of water quality model predictions. However, the hypothetical studies cited previously clearly demonstrate the necessity for accounting for the potentially important effect of input correlation in uncertainty analysis. Thus, the first project task was to modify the QUAL2E-UNCAS code to accommodate correlation among input parameters and variables. The second was to perform the case study analyses.

The original strategy in developing QUAL2E-UNCAS was to keep user input coding requirements to a minimum. Supplying an input correlation matrix for the large number of QUAL2E inputs (potentially over 100) is a daunting task, from both a data gathering and an input coding viewpoint. The work of Song and Brown (1990) showed that the important correlation contributions come from just a small sub-set of the total set of input pairs. Based on these findings, it seemed unnecessary and unreasonable to require the user to specify a large, complete, rigidly formatted correlation matrix to perform an UNCAS simulation. It was decided that QUAL2E-UNCAS simulations with correlated inputs would be done simply by having the user specify the correlation coefficients only for the pairs of inputs for which correlations were desired. This input is formatted as a simple list identifying the input pair and its corresponding correlation coefficient. The list is supplied in any order, and appears at the end of the data file containing the variances of the input parameters and variables used in the uncertainty analysis. All input pairs not appearing in this list are assumed independent by default.

UNCERTAINTY ANALYSIS TECHNIQUES

Two of the uncertainty analysis methodologies in QUAL2E-UNCAS were compared in this study: first order error analysis (FOEA) and monte carlo simulation (MCS). Both techniques offer unique insight into the manner and extent to which uncertainty in model inputs propagates through a model and is manifested as uncertainty in the output water quality variables. First order error analysis provides the user with a components of variance analysis, while monte carlo simulations supply the frequency distributions of the output water quality variables.

In first order error analysis, uncertainties in model input parameters and variables are propagated through the model using the first order terms in a Taylor series expansion about the mean value of each input. The mean and the variance of the output variable can be obtained from the linearized equation as follows (Benjamin and Cornell, 1970):

$$V(Y_k) = \sum_{i=1}^{n}\left(\frac{\Delta Y_k}{\Delta X_i}\right)^2 + 2\sum_{i=1}^{n}\sum_{j=1}^{i-1}\left(\frac{\Delta Y_k}{\Delta X_i}\right)\left(\frac{\Delta Y_k}{\Delta X_j}\right) cov(X_i,X_j) \qquad (1)$$

where; $V(Y_k)$ denotes the variance of the output variable Y_k, n is the number of input variables and parameters, $V(X_i)$ is the variance of the input variable X_i, and $cov(X_i,X_j)$ represents the covariance between inputs X_i and X_j. The derivative approximations, $\Delta Y/\Delta X$ represent the sensitivities of the model output to the model inputs. They are computed in QUAL2E-UNCAS as the difference in the function values evaluated at the mean values of the input variables and at the mean values with X_i perturbed by an amount ΔX_i. The first term in Eq. 1 represents the contributions to output variance from the variance of each input variable acting independently. The second term in Eq. 1 denotes the contributions to the output variance from correlation among the different pairs of input variables.

In monte carlo simulation, a probability distribution is identified or assumed for each uncertain model input parameter or variable. Repeated model simulations are performed for a predetermined number of times, with each simulation using a value for each uncertain input randomly selected from the assumed probability distribution. The resulting distribution of simulated output values can be analyzed statistically to compute an output variance that represents the combined effects of all model input uncertainties. Several references describe methods for determining an adequate number of simulations to provide information on model output frequency distributions. Song and Brown (1990) and Brown (1990) used 1000 simulations, and Burgess and Lettenmaier (1975) used 2000. Considering the reliability of the output results and the computational time requirements, 1000 simulations were chosen for the monte carlo analysis in this study. The multivariate normal probability distribution was used for all monte carlo simulations.

EXPERIMENTAL DESIGN

Two data sets were used to assess the effects of correlation among model inputs on the output variable uncertainty in the QUAL2E model. The first is from the Withlacoochee River in north Florida, a small stream with a single industrial discharge. The second is from the Ouachita River in Louisiana, a large slowly moving navigation stream with multiple industrial and municipal inputs. Two representative correlation matrices were selected for study. One is typical of correlations found while using the model in a calibration mode, the other is representative of a forecast mode.

The correlation among water quality model input variables and parameters has not been well studied. Attempts to locate appropriate field data on which to base quantitative estimates were met with limited success. Even the extensive field and laboratory data from the two case studies used in this paper were not sufficient to extract reliable quantitative estimates of these correlations. Thus, correlations were assigned (assumed) on a qualitative basis using two criteria; sign (positive and negative) and strength (strong (0.7-1.0), moderate (0.4-0.6), weak (0.1-0.3), or negligible(0.0)). For example, assigning a correlation coefficient of 0.6 between a CBOD rate coefficient and an ammonia (nitrification) rate coefficient means that these two inputs have a moderately positive correlation.

Table 1. Input Correlation Coefficients for Uncertainty Analysis Using QUAL2E-UNCAS

Input Parameter/Variable Pair		Forecast Mode	Calibration Mode
CBOD rate coefficient	- NH3 rate coefficient	0.4	-0.3
CBOD rate coefficient	- Point load CBOD	0.6	-0.5
CBOD rate coefficient	- Point load NH3	0.3	0.2
CBOD rate coefficient	- Algal max. growth rate	-	0.3
CBOD rate coefficient	- Algal respiration rate	-	0.3
CBOD rate coefficient	- SOD rate coefficient	0.5	-
NH3 rate coefficient	- Point load CBOD	0.3	0.2
NH3 rate coefficient	- Point load NH3	0.6	-0.5
NH3 rate coefficient	- Algal max. growth rate	0.2	-0.1
NH3 rate coefficient	- Algal respiration rate	0.2	-0.1
NH3 rate coefficient	- SOD rate coefficient	0.2	-
Point load CBOD	- Point load NH3	0.8	-0.6
Point load CBOD	- Algal max. growth rate	0.3	-0.4
Point load CBOD	- Algal respiration rate	0.3	-0.4
Point load CBOD	- SOD rate coefficient	0.4	-
Point load CBOD	- Point load DO	0.2	-
Point load NH3	- Algal max. growth rate	0.5	0.2
Point load NH3	- Algal respiration rate	0.5	0.2
Point load NH3	- SOD rate coefficient	0.4	-
Point load NH3	- Point load DO	0.1	-
Algal max. growth rate	- Algal respiration rate	0.7	0.7
Algal max. growth rate	- SOD rate coefficient	0.2	-0.3
Algal respiration rate	- SOD rate coefficient	0.4	-0.3
SOD rate coefficient	- Point load DO	0.1	-
Reaeration rate coef.	- Algal max. growth rate	-0.5	-
Reaeration rate coef.	- Algal respiration rate	-0.4	-
Reaeration rate coef.	- SOD rate coefficient	-0.4	-

In a manner consistent with prior work (Brown, 1990), two different correlation matrices were constructed for this study (see Table 1). The first set of values was designed to represent the input correlation structure for the case of applying the model in a forecast mode. The second set was designed to represent a calibration mode. The primary difference between these two sets is that in the forecast mode, values of model inputs are generally assumed or fixed by regulation to represent some unknown future condition; while in the calibration mode, many of the model inputs are measured and the remaining ones are adjusted (within acceptable ranges) to match an observed set of state variables. The correlation relationships for these two cases are quite different.

For the forecast mode correlation matrix, 25 pairs of inputs were assumed to have non-zero correlation coefficients. The values assigned represent the general tendency for the mean of one input parameter to increase or decrease as the mean of the second input increases. For example, the correlations between CBOD rate coefficient and CBOD point load, and between ammonia (nitrification) rate and ammonia point load were modeled as moderately positive (+0.6). Strong correlations were assumed between CBOD and ammonia point loads (+0.8), and between algal maximum growth and respiration rates (+0.7). The correlations between headwater conditions and all

other inputs were assumed to be negligible (0.0), as were those between climatological factors and all other inputs. The correlations between reaeration rate coefficients and the algal growth and respiration parameters was assumed moderate to strong and negative (-0.4 to -0.5).

For the calibration mode correlation matrix, 17 pairs of inputs were assumed to be correlated. The major differences from the correlations in the forecast mode are in assessing whether measurement of these particular inputs can be achieved independently of each other. For example, the value of the reaeration rate coefficient, if measured by a tracer technique, can be obtained independently of all other input measurements. On the other hand, the value of the CBOD load cannot be measured independently from the value of the organic nitrogen or ammonia load, nor independently from the CBOD or nitrification rate coefficients, if a standard long term BOD test is performed to obtain values of these inputs. Furthermore, because many input values are often obtained by taking differences (e.g.CBOD = Total BOD - NBOD) or by regression analyses (e.g. fitting first order kinetic expressions to obtain estimate of ultimate CBOD and CBOD rate coefficient), the sign of these correlation coefficients is usually negative. Thus the calibration correlation matrix has many more negative entries than the forecast correlation matrix.

The values of the correlation coefficients used in this study are similar to the set of input correlations was developed by Brown (1990) for the uncertainty analysis simulations with the Streeter-Phelps model. Because the kinetic formulations and model structure in QUAL2E are not exactly the same as those in the Streeter-Phelps model, these values were assigned to the input parameter or variable in QUAL2E that most closely matched the inputs in the analytical model (e.g. NBOD in the Streeter-Phelps model was matched to ammonia point load in QUAL2E). Every attempt was made to keep the correlation matrices as close as possible to those of the analytical simulation in order to provide a common basis for comparing the results of the two studies.

Finally, it should be noted that these correlation coefficient assignments are subjective and are likely to be refined with further study. However, in the absence of extensive, replicate field data, the two sets of values in this study are believed to represent a reasonable starting point for assessing the effects of input parameter correlation on the precision of model predictions.

CASE STUDY - WITHLACOOCHEE RIVER

The first data set used in this study of input parameter correlation was obtained from a USEPA survey of the Withlacoochee River during October 1984 (Koenig, 1986). The river is subjected to both municipal and industrial waste loads and there is a significant accretion of flow from groundwater inputs (50%). The river has a uniform low slope, but is characterized by alternating shoals and pools (occasionally in excess of 25 ft deep). Average depths during the survey periods were from 5.2 to 14.8 feet, widths ranged from 90 to 140 feet, and flows varied from 150 cfs at the headwater to 660 cfs at the end of the system. Water quality is affected by algal activity resulting from municipal waste discharges above the section of stream studied. The addition of industrial waste at river mile (RM) 24 dramatically reduces light penetration to the extent that the algal population diminishes rapidly in the downstream direction.

Ten state variables were simulated in this study; temperature, dissolved oxygen (DO), carbonaceous BOD (CBOD), four nitrogen forms (organic, ammonia, nitrite, and nitrate), two phosphorus forms (organic and dissolved), and algae as chlorophyll-a. A summary of the calibrated inputs and their relative standard deviations (standard deviation divided by the mean) is shown in Table 2. The calibrated values in general were obtained by adjusting field and/or laboratory measurements of the model inputs. The variance estimates were computed from replicate data taken

during the survey period and by inference from other published data. (McCutcheon, 1985 and Tetra Tech, 1985). Detailed results of the uncertainty analysis without correlation is presented elsewhere (Brown, 1987).

Table 2. Summary of Input Data for QUAL2E-UNCAS Simulations
Withlacoochee River Survey 1984

Input Parameter or Coefficient	Calibrated (Mean) Values	Relative Standard Deviation (%)
Hydraulic Data (7)*		
Flows (cfs)	150 - 660	3%
Depths (ft)	5.2 - 14.8	8%
Velocities (fps)	.1 - .8	10%
Others	a,b	10 - 20%
Reaction Coefficients (8)*		
CBOD Decay (1/day)	.04 - .10	15%
Reaeration (1/day)	.08 - .80	13%
SOD (gm/ft^2-day)	.04 - .13	20%
N, P, Algae	a,b	15 - 25%
Algae, Nutrient, Light Coefficients (17)*		
Maximum Growth Rate (1/day)	1.3	10%
Respiration Rate (1/day)	.15	10%
Others	a,b	10%
Climatology, Temperature Variables (23)*		
Wet, Dry Bulb Air Temps (C)	18.0 - 23.5	2%
Temperature Coefficients	1.00 - 1.083	3%
Others	a,b	1 - 15%
Headwater, Incremental, Point Loads (27)*		
DO, Temperature	a	1 - 3%
CBOD, N, P, Algae	a	8 - 25%

( )* Value indicates number of inputs in each category.
(a) Basin specific values from Koenig, 1986.
(b) Typical values from Table III-3, Brown and Barnwell, 1985

The QUAL2E-UNCAS model provides uncertainty output for all simulated variables. However, only the results for dissolved oxygen will be discussed in this paper. A plot of observed and modeled dissolved oxygen concentrations is shown in Figure 1. There is a pronounced minimum in the DO profile (sag point) a few miles downstream from the industrial waste discharge. The dilution from the spring water at RM 12 doubles the flow and depresses the dissolved oxygen only slightly because of its lower DO content. The results of the uncertainty analysis using the forecast and calibration mode correlation matrices is presented in Table 3 for both first order error analysis (FOEA) and monte carlo simulation (MCS). The results are reported at three locations: RM 26, near the beginning of the river section; RM 20 at the dissolved oxygen sag point below the industrial waste discharge; and at RM 2 near the end of the section studied.

Table 3. Effect of Input Correlation
Simulated Dissolved Oxygen Standard Deviation (mg/L)
Withlacoochee River Basin - 1984 Survey

	First Order Error Analysis			Monte Carlo Simulation		
Inputs	RM 26	RM 20	RM 2	RM 26	RM 20	RM 2
Independent	.18	.27	.30	.18	.28	.31
Correlated - Forecast Mode	.18	.34	.32	.18	.34	.34
Correlated - Calibration Mode	.17	.26	.30	.18	.27	.31
Ratios						
Forecast / Indep	1.00	1.26	1.07	1.00	1.21	1.10
Calibration / Indep	.94	.96	1.00	1.00	.96	1.00

A number of important observations can be made from examining the results in Table 3. First, the simulated DO standard deviation using the forecast mode correlation matrix is larger than that from the base case analysis which assumes independence among the inputs. At RM 20, for example, the increase is the largest, and is from .27 to .34 mg/L (26%) for FOEA and from .28 to .34 mg/L (21%) for MCS. The increases are smaller in the upper reaches because the inputs with strong correlation coefficients (e.g. the loads) do not have a strong impact on the simulated dissolved oxygen. Likewise, the increases are diminished near the end of the system because the DO is recovering from the effects of the upstream industrial waste input.

The second observation is that the effect of input correlation using the calibration mode matrix is to decrease the simulated DO standard deviation relative to the case of independent inputs. The magnitude of the decrease averages about 0.01 mg/L or about 5%, and is not as great as the increase computed in the case of the forecast correlation matrix. The positive and negative correlation coefficients in the calibration scenario apparently tend to offset one another, resulting in only a small net change (decrease) in the magnitude of the simulated DO standard deviation.

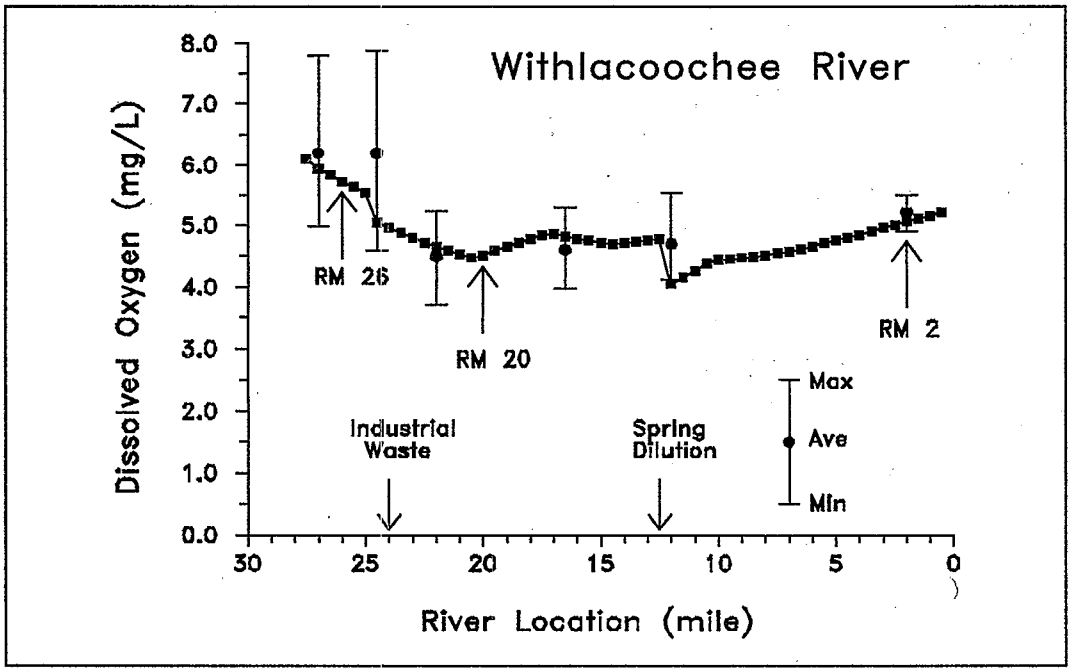

Figure 1 - Observed and QUAL2E Simulated Dissolved Oxygen Concentrations - Withlacoochee River Survey 1984.

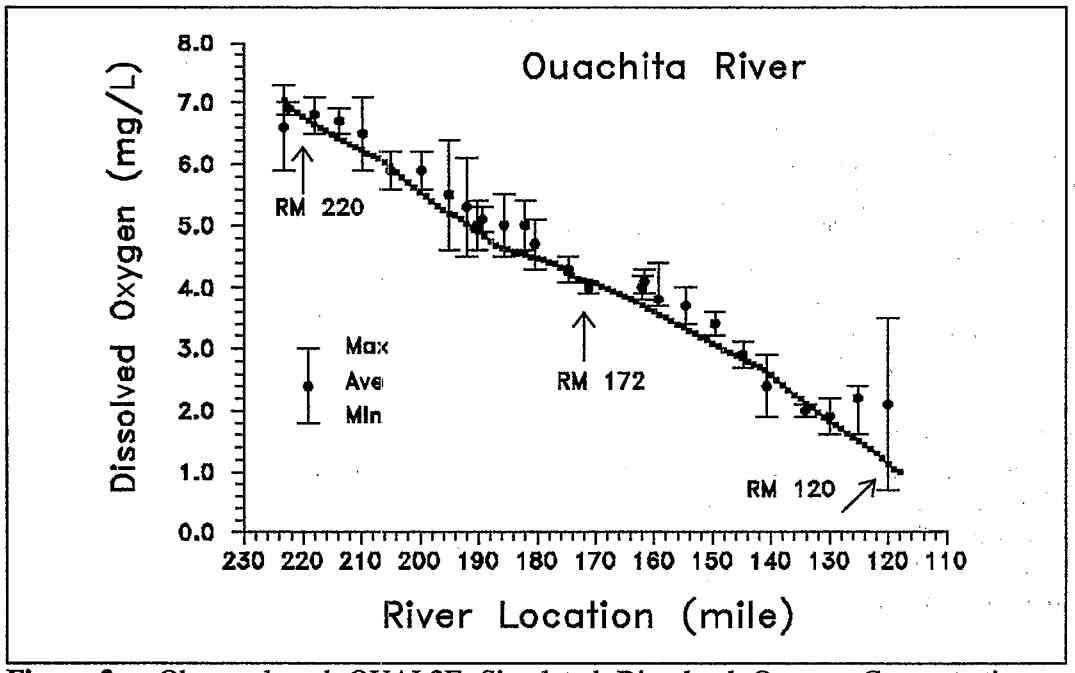

Figure 2 - Observed and QUAL2E Simulated Dissolved Oxygen Concentrations - Ouachita River Survey 1980.

Thirdly, the DO standard deviations from the monte carlo simulations are 3-5 % larger than those from the first order error analysis. This observation suggests that model non-linearities are not severe for these simulations, which is consistent with prior findings (Brown, 1985 and 1987). It is interesting to note that this difference is about the same magnitude as the error in estimating the DO standard deviation from 1000 monte carlo simulations as reported by Brown (1987).

These findings from the Withlacoochee case study are generally supportive of those by Brown (1990) using similar correlation matrices and the hypothetical data sets of the Streeter-Phelps model. The major difference is in the magnitude of the effects. The increases using the forecast mode matrix and the decreases using the calibration mode matrix are smaller by about one-half in the QUAL2E-UNCAS simulations than in the Streeter-Phelps ones. This attenuation is likely attributable to the averaging effects of a large multi-reach simulation characteristic of QUAL2E applications.

CASE STUDY - QUACHITA RIVER

The second data set is from an NCASI survey of the Ouachita river during July 1980 (NCASI, 1982). The length of river studied is a navigational pool located between two Corps of Engineers lock and dams. The section is just over 100 miles in length and receives waste discharge from a variety of industries and a number of municipalities. The summer of 1980 was a particularly dry period, with little or no precipitation occurring in the basin during the two months prior to the survey. Thus flows, while controlled by the lock and dams, was low and relatively steady. Time of flow in the pool was just over two weeks.

All but one of the 11 discharges and tributaries are located in the upper two-thirds of the basin. Average depths during the survey period were from 15 to 45 feet, and widths varied from 380 to 515 feet. Flow ranged from 2450 cfs at the head of the section to 3170 cfs at the end. The river has a very low uniform slope, with gradual meanders. Velocities were low, averaging no more than 0.25 fps. Mixing action of large eddy roll cells and stream meanders keep the water column fairly well mixed. This was confirmed by periodic sampling traverses of the stream cross section as indicated by the range of DO concentrations shown in Figure 2. The river water quality is affected by algal activity, but light penetration into the turbid water is poor, limiting this activity to the upper foot or so of the water column.

Six state variables were simulated in this study; DO, CBOD, three nitrogen forms (ammonia, nitrite, and nitrate), and algae as chlorophyll-a. A summary of the calibrated inputs and their standard deviations is given in Table 4. The calibrated values and variance estimates were obtained in a manner similar to that used in the Withlacoochee study. Detailed results of the uncertainty analysis without correlation has been discussed by Brown (1985).

As with the Withlacoochee example, only the dissolved oxygen uncertainty results will be evaluated. A plot of the observed and modeled dissolved oxygen concentrations is shown in Figure 2. Note that there is a steady decline in the DO concentration, with little evidence of a recovery from the applied waste loads. The results of the uncertainty analysis using the forecast and calibration mode correlation matrices is presented in Table 5 for both first order error analysis and monte carlo simulation. Again, the results are reported at three locations in the basin; near the headwater at RM 220, in the middle of the modeled section at RM 172, and at the downstream end of the river at RM 120.

Table 4. Summary of Input Data for QUAL2E-UNCAS Simulations
Ouachita River Survey 1980

Input Parameter or Coefficient	Calibrated (Mean) Values	Relative Standard Deviation (%)
Hydraulic Data (4)*		
Flows (cfs)	2450 - 3170	6%
Depths (ft)	15 - 45	5%
Velocities (fps)	.15 - .45	10%
Others	a,b	10 - 20%
Reaction Coefficients (10)*		
CBOD Decay (1/day)	.01 - .02	20%
Reaeration (1/day)	.02 - .20	12%
SOD (gm/ft^2-day)	.07 - .14	20%
N, Algae	a,b	10 - 20%
Algae, Nutrient, Light Coefficients (11)*		
Maximum Growth Rate (1/day)	2.5	10%
Respiration Rate (1/day)	.13	10%
Others	a,b	5 - 10%
Climatology, Temperature Variables (10)*		
Temperature Coefficients	1.00 - 1.047	3%
Others	a,b	2 - 5%
Headwater, Point Loads (14)*		
DO, Temperature, CBOD	a	2 - 10%
N, Algae	a	10 - 20%

( )* Value indicates number of inputs in each category.
(a) Basin specific values from NCASI, 1982.
(b) Typical values from Table III-3, Brown and Barnwell, 1985

Inspection of the results in Table 5 show a pattern remarkably similar to that obtained for the Withlacoochee River. Using correlated inputs in a forecast mode increased the DO standard deviations about the same amount (3 - 28%). There is also a clear pattern of the correlation effect increasing as the DO gets lower and closer to zero. The decrease in DO standard deviation from using the calibration mode correlations is also repeated in this case study. While smaller than the increase with the forecast mode, it is almost twice as large as that found in the Withlacoochee example.

Table 5. Effect of Input Correlation
Simulated Dissolved Oxygen Standard Deviation (mg/L)
Ouachita River Basin - 1980 Survey

	First Order Error Analysis			Monte Carlo Simulation		
Inputs	RM 220	RM 172	RM 120	RM 220	RM 172	RM 120
Independent	.32	.38	.71	.32	.41	.70
Correlated - Forecast Mode	.32	.45	.91	.33	.46	.81
Correlated - Calibration Mode	.32	.37	.67	.33	.38	.65
Ratios						
Forecast / Indep	1.00	1.18	1.28	1.03	1.12	1.16
Calibration / Indep	1.00	.97	.94	1.03	.93	.93

calibration mode correlations is also repeated in this case study. While smaller than the increase with the forecast mode, it is almost twice as large as that found in the Withlacoochee example.

The primary difference between the two case studies is the smaller DO standard deviations predicted in the Ouachita monte carlo simulations at RMs 172 and 120 with the forecast mode matrix, and at RM 120 with the calibration mode matrix. This observation is contrary to most QUAL2E applications which have shown that monte carlo simulation produces larger standard deviations than first order analysis. The reason for this unexpected finding in this case study is not clear. In two instances, the differences are small (less than 3%), and thus can be attributed to the error in estimating DO standard deviations from only 1000 monte carlo simulations. (This factor may also explain the 3% increase in simulated DO standard deviation at RM 220 when using the calibration mode correlation matrix.) In the remaining instance (RM 120 -forecast mode), the discrepancy occurs at a low DO (less than 2 mg/L) and after a long travel time (greater than 20 days). The difference is over 10%, and may indicate an important, yet unidentified characteristic of uncertainty analysis under these extreme conditions.

CONCLUSIONS

In general, the results from the QUAL2E-UNCAS simulations compare favorably with those reported by Brown (1990) for the Streeter-Phelps simulations. Input parameter correlation was found to both increase and decrease the dissolved oxygen standard deviation, depending on the form of the parameter correlation matrix used in the uncertainty simulation. The impact of correlated inputs in a forecast mode was to increase the simulated dissolved oxygen standard deviation on average about 15 percent (range 0 to 30%). Accounting for correlation among input parameters in a calibration mode decreases the simulated dissolved oxygen standard deviation on average about 5% percent (range -3 to 7%). The effect of input parameter correlation estimated in the QUAL2E-UNCAS case studies, while similar in sign, is about one-half that determined for a hypothetical, single reach Streeter-Phelps example (Brown, 1990). In all simulations, except those with long travel times (greater than 20 days), monte carlo analysis showed slightly larger (2-6%) output standard deviations for dissolved oxygen than those from first order error analysis. Thus the effect of model non-linearity on the results of dissolved oxygen uncertainty analysis is not believed to be severe for these simulations.

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*U.S. GOVERNMENT PRINTING OFFICE:1994-550-001/00184