ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 10, 40–52 (1985)

Sewage Effluent Biomonitoring

II. Biochemical Indicators of Ammonia Exposure in Channel Catfish

STEPHEN V. MITZ AND JOHN P. GIESY

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 10, 40–52 (1985)

Sewage Effluent Biomonitoring

II. Biochemical Indicators of Ammonia Exposure in Channel Catfish

STEPHEN V. MITZ[1] AND JOHN P. GIESY

Pesticide Research Center, Department of Fisheries and Wildlife, and Center for Environmental Toxicology, Michigan State University, East Lansing, Michigan 48824

Received October 3, 1984; revised December 26, 1984

Channel catfish (*Ictalurus punctatus*) were exposed, *in situ*, to sewage effluent for 17 days to determine the effect of unionized ammonia (UIA) on concentrations of glutamate, glutamine and α-ketoglutarate (α-KGA) in brain tissue, and activity of glutamate dehydrogenase (L-GDH) in liver tissue. Fish were held in cages either 600 m upstream (0.005 ± 0.001 mg/liter as UIA, $\bar{X}$ ± SE, n = 6) or 9 km downstream (0.032 ± 0.004 mg/liter, $\bar{X}$ ± SE, n = 6). The mean concentrations in micromoles per gram wet weight ($\bar{X}$ ± SE, n = 27–30) for glutamate, glutamine, and α-KGA at the upstream location were 3.04 ± 0.29, 5.76 ± 0.29, and 0.003 ± 0.01, respectively. Mean concentrations in micromoles per gram wet weight ($\bar{X}$ ± SE, n = 27–30) for glutamate, glutamine, and α-KGA at the downstream location were 3.03 ± 0.29, 4.60 ± 0.37, and 0.02 ± 0.004, respectively. Mean L-GDH activity in units per milligram protein ($\bar{X}$ ± SE, n = 28–30) at the upstream and downstream locations were 0.095 ± 0.003 and 0.092 ± 0.003, respectively. Neither the concentrations of these three brain tissue substrates, nor L-GDH activity were significantly different between fish at the two locations even though the observed UIA concentrations were equivalent to concentrations which have been observed to increase glutamine concentration in brain tissue of catfish during exposures under laboratory conditions. Therefore, under the observed field conditions these parameters were not useful biochemical indicators of exposure to potentially detrimental concentrations of UIA.

INTRODUCTION

Ammonia is a major toxic component of sewage treatment plant effluents (EIFAC, 1973; Tsai, 1975; Thurston *et al.*, 1981). Unionized ammonia (UIA) is extremely toxic to fish, although the mechanism of its toxicity is not fully understood. The recommended maximum, safe, chronic UIA exposure concentration for fish is 0.020 mg/liter as NH_3 (U.S. EPA, 1976; Willingham *et al.*, 1979). Ammonia toxicity has been studied in the laboratory for many years; however, the affects of the toxicant on the total lotic environment have not been clearly demonstrated under field conditions (Pallar *et al.*, 1983). This is especially true of sublethal effects.

Ammonia is the predominate form of nitrogenous waste produced by ammonotelic freshwater teleosts (Hillaby and Randall, 1979). Endogenous UIA in freshwater teleosts must either be excreted or converted to a less toxic form by biochemical detoxification reactions. Endogenous UIA in ammonotelic freshwater teleosts normally is excreted via passive diffusion through the gills. This is possible because of the small molecular size and lipid solubility of UIA, as well as an UIA concentration

[1] Present address: Savannah River Ecology Laboratory, Drawer E, Aiken, S.C. 29801.

0147-6513/85 $3.00

gradient between the ambient water and the blood. However, excretion of UIA via passive diffusion is disrupted when ambient UIA concentrations exceed blood UIA concentrations (Fromm and Gillette, 1968), thereby resulting in hyperammonemia.

In laboratory studies, researchers have observed two biochemical reactions which fix ammonia into less toxic organic compounds during hyperammonemia (Berl *et al.*, 1962; Cooper *et al.*, 1979; Subcommittee on Ammonia, 1979). The linking of ammonium ion to α-ketoglutarate (α-KGA) is catalyzed by glutamate dehydrogenase-GDH (EC 1.4.1.3) to form glutamate [Fig. 1, Eq. (1)]. This occurs predominately in the liver but also takes place in the brain (Wu, 1963). At physiological pH, the equilibrium constant (K_{eq}) for this reaction is approximately 6×10^{14}, thus strongly favoring the reductive amination of α-KGA to glutamate. The amination of glutamate to glutamine [Fig. 1, Eq. (2)] takes place only in the brain (Wu, 1963).

In laboratory studies, glutamine concentration in the brain is a useful indicator of ammonia exposure (Berl *et al.*, 1962; Levi *et al.*, 1974; Arillo *et al.*, 1981). However, we are unaware of any studies that examined brain glutamine concentration in fish under field situations such as sewage effluent exposures. The objective of the present study was to ascertain whether changes in brain glutamine concentrations or other parameters in the two ammonia detoxification reactions in fish are affected by ammonia exposure in a river receiving sewage effluent. If a concentration-response relationship could be established between ambient environmental UIA concentrations and the brain concentrations of either glutamate, glutamine, or α-ketoglutarate, and/or changes in the activities of enzymes responsible for ammonia detoxification in fish, then aquatic toxicologists would possess a powerful diagnostic tool to assess *in situ*, sublethal ammonia exposure.

The work reported here is part of a larger study in which a number of histological, biochemical, and organismal-level responses were measured to determine if the effluent from a wastewater treatment plant had an adverse effect on caged fish (Mitz, 1984; Mitz and Giesy, 1985). In the summer of 1982, a 17-day, in-stream, caged-fish biomonitoring study was conducted at sites 600 m upstream and 9 km downstream from the Anthony Ragnone Wastewater Treatment Plant (WWTP) on the Flint River at Montrose, Michigan. This effluent was selected because UIA

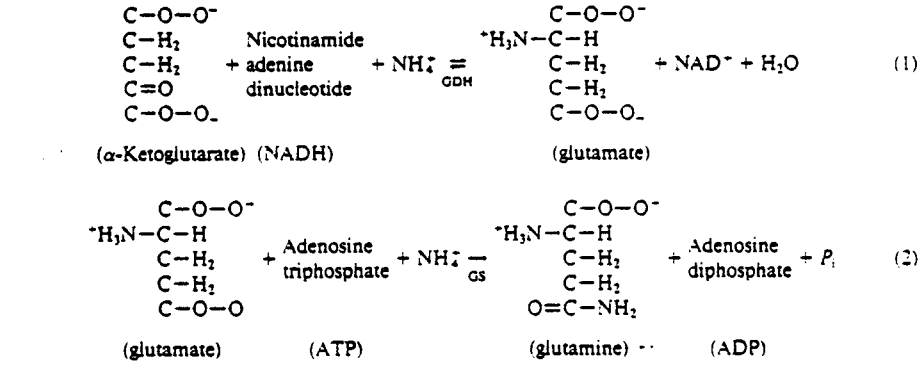

FIG. 1. Two biochemical reactions responsible for ammonia detoxification in vertebrates. GDH = glutamate dehydrogenase. GS = glutamate synthetase.

concentrations exceeded 0.020 mg/liter as far as 30 km downstream from the outfall (Roycraft and Buda, 1979). Furthermore, fish mortality had been observed downstream from the WWTP (J. Wuycheck, personal communication). The field study was conducted to determine if UIA was the toxic agent so that subsequent changes in the treatment process could be instituted in the most cost-effective manner. In this paper we report concentrations of glutamate, glutamine and α-KGA in brain tissues, as well as GDH specific activity in liver tissue (L-GDH) and discuss their relative sensitivity as biomonitors of sublethal exposure to UIA under field conditions.

MATERIALS AND METHODS

Fish

The channel catfish (*Ictalurus punctatus*) was chosen as the test organism because it is a typical warmwater stream fish occurring in the Flint River (Odin, 1981), and information is available on the physiology of ammonia metabolism and ammonia toxicity for this species. Juvenile channel catfish were obtained from Aquatic Control Inc., Seymore, Indiana, 12 days before commencement of the field exposure. Holding facilities, feeding regimes and tagging procedures have been described previously (Mitz, 1984; Mitz and Giesy, 1985). Five days before the field exposure began, fish were weighed (mean wt 23.1 $\pm$ 0.6 g SE, n = 112) and tagged.

Study Sites

Two study sites were located on the Flint River near the Anthony Ragnone WWTP at Montrose, Michigan (Mitz, 1984; Mitz and Giesy, 1985). A control site was located approximately 600 m upstream from the Ragnone WWTP outfall, while the second site was located approximately 9 km downstream from the Ragnone WWTP outfall. The two sites will henceforth be referred to as "upstream" and "downstream," respectively.

Field Exposure

The 17-day field exposure was from July 21–August 6, 1982. Test fish stocking procedures, cage description, feeding schedules, and fish and water quality sampling schedules have been described previously (Mitz, 1984; Mitz and Giesy, 1985).

Tissue Sample Preservation

Each fish was rapidly weighed to the nearest 0.1 g in a tared, 1000-ml glass beaker. Weighing was performed on an Ohaus platform scale. The liver was quickly excised and the gall bladder removed. A portion of the liver was frozen in a liquid nitrogen-cooled, aluminum tissue smasher to halt enzyme activity. The brain was then excised and frozen by the same method. All frozen tissues were stored in liquid nitrogen (−196°C).

Glutamate Dehydrogenase Assay

Liver GDH activity was assayed kinetically on a Varian 634 dual beam UV-Vis spectrophotometer according to a method modified from that of Wilson (1973).

Each frozen liver sample was weighed to the nearest 0.001 g and placed in ice-cold 0.2 *M* potassium phosphate buffer at pH 7.6. The volumes were adjusted to obtain approximately 100 mg wet wt tissue/ml buffer solution. Samples were homogenized with a motor-driven TRI-R Instruments Model K43 Teflon tissue grinder, then sonicated 2 min at 130–140 W with a Braunsonic 1510 sonicator. The homogenate was centrifuged 30 min at 14,000 rpm and 4°C. The supernatant was used directly for the GDH analysis. The assay mixture contained 1.85 ml of 0.2 *M* potassium phosphate buffer, pH 7.6; 0.15 ml of 3 *M* NH_4Cl in the phosphate buffer; 0.6 ml of 0.45 m*M* NADH in the phosphate buffer; 0.2 ml of 0.167 *M* potassium α-KGA in the phosphate buffer; 0.2 ml of GDH standard or supernatant. Potassium α-KGA, NADH, and GDH stock solution Type II (703 units/ml) were obtained from Sigma Chemical Company, St. Louis, Mo.

Oxidation of NADH to NAD^+ was proportional to the amount of GDH present and was recorded on a Beckman chart recorder as the decrease in absorbance at 340 nm. The line segment between 30–90 sec after addition of GDH standard or supernatant to the reaction cuvette was used to calculate the slope, which was a measurement of conversion rate. GDH activity was calculated from a GDH standard curve, of conversion rate as a function of GDH activity. Results were expressed as units GDH per milligram protein, with one unit of GDH converting 1 μmol of α-KGA to glutamate per minute at 25°C.

Protein

Soluble protein was determined by a modification of the method described by Bergmeyer (1974) on a 40-fold diluted portion of the GDH assay supernatant. Folin–Ciocalteu Reagent (Sigma Chemical Company) was diluted 1:1 with deionized H_2O. Bovine serum albumin (Sigma Chemical Company) was used as the standard. Absorbance was measured at 660 nm on a Varian 634 UV-Vis spectrophotometer.

Brain Tissue Assays

Deproteinization. Brain tissue was deproteinized according to a modification of the method of Dagley (1974). Each brain was rapidly weighed and ground to a powder in a stainless steel tissue grinder cooled in liquid nitrogen. Five hundred microliters of 2 *N* $HClO_4$ was frozen and ground in a separate stainless steel tissue grinder cooled in liquid nitrogen. The two frozen powders were combined in a plastic centrifuge tube and allowed to warm to 4°C. One and a half to two milliliters of ice-cold deionized H_2O was added. Ten minutes later, the contents were homogenized for 60 sec with a motorized, Teflon tissue grinder. The homogenate was centrifuged at 3000*g* for 15 min at 0°C. The supernatant was removed, neutralized with 2 *N* $KHCO_3$, and centrifuged again under the same conditions. Half of the final supernatant was stored at −20°C for the glutamate and glutamine assays. The remaining supernatant for the α-KGA assay was stored on dry ice (−78.5°C).

Glutamate. Glutamate concentrations were determined by a modification of the method of Witt (1974). The principle of this enzymatic, spectrophotometric assay is that the reduction of 3-acetylpyridine adenine dinucleotide (APAD) to APADH is proportional to the amount of glutamate present. The amount of APADH formed during the assay was determined by measuring the increase in absorbance at 363

nm and comparison to a standard curve. The assay mixture contained 0.1 ml of standard or sample extract; 0.5 ml of 66 m*M* phosphate buffer, pH 8.2; 0.05 ml of 6.5 m*M* APAD solution; 0.330 ml of deionized H_2O; 10 μl GDH stock solution. Glutamate, GDH stock solution Type II (703 units/ml), and APAD were obtained from Sigma Chemical Company.

Glutamine. Glutamine concentrations were determined by a modification of the method of Lund (1974), which is based on the enzymatic hydrolysis of glutamine with purified glutaminase (EC 3.5.1.2) to yield glutamate, which is then quantified spectrophotometrically as described above. The tissue deproteinization procedure described by Lund (1974) was not followed. The assay mixture consisted of 0.4 ml of 0.5 *M* acetate buffer, pH 5.0; 0.1 ml of 20 m*M* hydroxylamine; 0.1 ml of 1 m*M* glutaminase in the acetate buffer; 0.05 ml of glutamine standard or 0.3 ml sample extract; deionized H_2O to make 1.0 ml. Glutamine, hydroxylamine, and glutaminase Type II were obtained from Sigma Chemical Company. The glutaminase contained glutamate decarboxylase (EC 4.1.1.15). Thus, 0.1 ml of 20 m*M* hydroxylamine was added to the hydrolysis reaction mixture to act as a powerful inhibitor of glutamate decarboxylase.

α-Ketoglutarate assay. α-KGA was assayed according to the method of Narins and Passonneau (1974), which is based on the principle that a decrease in fluorescence resulting from the oxidation of NADH is stochiometrically proportional to the amount of α-KGA present. Maximum fluorescence was obtained at excitation wavelength 346 nm, and emission wavelength 464 nm (Aminco SPF 500 spectrofluorometer). The assay mixture consisted of 1 ml of reagent mixture; 0.1 ml standard or sample extract; 10 μl of GDH stock solution. The reagent mixture contained 5 ml of 1.0 *M* phosphate buffer, pH 6.8; 4 ml of 1 *M* NH_4-Ac; 0.1 ml 0.1 *M* EDTA-ethylenediaminetetracetic acid; 0.1 ml 0.1 *M* ADP; 0.2 ml of 2.56 m*M* NADH in 0.1 *M* carbonate buffer, pH 10.6; deionized H_2O to make 100 ml. GDH stock solution Type II (703 units/ml), NADH, ADP, and α-KGA (free acid) were obtained from Sigma Chemical Company.

Experimental Design and Statistical Analyses

This experiment was a completely randomized design. "Treatments" were the site-specific water conditions to which the test fish were exposed at the upstream and downstream locations. Hereafter, "location" will represent the upstream and downstream groups of fish, and will be synonomous with treatment. Analysis of liver GDH activity, brain concentrations of glutamate, glutamine, and α-KGA were each unbalanced, threeway, nested designs. Summarization of data, analysis of variance (ANOVA), and calculation of the coefficients of the expected mean squares (EMS) were obtained by using the statistical analysis system (SAS) 79, PROC NESTED routine (Helwig and Council, 1979). The approximate test procedure of Satterthwaite (1946) was used to synthesize mean squares and degrees of freedom for the *F* test of significant location and analysis day effects.

RESULTS

Water Quality

Mean temperature, pH, conductivity, hardness, alkalinity, COD, TOC, and total dissolved solids were not significantly different ($P > 0.05$, Student's *t* test) between

the upstream and downstream study sites (Table 1). However, mean total ammonia, UIA, and Fe concentrations were significantly greater ($P < 0.001$, Student's t test) at the downstream location. In contrast, mean DO concentrations were significantly greater ($P < 0.001$, Student's t test) at the upstream site. TRC, Cd, Cr, Cu, Ni, Pb, Zn, PCB, and chlorinated hydrocarbons concentrations were below detection limits at both locations (Mitz and Giesy, 1985).

Liver Glutamate Dehydrogenase (L-GDH)

Mean L-GDH specific activity from fish at the upstream station was 0.095 ± 0.003 units/mg protein. (±SE, $n = 28$). Mean L-GDH specific activity for fish from downstream was 0.092 ± 0.003 units/mg protein. (±SE, $n = 30$). Mean L-GDH specific activities were not significantly different ($P > 0.5$, threeway, Nested ANOVA) between the two locations.

Brain Substrates

Glutamate and glutamine. Neither mean glutamate nor mean glutamine concentrations in brain tissue were significantly different (threeway Nested ANOVA, $P > 0.75$, and $P > 0.1$, respectively) between fish held at the two locations (Table 2).

α-Ketoglutarate. Mean α-KGA concentrations in brain tissue were 0.03 ± 0.01 μmol/g wet wt (±SE, $n = 26$), and 0.02 ± 0.004 μmol/g wet wt (±SE, $n = 28$) in the upstream and downstream fish, respectively. Mean α-KGA concentrations in brain tissue from fish from the two locations were not significantly different ($P > 0.25$, threeway Nested ANOVA).

TABLE 1

WATER QUALITY CHARACTERISTICS AT THE IN-STREAM LOCATIONS DURING THE ANTHONY RAGNONE WWTP BIOMONITORING STUDY

Characteristic	Location	
	Upstream	Downstream
Temperature (°C)	23.1 ± 0.6	21.8 ± 0.5
pH	8.2	7.9
Dissolved oxygen (DO)	8.6 ± 0.7	4.9 ± 0.3
Total residual chlorine (TRC)[a]	bdl	bdl
Total NH_3-N[b]	0.06 ± 0.01	0.94 ± 0.07
Unionized ammonia[b]	0.005 ± 0.001	0.032 ± 0.004
Conductivity (μmhos)	461 ± 18	510 ± 22
Hardness (as $CaCO_3$)	265 ± 5	274 ± 5
Alkalinity (as $CaCO_3$)	197 ± 4	203 ± 3
Fe	440 ± 64	1273 ± 175
Chemical oxygen demand (COD)	34 ± 3	43 ± 2
Total organic carbon (TOC)	10 ± 0	11 ± 1
Total cyanide	bdl	0.019 ± 0.006
Total dissolved solids	425 ± 15	460 ± 10

Note. Values are means ± SE and are expressed as mg/l, $n = 8$.
[a] bdl = below detection limit (0.006 mg/l).
[b] $n = 6$.

TABLE 2

MEAN CONCENTRATIONS OF GLUTAMATE AND GLUTAMINE IN BRAIN TISSUE OF CHANNEL CATFISH FROM THE ANTHONY RAGNONE WWTP BIOMONITORING STUDY

	Location	
	Upstream	Downstream
Glutamate		
$\bar{X} \pm SE$	3.04 ± 0.29	3.03 ± 0.29
(Range)	(0.70–6.34)	(1.46–6.30)
n	30	28
Glutamine		
$\bar{X} \pm SE$	5.76 ± 0.29	4.60 ± 0.37
(Range)	(3.00–8.53)	(0.66–9.08)
n	28	27

Note. Concentrations are in μmol/g wet wt.

DISCUSSION

Liver Glutamate Dehydrogenase (L-GDH)

Wilson (1973) measured L-GDH specific activities of 0.0294 and 0.0358 unit/mg protein in native and cultured channel catfish, respectively. Korsgaard (1982) reported 0.0307 unit/mg protein in the marine eelpout (*Zoarces viviparus*). Iwata *et al.* (1981) determined that the specific activity of L-GDH ranged from approximately 0.2 to 0.6 unit/mg protein in liver of the mudskipper (*Periophthalmus cantonensis*) acclimatized to different salinites or kept out of water. In a study on the effects of dietary dieldrin to rainbow trout, Mehrle and Bloomfield (1974) stated that L-GDH specific activities ranged from 0.20 to 0.36 unit/mg protein.

The L-GDH specific activities observed in this study were within the range of values obtained by other researchers investigating fish L-GDH activities, even though the present results were slightly greater than those reported for channel catfish by Wilson (1973). Nevertheless, we found L-GDH specific activity in channel catfish to be an ineffective biochemical indicator of *in situ* ammonia exposure when mean UIA concentrations were 0.032 mg/liter or less. We know of no literature describing the effect of direct ammonia exposure on L-GDH activity in fish. However, Mehrle and Bloomfield (1974) reported that L-GDH activity in rainbow trout increased significantly after chronic dietary ingestion of dieldrin. The authors suggested that the increased L-GDH activity was a response to increased endogenous ammonia, which resulted from decreased ammonia detoxification in the brain. In another study, Iwata *et al.* (1981) observed greater L-GDH activity when mudskippers were kept out of water or exposed to osmotic shock. The authors attributed this to synthesis of amino acids as a method of removing excess endogenous ammonia.

A possible explanation for the similar L-GDH specific activities in fish from the two locations is that the UIA concentrations were not sufficiently great to affect L-GDH activity. The present body of literature on the role of L-GDH in ammonia detoxification is insufficient to evaluate the metabolic role of this enzyme in animal tissues (Storey *et al.*, 1978).

Brain Substrates

Glutamate and glutamine. Other researchers have concluded that during hyperammonemia, ammonia in the cerebrum is detoxified, primarily via the amination of glutamate to glutamine (Berl *et al.*, 1962; Levi *et al.*, 1974; Cooper *et al.*, 1979; Arillo *et al.*, 1981). Because most of the glutamate comes from other metabolic pathways, only a small amount of the total cerebral ammonia is combined with α-KGA to form the glutamate precursor of glutamine (Berl *et al.*, 1962; Cooper *et al.*, 1979). The accepted explanation for this phenomenon is that there are two, metabolically separate, cellular pools of glutamate in brain tissue, thereby resulting in compartmentalization of ammonia metabolism (Berl *et al.*, 1962; Van den Berg *et al.*, 1974; Cooper *et al.*, 1979). The glutamate pool, which is the major precursor for glutamine synthesized in response to blood-borne ammonia, appears to be located in the astrocytes (Cooper *et al.*, 1979). This glutamate pool is small and has a rapid turnover rate. Glutamine synthetase (EC 6.3.1.2) activity is confined to the astrocytes *in vivo* (Norenberg and Martinez-Hernandez, 1979), thus providing supportive evidence of the smaller glutamate pool location. A larger glutamate pool appears to be located in the neurons. The purpose of the larger glutamate pool seems to be to provide the glutamate which serves a neurotransmitter function and is a precursor for the synthesis of the neuroinhibitor, γ-aminobutyric acid (GABA). Compartmentalization of glutamate synthesis may explain the relatively constant brain concentrations of glutamate we observed in fish from the two locations. Our results agree with those of other researchers who also observed no change in brain glutamate concentration following ammonia exposure (Berl *et al.*, 1962; Cooper *et al.*, 1979; Levi *et al.*, 1974; Arillo *et al.*, 1981).

Glutamate compartmentalization may result in increased glutamine brain concentrations during hyperammonemia. Levi *et al.* (1974) reported brain glutamine concentration increased 10-fold in carp (*Cyprinus carpio*) exposed to 0.75 m*M* (sic) (12.75 mg/liter) total ammonia for 24–48 hr. Arillo *et al.* (1981) reported that brain glutamine concentrations were significantly greater in rainbow trout which had been exposed to 0.020 mg/liter UIA for 48 hr. Berl *et al.* (1962) performed intracarotid infusion of $^{15}NH_3$ in adult cats, and concluded that [^{15}N]glutamine was the only cerebral amino acid concentration that increased significantly. Cooper *et al.* (1979) performed intracarotid infusion of $^{13}NH_3$ in rats, and reported that 84% of the label recovered in the brain was in a metabolized form, 95% of which was in the amide group of glutamine.

The glutamine concentrations observed in brain tissues from the present study were similar to those observed in channel catfish by Wilson and Poe (1974; Table 3). However, we did not observe increased glutamine concentrations in brain tissue due to chronic exposure to the greater ammonia concentration. This is contrary to the results of Levi *et al.*, 1974; Arillo *et al.*, 1981; Berl *et al.*, 1962; Cooper *et al.*, 1979. The lack of significant difference between the glutamine concentrations in brain tissue of fish from the two locations may be due to several causes. One is that antagonistic interactions occurred because of the dynamic biotic and abiotic conditions, and the multiplicity of toxicants in the effluent. The resulting antagonistic interactions could have lessened the toxic effect of ammonia at the downstream station. Second, the UIA concentration may simply not have been great enough to induce hyperammonemia in the downstream fish. Third, the glutamine concentrations

TABLE 3

LITERATURE VALUES OF BRAIN TISSUE CONCENTRATIONS OF GLUTAMATE IN FISH

Organism	Ammonia exposure (if applicable)	Glutamate conc.	Glutamine conc.	Reference
Ictalurus punctatus	—	2.99	4.09	Wilson and Poe (1974)
Cyprinus carpio	Controls	5.75	2.25	Levi *et al.* (1974)
	Treated[a]	5.47	22.09*	
Salmo gairdneri	Controls (48 hr)	4.88	3.48	Arillo *et al.* (1981)
	20 μg/l UIA (48 hr)	4.74	4.80*	
	40 μg/l UIA (48 hr)	3.55	7.10*	
	500 μg/l UIA (24 hr)	2.67	8.80*	

Note. Values are in μmol/g wet wt tissue. In studies that involved exposure to ammonia, mean ambient UIA concentrations are presented.

[a] Exposure was for 24–48 hr at 0.75 m*M* NH (sic); no pH data given, therefore calculation of UIA concentration was impossible.

* Significantly greater than controls.

in brain might only increase initially upon exposure to ammonia after which the glutamine concentrations may decrease to their initial values. Previous investigations on the effects of ammonia on glutamate and glutamine concentrations in brain tissue (Berl *et al.*, 1962; Levi *et al.*, 1974; Van den Berg *et al.*, 1974; Hawkins *et al.*, 1973; Cooper *et al.*, 1979; Arillo *et al.*, 1981) ranged from 5 min to 48 hr in duration. Therefore, it may be erroneous to assume that the conclusions from these acute studies can be applied in interpreting results from the present 17-day study.

α-Ketoglutarate. We are unaware of any other data describing the free concentration of α-KGA in fish brain tissue. However, we have summarized the results of investigations on α-KGA concentrations in brain tissue from rats that were administered ip-intraperitoneal injections of NH_4-Ac (Table 4). The mean brain α-KGA concentrations in fish from the present study were approximately three- to sevenfold less than the brain α-KGA concentrations reported in rat studies. The greater brain tissue concentration of free α-KGA in rats may have been attributable to interspecies physiological differences between channel catfish and the rat. Interestingly, α-KGA concentrations in brain tissue from control rats were not significantly different from α-KGA concentrations in brain tissue from rats administered ip injections of NH_4-Ac (Shorey *et al.*, 1967; Hindfelt and Siesjo, 1971; Hawkins *et al.*, 1973). The present results indicate the need for additional research on fish brain α-KGA concentrations, including ammonia concentration–response investigations.

Comparison of Sublethal UIA Effects

The mean UIA concentration of 0.032 mg/liter at the downstream site was approximately 50- to 100-fold less than the 96-hr LC_{50} of UIA for channel catfish (Colt and Tchobanoglous, 1976 and 1978; Roseboom and Richey, 1975). However, the mean UIA concentrations at the downstream site were similar to UIA concentrations which have been observed to cause sublethal, deleterious effects to fish. For

TABLE 4

LITERATURE VALUES OF BRAIN α-KGA CONCENTRATIONS IN RATS GIVEN ip-INJECTED NH_4-Ac SOLUTION

NH_4-Ac Dosage (mmol/kg)	Time after injection (min)	α-KGA concentration	Reference
Control	2.5	0.0929	Shorey *et al.* (1967)
	15.0	0.0973	
7.9	2.5	0.0887	
	15.0	0.0911	
Control	15	0.131	Hindfelt and Siesjo (1971)
2.6	15	0.130	
5.2	15	0.151	
7.8	15	0.152	
Control	5	0.208	Hawkins *et al.* (1973)
10.0	5	0.204	

Note. Values are in μmol/g wet wt.

example, Colt and Tchobanoglous (1978) reported that growth of channel catfish was reduced after exposure to 0.048 mg/liter UIA for 31 days. Smith and Piper (1975) observed significantly less growth in trout after 6 months exposure to 0.017 mg/liter UIA. Burrows (1964) reported that growth of chinook salmon (*Oncorhynchus tschawytscha*) was reduced after 6 weeks exposure to 0.002 mg/liter UIA, 12 hr/day.

UIA concentrations less than 0.032 mg/liter have caused severe histopathological effects in fish. Smith and Piper (1975) observed severe histopathological damage to gill and liver tissue in trout chronically exposed to 0.017 mg/liter UIA. Similarly, Larmoyuex and Piper (1973) observed extensive histopathological gill damage in rainbow trout exposed to 0.014 mg/liter UIA for 4 weeks. Burrows (1964) reported that chinook salmon developed gill hyperplasia after 6 weeks of exposure to 0.006 mg/liter UIA. Mitz and Giesy (1985) reported severe gill histopathological effects in juvenile channel catfish exposed *in situ* for 17 days to sewage effluent containing a mean UIA concentration of 0.032 mg/liter. The significance of gill histopathological injury is that it may increase the susceptibility to bacterial gill disease (Burrows, 1964; Smith and Piper, 1975; Smart, 1976) and also result in a decreased scope of activity.

The duration of UIA exposure in the present study was briefer than that which would be expected to cause chronic sublethal effects on growth. Nevertheless, the fact that no significant changes were observed in any of the substrates or L-GDH activity indicates that these measures of short-term biochemical changes would not be related to chronic effects under the field conditions of this study.

CONCLUSIONS

1. The specific activity of L-GDH and concentration of glutamine observed in the present study were similar to those reported for channel catfish during laboratory studies. No literature was found describing the concentration of α-KGA in brain

tissue of fish. However, the α-KGA concentrations observed in the present study were approximately sevenfold greater than those reported in rats.

2. Laboratory studies of fish exposed to UIA suggest that one would expect to observe increased glutamine concentrations in brain tissue due to amination of glutamate. However, no significant differences were observed in the concentrations of glutamate, glutamine, or α-KGA, or specific activity of L-GDH between fish at the two, *in situ* study locations, including exposure to 0.032 mg UIA/liter for 17 days.

3. Therefore, neither these substrates nor L-GDH activity were useful biochemical indicators of exposure to 0.032 mg UIA/liter for 17 days even though this concentration of UIA is sufficient to cause reduction in growth and/or severe histopathological effects in fish after 4 weeks to 6 months exposure.

ACKNOWLEDGMENTS

Supported in part by the Michigan Agricultural Experiment Station (Journal Article No. 11309). Manuscript preparation was supported by Contract DE-AC09-76SR00819 between the U.S. Department of Energy and the University of Georgia's Savannah River Ecology Laboratory.

REFERENCES

ARILLO, A., MARGIOCCO, C., MELODIA, F., MENSI, P., AND SCHENONE, G. (1981). Ammonia toxicity mechanism in fish: Studies on rainbow trout (*Salmo gairdneri* Rich). *Ecotoxicol. Environ. Safety* 5, 316–328.

BERGMEYER, H. U. (ed.) (1974). *Methods of Enzymatic Analysis.* Vol. 1, pp. 172–174. Verlag Chemie, Weinheim; Academic Press, New York/London.

BERL, S., TAKAGAKI, G., CLARKE, D. D., AND WAELSCH, H. (1962). Metabolic compartments *in vivo*. Ammonia and glutamic acid metabolism in brain and liver. *J. Biol. Chem.* 237, 2562–2569.

BURROWS, R. E. (1964). *Effects of Accumulated Excretory Products on Hatchery-Reared Salmonids*, pp. 1–11. U.S. Fish. Wildl. Serv. Res. Rep. 66.

COLT, J., AND TCHOBANOGLOUS, G. (1976). Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish. *Ictalurus punctatus*. *Aquaculture* 8, 209–224.

COLT, J., AND TCHOBANOGLOUS, G. (1978). Chronic exposure of channel catfish, *Ictalurus punctatus*, to ammonia: Effects on growth and survival. *Aquaculture* 15, 353–372.

COOPER, A. J. L., MCDONALD, J. M., GELBARD, A. S., GLEDHILL, R. F., AND DUFFY, T. E. (1979). The metabolic fate of ^{13}N-labeled ammonia in rat brain. *J. Biol. Chem.* 254, 4982–4992.

DAGLEY, S. (1974). Citrate. UV Spectrophotometric determination. In *Methods of Enzymatic Analysis* (H. U. Bergmeyer, ed.), Vol. 3, pp. 1568. Academic Press, New York/London.

EIFAC. (1973). Water quality criteria for European freshwater fish. Report on ammonia and inland fisheries. *Water Res.* 7, 1011–1022.

FROMM, P. O., AND GILLETTE, J. R. (1968). Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* 26, 887–896.

HAWKINS, R. A., MILLER, A. L., NIELSEN, R. C., AND VEECH, R. L. (1973). The acute action of ammonia on rat brain metabolism *in vivo*. *Biochem. J.* 134, 1001–1008.

HELWIG, J. T., AND COUNCIL, K. A. (1979). *SAS User's Guide*, pp. 313–315. SAS Institute, Inc., Raleigh, N.C.

HILLABY, B. A., AND RANDALL, D. J. (1979). Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Canad.* 36, 621–629.

HINDFELT, B., AND SIESJO, B. K. (1971). Cerebral effects of acute ammonia intoxication. II. The effect upon energy metabolism. *Scand. J. Clin. Lab. Invest.* 28, 365–374.

IWATA, K., KAKUTA, I., IKEDA, M., KIMOTO, S., AND WADA, N. (1981). Nitrogen metabolism in the

mudskipper, *Periophthalmus cantonensis*: A role of free amino acids in detoxification of ammonia produced during its terrestial life. *Comp. Biochem. Physiol. A* **68**, 589–596.

KORSGAARD, B. (1982). Changes in activity levels of glutamate dehydrogenase and two transaminases of maternal liver during reproduction and after administration of oestradiol and progesterone in *Zoarces viviparus* (L). *J. Fish Biol.* **20**, 445–454.

LARMOYEUX, J. D., AND PIPER, R. G. (1973). Effects of water reuse on rainbow trout in hatcheries. *Progr. Fish. Cult.* **35**, 2–8.

LEVI, G., MORISI, G., COLETTI, A., AND CATANZARO, R. (1974). Free amino acids in fish brain: Normal levels and changes upon exposure to high ammonia concentrations *in vivo*, and upon incubation of brain slices. *Comp. Biochem. Physiol. A* **49**, 623–636.

LUND, P. (1974). Determination with glutaminase and glutamate dehydrogenase. In *Methods of Enzymatic Analysis* (H. U. Bergmeyer, ed.), Vol. IV, pp. 1719–1722. Academic Press, New York/London.

MEHRLE, P. M., AND BLOOMFIELD, R. A. (1974). Ammonia detoxifying mechanisms of rainbow trout altered by dietary dieldrin. *Toxicol. Appl. Pharmacol.* **27**, 355–365.

MITZ, S. V. (1984). *Ammonia Detoxification and Histopathology in Channel Catfish From An In Situ Sewage Treatment Effluent Bioassay*. pp. 1–83. Masters Thesis, Michigan State University, East Lansing.

MITZ, S. V., AND GIESY, J. P. (1985). Sewage effluent biomonitoring. I. Survival, growth, and histopathological effects in channel catfish. *Ecotoxicol. Environ. Safety* **10**, 22–39.

NARINS, R. G., AND PASSONNEAU, J. V. (1974). Fluorimetric determination. In *Methods of Enzymatic Analysis* (H. U. Bergmeyer, ed.), Vol. III, pp. 1580–1584. Academic Press, New York/London.

NORENBERG, M. D., AND MARTINEZ-HERNANDEZ, A. (1979). Fine structure localization of glutamine synthetase in astrocytes of rat brain. *Brain Res.* **161**, 303–310.

ODIN, C. (1981). *Report on the Flood Control and Fish and Wildlife Management Project at Shiawassee Flats, Saginaw County, Michigan*. pp. 45–50. U.S. Fish Wildl. Serv. East Lansing Field Office (ES), East Lansing, Mich.

PALLER, M. H., LEWIS, W. M., HEIDINGER, R. C., AND WAWRONOWICZ, L. J. (1983). Effects of ammonia and chlorine on fish in streams receiving secondary discharges. *J. Water Pollut. Control Fed.* **55**, 1087–1097.

ROSEBOOM, D. P., AND RICHEY, D. L. (1975). *The Acute Toxicity of Chlorine on Bluegill and Channel Catfish in Illinois*. Illinois State Water Survey, Water Quality Section, Peoria.

ROYCRAFT, P. R., AND BUDA, S. G. (1979). *Flint River Study, May 23–24, 1978, August 1–2, 1978*. pp. 1–71. Mich. DNR Environ. Protect. Bur., Environ. Serv. Div. Pub. No. 4833-5762.

SATTERTHWAITE, F. E. (1946). An approximate distribution of estimates of variance components. *Biometrics* **2**, 110–114.

SHOREY, J., MCCANDLESS, D. W., AND SCHENKER, S. (1967). Cerebral α-ketoglutarate in ammonia intoxification. *Gastroenterology* **53**, 706–711.

SMART, G. (1976). The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). *J. Fish. Biol.* **8**, 471–475.

SMITH, C. E., AND PIPER, R. G. (1975). Lesions associated with chronic exposure to ammonia. In *The Pathology of Fishes* (W. E. Ribelin and G. Migaki, eds.), pp. 497–512. Univ. Wisconsin Press, Madison.

STOREY, K. B., GUDERLEY, H. E., GUPPY, M., AND HOCHACHKA, P. W. (1978). Control of ammoniagenesis in the kidney of water- and air-breathing osteoglossids: Characterization of glutamate dehydrogenase. *Canad. J. Zool.* **56**, 845–851.

Subcommittee on Ammonia. (1979). *Ammonia*. pp. 34–44. Univ. Park Press, Baltimore.

THURSTON, R. V., CHAKOUMAKOS, C., AND RUSSO, R. C. (1981). Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*). *Water Res.* **15**, 911–917.

TSAI, C.-F. (1975). *Effects of Sewage Treatment Plant Effluents on Fish: A Review of Literature*. CRC Pub. No. 36, pp. 63–104. Contribution No. 637, Centr. Environ. Estuarine Studies, University of Maryland.

U.S. Environmental Protection Agency. (1976). *Quality criteria for water*. pp. 10–13. Office of Water and Hazardous Materials, U.S. EPA, Washington, D.C.

VAN DEN BERG, C. J., MATHESON, D. F., RONDA, G., REIJNIERSE, G. L. A., BLOKHUIS, G. G. D., KROON, M. C., CLARKE, D. D., AND GARFINKEL, D. (1974). A model of glutamate metabolism in brain: A biochemical analysis of a heterogeneous structure. In *Metabolic Compartmentation and*

Neurotransmission (S. Berl, D. D. Clark, and D. Schneider, eds.), pp. 515–543. Plenum, New York/London.

WILLINGHAM, W. T., COLT, J. E., FAVA, J. A., HILLABY, B. A., HO, C. L., KATZ, M., RUSSO, R. C., SWANSON, D. L., AND THURSTON, R. V. (1979). Ammonia. EPA Criterion. In *A Review of the EPA Red Book: Quality Criteria for Water* (R. V. Thurston, R. C. Russo, C. M. Fetterhoff, Jr., T. A. Edsall, and Y. M. Barber, Jr., eds.), pp. 6–18. Water Quality Section, Amer. Fish. Soc., Bethesda, Md.

WILSON, R. P. (1973). Nitrogen metabolism in channel catfish, *Ictalurus punctatus*. I. Tissue distribution of aspartate and alanine amino-transferases and glutamate dehydrogenase. *Comp. Biochem. Physiol. B* **46**, 617–624.

WILSON, R. P., AND POE, W. E. (1974). Nitrogen metabolism in channel catfish, *Ictalurus punctatus*. III. Relative pool sizes of free amino acids and related compounds in various tissues of the catfish. *Comp. Biochem. Physiol. B* **48**, 545–556.

WITT, I. (1974). Determination with glutamate dehydrogenase and the 3-acetylpyridine analogue of NAD (APAD). In *Methods of Enzymatic Analysis* (H. U. Bergmeyer, ed.), Vol. IV, pp. 1713–1715. Academic Press, New York/London.

WU, C. (1963). Glutamine synthetase. I. A comparative study on its distribution in animals and its inhibition by DL-allo-6-hydroxylysine. *Comp. Biochem. Physiol.* **8**, 335–351.