Walter, W. M., Jr., Purcell, A. E., Hoover, M. W., White, A. G., J. Food Sci. 43, 407 (1978).
Wang, J., Kinsella, J. E., J. Food Sci. 40, 1156 (1975).

Received for review April 10, 1978. Accepted May 30, 1978. Paper No. 5596 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh. Presented at the 174th National Meeting of the American Chemical Society, Chicago, Illinois, August 28-September 2, 1977. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina State University, nor does it imply approval to exclusion of other products that may be suitable.

Uptake, Distribution, and Elimination of the Lampricide 2′,5-Dichloro-4′-nitro[^{14}C]salicylanilide (Bayer 2353) and Its 2-Aminoethanol Salt (Bayer 73) by Largemouth Bass

Donald P. Schultz* and Paul D. Harman

In two groups of largemouth bass exposed to a 0.05-μg/mL mixture of the lampricide 2′,5-dichloro-4′-nitro[^{14}C]salicylanilide (Bayer 2353) and its 2-aminoethanol salt (Bayer 73) for 24 or 144 h, radioactivity was found in all tissues and organs analyzed; the concentration was highest in the gallbladder and lowest in the muscle. The actual amount of ^{14}C material in the various tissues, when calculated as a percentage of the total in each fish, did not change significantly from one sampling interval to the next except for the bile and liver. Concentrations of ^{14}C material in tissues and organs were dependent on the loading rate of the fish (weight per unit volume of water) in the exposure solution. In a third group of fish, exposed to a 0.05-μg/mL mixture of the lampricide for 24 h and then transferred to lampricide-free flowing water for as long as 14 days, the concentration of ^{14}C material decreased with time in all organs and tissues except in the gallbladder bile. There was little biomagnification of lampricide in the edible portion of the fish.

Bayer 73, the 2-aminoethanol salt of Bayer 2353 (2′,-5-dichloro-4′-nitrosalicylanilide), has been widely used in Africa, South America, and the Near and Far East as a molluscicide to control certain species of water snails that serve as intermediate hosts for the trematodes causing schistosomiasis (Gonnert, 1962). In the Great Lakes region of the United States, Bayer 73 has been used to control the parasitic sea lamprey *Petromyzon marinus*. Complete registration for the use of Bayer 73 as a lampricide in the U.S. requires the collection of data concerning its fate in fish and water. Statham and Lech (1975) reported that glucuronide conjugation and biliary excretion was an important metabolic pathway for Bayer 73 in rainbow trout (*Salmo gairdneri*). In the present study we attempted to determine the uptake, distribution, and elimination of a mixture of [^{14}C]Bayer 2353 and Bayer 73 after different periods of exposure in largemouth bass (*Micropterus salmoides*).

MATERIALS AND METHODS

Largemouth bass were exposed to a mixture (hereafter referred to as B73-mix) of ca. 1:8 (w/w) of [^{14}C]Bayer 2353 and Bayer 73 in polyethylene tanks containing 75 L of water (pH 7.3, temperature 13.5 ± 1.0 °C). Constant water bath temperature was maintained with a chilling unit, and oxygen content of the treatment solution was sustained by aeration.

Stock solutions of [^{14}C]Bayer 2353 (uniformyl labeled in the chlorosalicylic acid ring, sp act. 10 mCi/mmol, American Radiochemical Corporation, Sanford, Fla.) and technical grade Bayer 73 (96% pure) in methanol were used to prepare the 0.05-μg/mL (50 ppb) treatment solutions.

Samples of three fish each were removed from the treatment solution at 2, 4, 8, 12, and 24 h in the 24-h uptake experiment (24-UP) and at 4, 24, 48, 72, and 144 h in the 144-h uptake experiment (144-UP). For the elimination experiment, fish were exposed in the treatment solution for 24 h and then placed in lampricide-free, flowing water (pH 6.8, temperature 14.0 ± 1.0 °C). The fish were not fed during the experimental time period. Samples of three fish each were then taken immediately after treatment and at 1, 3, 7, 10, and 14 days thereafter. Fish used in the three experiments had the following average total lengths (cm) and weights (g): 24-UP, 18.7 and 79; 144-UP, 25.0 and 213; and elimination, 20.4 and 123.

At each sampling period in each experiment, the head, viscera, bile, and following tissues were taken: muscle, blood, brain, spleen, liver, and kidney. Head and viscera from each fish were pooled. Blood samples were collected by caudal puncture with a hypodermic syringe. Blood and spleen were digested and decolorized in a scintillation vial by successive additions of 0.4 mL of 60% perchloric acid followed by 0.8 mL of 30% hydrogen peroxide. The vial was capped and incubated at 70–75 °C until the yellow color dissipated. Scintillation cocktail (10 mL, Scintiverse, Fisher Scientific Co., Pittsburgh, Pa.) was then added to each sample. Samples (ca. 100 mg) of muscle, brain, liver, and kidney were dissolved in 1–2 mL of a tissue solubilizer (Unisol, Isolab Inc., Akron, Ohio). Anhydrous methanol (0.5 mL) and scintillation cocktail (10–15 mL, Complement, Isolab Inc.) were then added to each sample.

Samples of 500 mL each from the treatment solutions were acidified with concentrated H_2SO_4 to pH 1.5–2.0 and

U.S. Department of the Interior, Fish and Wildlife Service, Southeastern Fish Control Laboratory, Warm Springs, Georgia 31830.

Table I. Average Concentrations (±SE) of ^{14}C Material in Exposure Water (μg/mL) and in Bile and Tissues (μg/g) from Largemouth Bass Exposed to a 0.05-μg/mL Mixture of [^{14}C]Bayer 2353 and Bayer 73 (1:8, w/w) for 2-24 h[a]

exposure time, h	sample								
	blood	brain	spleen	liver	kidney	bile	muscle	HV[b]	water
2	2.74	0.51	0.14	14.27	2.92	5.52	0.18	0.45	[c]
	(0.037)	(0.125)	(0.034)	(2.65)	(0.221)	(0.73)	(0.029)	(0.021)	
4	6.60	0.62	0.60	9.18	5.93	67.43	0.36	0.06	0.035
	(0.110)	(0.052)	(0.227)	(2.79)	(0.804)	(20.69)	(0.019)	(0.032)	
8	9.50	0.65	0.40	11.30	7.26	127.92	0.40	1.15	0.030
	(0.238)	(0.091)	(0.054)	(1.81)	(0.973)	(27.21)	(0.053)	(0.082)	
12	11.38	0.77	0.49	13.33	7.24	317.52	0.66	1.43	0.028
	(0.350)	(0.068)	(0.056)	(2.21)	(1.08)	(113.51)	(0.144)	(0.096)	
24	15.71	0.92	1.09	12.41	9.34	411.25	0.67	2.62	0.024
	(0.117)	(0.015)	(0.232)	(5.69)	(1.41)	(199.51)	(0.054)	(0.171)	

[a] Each value is the average for three fish. [b] HV = head plus viscera. [c] No sample taken.

Table II. Average Concentrations (±SE) of ^{14}C Material in Exposure Water (μg/mL) and in Bile and Tissues (μg/g) from Largemouth Bass Exposed to a 0.05-μg/mL Mixture of [^{14}C]Bayer 2353 and Bayer 73 (1:8, w/w) for 4-144 h[a]

exposure time, h	sample								
	blood	brain	spleen	liver	kidney	bile	muscle	HV[b]	water
4	0.43	0.09	0.33	0.97	1.00	7.14	0.06	0.14	0.025
	(0.045)	(0.012)		(0.239)	(0.138)	(2.10)	(0.002)	(0.001)	
24	1.40	0.16	0.41	1.12	2.08	44.36	0.13	0.37	0.014
	(0.361)	(0.038)	(0.091)	(0.062)	(0.074)	(1.64)	(0.008)	(0.030)	
48	2.32	0.22	0.74	1.32	3.20	58.91	0.25	0.72	0.004
	(0.168)	(0.010)	(0.355)	(0.021)	(0.141)	(9.17)	(0.020)	(0.054)	
72	3.17	0.38	0.80	1.93	3.63	63.62	0.29	0.93	0.005
	(0.546)	(0.031)	(0.426)	(0.876)	(0.332)	(21.97)	(0.026)	(0.047)	
144	4.15	0.44	1.73	2.32	4.95	141.94	0.35	1.14	0.006
	(0.438)	(0.040)	(0.568)	(0.359)	(0.585)	(11.03)	(0.028)	(0.041)	

[a] Each value is the average for three fish. [b] HV = head plus viscera.

extracted in a separatory funnel with three volumes (50 + 25 + 25 mL) of hexane–ethyl ether (50:50, v/v). From each extract, 5-mL portions were evaporated to about 0.5 mL; 10 mL of scintillation cocktail (TLA, Beckman Inc., Fullerton, Calif.) were added; and the radioactivity was determined.

Radioactivity was measured in a Beckman LS-230 liquid scintillation spectrometer. Each sample was counted at least three times for 10 min or longer each time. All radioactivity data were corrected for background, quench, and machine efficiency and were converted to μg/g or μg/mL on the basis of the specific activity of the [^{14}C]-B73-mix.

RESULTS

24-h Uptake Experiment. The concentration (μg/g) of ^{14}C material increased at each sampling period in blood, brain, bile, muscle, head–viscera, and (with one exception) kidney (Table I). Radioactivity in the spleen was variable at 4, 8, and 12 h but more than doubled from 12 to 24 h. Variability was greatest in the liver: The concentration of radioactivity peaked at 14.27 μg/g at 2 h, dropped to 9.18 μg/g after 4 h, and then remained static from 8 to 24 h. At 24 h the concentration of radioactive material in various tissues ranged from a low of 0.67 μg/g in the muscle to a high of 411.25 μg/g in the bile.

Bayer 73 is used for selective killing of the sea lamprey by treating rivers which flow into the Great Lakes. Since other fish in the rivers are not killed by the lampricide, they may be harvested for human consumption. Hence it is essential to know the amount of Bayer 73 an individual might ingest by eating fish from a treated river. Therefore we determined the total amount of radioactive material in the fish by multiplying the concentration found in each tissue or organ by the weight of the individual tissues or organs and totaling the individual amounts. The highest amount was found in the head–viscera at all time intervals (Figure 1). Bile, muscle, blood, and liver contained less radioactivity than head–viscera, but much more than the brain, spleen, or kidney, which never contained as much as 5 μg total radioactive material.

The concentration of B73-mix in the exposure solution dropped from an initial concentration of 0.050 to 0.024 μg/mL during the 24-h experiment due (as shown later) to uptake by the fish.

The 144-h Uptake Experiment. The concentration of ^{14}C material increased in all tissues at each sampling period (Table II). Concentrations of radioactive material at 144 h ranged from a low of 0.35 μg/g in the muscle to a high of 141.94 μg/g in the bile. Concentrations (μg/g) in other tissues were as follows: brain, 0.44; head–viscera, 1.14; spleen, 1.73; liver, 2.32; blood, 4.15; and kidney, 4.95.

We again determined the total amount of radioactive compounds (μg) in the fish by calculating the individual tissue totals (Figure 2). After 144 h of exposure, the head–viscera contained 38% (275 μg) of the radioactive materials, muscle 23% (165 μg), blood 20% (144 μg), and bile 15% (112 μg). Brain, spleen, and kidney contained no more than 1% of the total amount of radioactive compounds.

Elimination Experiment. The concentration of radioactive material (μg/g) decreased progressively in the spleen, kidney, muscle, and (with one exception) head–viscera and blood throughout the experiment (Table III). Concentrations of radioactive materials in the brain generally decreased with time; however, in the liver they decreased from 0 to 7 days, but increased from 10 to 14 days. Except for the 1-day sample, concentrations of radioactive material in the bile were not greatly different throughout the experiment. All gallbladders were full of

Table III. Average Concentrations (μg/g; ±SE) of ^{14}C Materials in Bile and Tissues of Largemouth Bass Exposed to a 0.05-μg/mL Mixture of [^{14}C]Bayer 2353 and Bayer 73 (1:8, w/w) for 24 h and then Transferred to Lampricide-Free, Flowing Water for up to 14 Days[a]

withdrawal interval, days	sample							
	blood	brain	spleen	liver	kidney	bile	muscle	HV[b]
0[c]	4.92	0.54	0.80	3.67	4.44	241.53	0.39	1.10
	(0.26)	(0.120)	(0.129)	(1.383)	(0.686)	(162.2)	(0.051)	(0.026)
1	4.75	0.62	0.59	1.97	3.59	126.67	0.31	1.12
	(0.31)	(0.119)	(0.135)	(0.290)	(0.293)	(27.4)	(0.032)	(0.107)
3	3.56	0.44	0.45	1.23	3.31	293.31	0.25	0.82
	(0.77)	(0.088)	(0.045)	(0.124)	(0.514)	(142.8)	(0.039)	(0.038)
7	3.68	0.37	0.45	0.90	3.15	273.13	0.24	0.69
	(0.20)	(0.011)	(0.016)	(0.202)	(0.124)	(49.3)	(0.020)	(0.016)
10	3.25	0.16	0.32	1.34	2.78	270.82	0.18	0.68
	(0.23)	(0.004)	(0.034)	(0.598)	(0.093)	(108.5)	(0.010)	(0.032)
14	2.69	0.24	0.18	1.59	2.67	249.03	0.16	0.53
	(0.18)	(0.020)	(0.042)	(0.034)	(0.357)	(58.6)	(0.012)	(0.036)

[a] Each value is the average for three fish. [b] HV = head plus viscera. [c] Time at which fish were removed from treatment solution. Concentration of ^{14}C material in exposure solution at this time was 0.013 μg/mL.

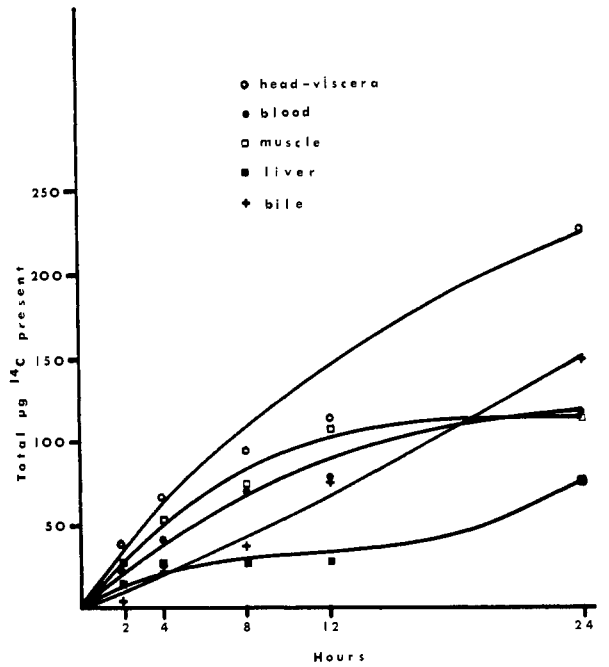

Figure 1. Total amount (μg) of radioactive material found in bile and tissues of largemouth bass exposed to a 0.05-μg/mL mixture of [^{14}C]Bayer 2353 and Bayer 73 (1:8, w/w) for up to 24 h (amounts found in brain, spleen, and kidney were too low to be graphed).

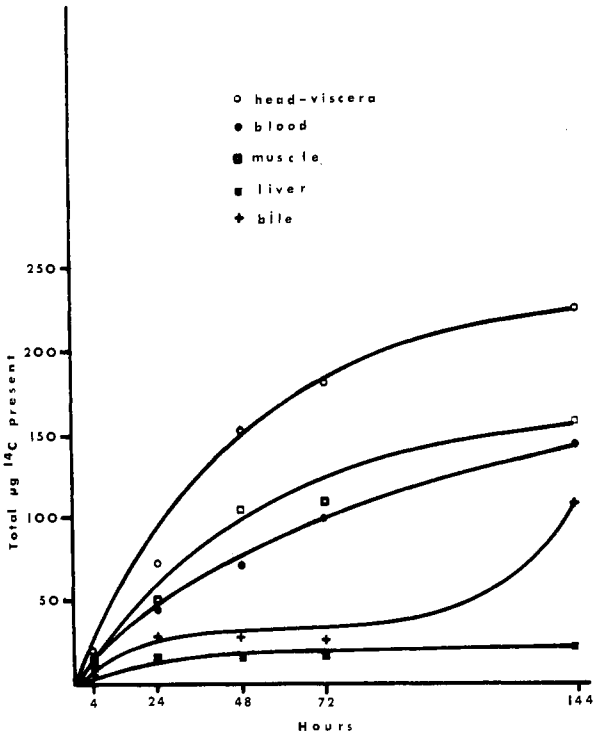

Figure 2. Total amounts (μg) of radioactive material found in bile and tissues of largemouth bass exposed to a 0.05-μg/mL mixture of [^{14}C]Bayer 2353 and Bayer 73 (1:8, w/w) for up to 144 h (amounts found in brain, spleen, and kidney were too low to be graphed).

bile. Since the fish were not fed during the experiment, they evidently retained the bile.

The total amounts of radioactive compounds (μg) were highest in the bile, followed by the head–viscera, blood, muscle, and liver (Figure 3).

The concentration of B73-mix in the treatment solution decreased from the initial 0.05 to 0.013 μg/mL during the 24-h exposure period.

DISCUSSION

We accounted for 101, 94, and 112% of the initial B73-mix from the three experiments (Table IV). The fish contained 48, 80, and 73% of the total radioactivity recovered during the 24-UP, 144-UP, and elimination experiments, respectively. The remaining radioactivity was found in the exposure solutions.

In the two uptake experiments, the total amount of ^{14}C material (μg) in the various tissues, as a percentage of the total uptake, did not vary greatly from one sampling interval to the next. For example, in the 24-h study, the percentages of the total content of radioactivity at 2, 4, 8, 12, and 24 h were 20, 18, 23, 19, and 17 for the blood at each successive interval and 34, 31, 30, 28, and 33 for the head–viscera. In the 144-UP experiment, the values at 4, 24, 48, 72, and 144 h were 16, 21, 19, 22, and 20% for blood, and 32, 32, 39, 40, and 38% for the head–viscera. These values indicate that there was little movement of radioactive material from one tissue to another. There were two exceptions to this pattern—in the bile and the liver. At the five successive sampling intervals of each experiment, the bile had values of 3, 9, 12, 18, and 22% in the 24-UP study and 5, 15, 9, 7, and 15% in the 144-UP study. The high concentrations of ^{14}C materials in the bile correlate well with the biliary concentration mechanisms observed by other workers (Rodgers and Stalling, 1972; Schultz,

Table IV. Total B73-Mix (μg) Found in Fish and Water from Three Experiments[a]

source	experiment 24-h uptake	144-h uptake	elimination
fish[b]	1722	1862	3097[c]
water	1864	712	1144
fish plus water	3586	3538[d]	4241
initial	3563	3755	3791
recovered, %	101	94	112

[a] B73-mix = [^{14}C]Bayer 2353 + Bayer 73 (1:8, w/w). [b] From Figure 1-3. [c] This value is not found in Figure 3. It was derived by dividing the total μg at 0 days (442.47) by three to obtain the average μg per fish and multiplying this value (147.49) by the total number of fish (21) in the experiment. The three extra fish were removed at the 14-day sampling period for residue analyses; values for them were not included in Table III or Figure 1. [d] This total includes values for extra fish which were removed at several time intervals for separate analyses and not included in Figure 1.

1973; Statham and Lech, 1975; Schultz and Harman, 1976; and Statham et al., 1976). In contrast to the bile, the liver had values at the successive sampling intervals of 14, 13, 10, 8, and 11% in the 24-UP experiment, and 15, 8, 5, 4, and 3% in the 144-UP experiment.

In the elimination experiment, radioactive content and percentage of total uptake decreased with time in most tissues. This decrease would be expected since the fish were in lampricide-free water. The major exception to the decreasing trend was in the bile. We found a total content of 86, 84, 58, 94, 139, and 153 μg at 0, 1, 3, 7, 10, and 14 days (Figure 3), whereas the percentage of the total uptake was 19, 22, 18, 33, 40, and 48%, at the same time intervals. Thus, when the fish were no longer exposed to the lampricide, they eliminated the radioactive material from most tissues but concentrated it in the bile.

Statham et al. (1976) reported a biliary concentration factor of 10 100 (^{14}C material in bile:^{14}C material in water) in rainbow trout after a 24-h exposure to a 0.05-μg/mL solution of [^{14}C]Bayer 2353. We found ratios of 8200, 900, and 5000 in the 24-UP, 144-UP, and elimination studies, respectively.

Statham and Lech (1975), who exposed rainbow trout to a 0.05-μg/mL solution of [^{14}C]Bayer 2353 for 12 h, reported values of 4.2, 13.4, and 0.08 μg/g in the blood, liver, and muscle, respectively. In our three experiments (24-UP, 144-UP, and elimination), we found values at 24

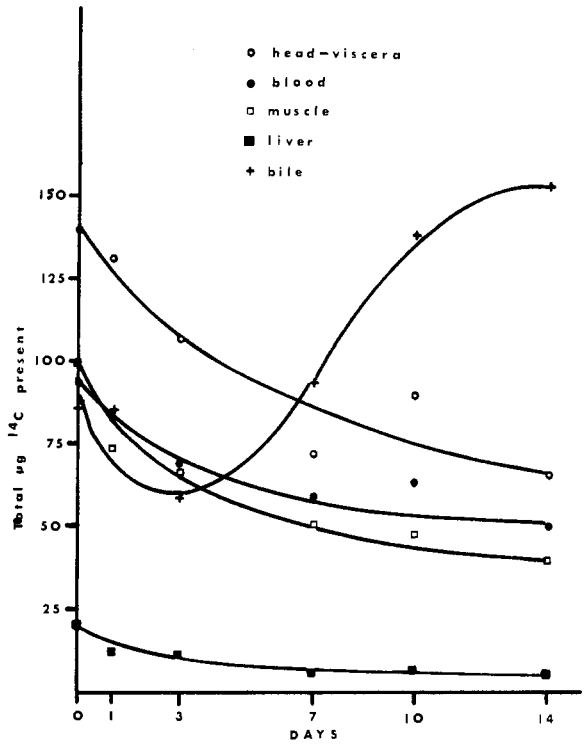

Figure 3. Total amounts (μg) of radioactive material found in bile and tissues of largemouth bass exposed to a 0.05-μg/mL mixture of [^{14}C]Bayer 2353 and Bayer 73 (1:8, w/w) for 24 h and then transferred to lampricide-free, flowing water for up to 14 days (amounts found in brain, spleen, and kidney were too low to be graphed).

h of 15.7, 1.4, and 4.9 μg/g in blood; 12.4, 1.12, and 3.67 μg/g in liver; and 0.67, 0.13, and 0.39 μg/g in muscle. To explain these differences, we calculated the micrograms of B73-mix taken up and remaining in the fish (Table V). One obvious difference was in the loading factor (i.e., weight of fish per unit of solution) in the three experiments. We used 1181, 3202, and 2507 g of fish in the 24-UP, 144-UP, and elimination studies. At these loading rates we had ratios (μg of B73-mix:g of fish) of 3.02, 1.17, and 1.50 for the corresponding studies. In addition to the higher initial ratio in the 24-UP study, as fish were removed at the successive sampling intervals, the ratio increased so that after the 12-h sample the ratio was 8.13. Thus, there were 8.13 μg of available B73-mix in the treatment solution per gram of fish remaining in the 24-UP

Table V. Amount of B73-Mix (μg) Absorbed by Fish during Specified Exposure Periods and the Amount of B73-Mix Left per Gram of Fish Remaining after Each Sampling Time[a]

experiment and time interval, h	B73-mix absorbed, μg/g of fish	weight of fish, g	μg of B73-mix taken up	μg of B73-mix remaining	weight of fish remaining, g	μg of B73-mix remaining/g of fish remaining
24-h uptake						
0				3563	1181	3.02
2	0.45	1181	531	3032	937	3.24
4	1.03	937	543	2489	727	3.42
8	1.28	727	182	2307	491	4.70
12	1.74	491	226	2081	256	8.13
24	2.67	256	238	1843	0	
144-h uptake						
0				3755	3202	1.17
4	0.14	3202	448	3307	2622	1.26
24	0.34	2622	524	2783	1949	1.43
elimination						
0				3791	2507	1.51
24	1.15	2507	2883	908	2123	0.43

[a] B73-mix = [^{14}C]Bayer 2353 + Bayer 73 (1:8, w/w).

study whereas there were only 1.43 and 0.41 μg/g of fish after 24 h in the 144-UP and elimination studies. We believe that the differences in concentrations between the three studies are correlated with the differences in loading ratios and the rate at which the fish were removed from the treatment solutions.

Statham and Lech (1975) reported that [^{14}C]Bayer 2353 was taken up and metabolized by rainbow trout to a glucuronide. We found that after β-glucuronidase treatment of bass bile, the ether extracts contained a compound which had the same R_f as B73-mix and co-chromatographed with it. We were unable to perform spectral analyses because the quantity of the compound available for study was extremely limited, thus we cannot prove the presence of a glucuronide in bass bile, but presume this is the case, based on the work of Statham and Lech (1975).

We also attempted to quantify the amount of B73-mix in the fish muscle by gas chromatography. Although a precise method has not been developed, we ascertained that 60 to 80% of the radioactive materials found in muscle tissue consisted of B73-mix. This finding was confirmed by thin-layer chromatography. We did not analyze other tissues or organs because of the inadequacies of the method.

Our experiments show that B73-mix is taken up and distributed throughout the various organs and tissues of fish; that the bile contained the greatest concentration of B73-mix and that the highest concentration of B73-mix was found in fish from the experiment which had the lowest loading rate (24-UP).

LITERATURE CITED

Gonnert, R., *Pflanzenschutz-Nachr.* **15**, 4–24 (1962).
Rodgers, C., Stalling, D. L., *Weed Sci.* **20**, 101–105 (1972).
Schultz, D. P., *J. Agric. Food Chem.* **21**, 186–192 (1973).
Schultz, D. P., Harman, P. D., *J. Fish. Res. Board Can.* **33**, 1121–1129 (1976).
Statham, C. N., Lech, J. J., *J. Fish. Res. Board Can.* **32**, 515–522 (1975).
Statham, C. N., Melancon, M. J., Jr., Lech, J. J., *Science* **193**, 680–681 (1976).

Received for review March 6, 1978. Accepted June 6, 1978. Reference to brand names are for identification only and do not imply endorsement by the U.S. Department of the Interior, Fish and Wildlife Service.

Nutritive Value of the Nitrogen-Fixing Aquatic Fern *Azolla filiculoides*

Kent W. Buckingham,*[1] Stephen W. Ela, James G. Morris, and Charles R. Goldman

Azolla filiculoides had a low nutritive value for growing rats when given as the sole source of protein in the diet. The addition of lysine, methionine, and histidine gave a marked increase in rat growth and PER. The high neutral detergent fiber of *Azolla* (39%) is a major limiting factor for the efficient utilization of *Azolla* as a protein source for simple-stomached animals. In vitro digestability data showed that *Azolla* was readily degraded by rumen microorganisms and indicated its possible usefulness as a protein source for ruminants.

Floating fresh water ferns of the genus *Azolla* have a wide geographic distribution (Ashton and Walmsley, 1976; Stewart and Pearson, 1970). Several species of *Azolla* are cultivated in Southeast Asia as a green manure to fertilize rice paddies and as food for animals (Moore, 1969). Current interest in *Azolla* arises from its symbiotic relationship with a nitrogen-fixing blue-green algae, genus *Anabaena*. The algae live in leaf cavities of *Azolla* and are capable of using their own photosynthetic energy to reduce atmospheric nitrogen and produce ammonia which can be used by the fern to meet all its nitrogen requirements (Ashton and Walmsley, 1976; Newton and Cavins, 1976; Marx, 1977). This attribute is of considerable potential importance to agricultural regions with inadequate energy for the production of synthetic nitrogen fertilizers (Marx, 1977). This symbiotic relationship also permits the fern to be relatively independent of fixed nitrogen in its environment. Thus *Azolla* tends to be high in nitrogen and is a potentially attractive source of protein for animal rations.

A tropical species of *Azolla* has reportedly been used as a feed for pigs and ducks in Indochina; for cattle, fish, and poultry in Vietnam; and for pigs in Singapore and Formosa (Moore, 1969). The North Vietnamese (Thuyet and Tuan, 1973) describe *Azolla* as an excellent substitute for green forage for cattle and suggest that it may replace 50% of the rice bran used as feed for pigs. They also reported that the crude protein of *Azolla* was 13% of dry matter and that lysine and tryptophan were low compared to rice protein. Fujiwara et al. (1947) reported 23.8% crude protein in *Azolla*. However, no substantiating reports of complete proximate analysis of *Azolla* could be found in the literature. Data from animal feeding trials in which *Azolla* was compared to common foodstuffs are also lacking. This information is requisite to the consideration of *Azolla* as a feedstuff, and so the present study was undertaken to determine the chemical composition of *Azolla*; compare the growth of weanling rats given protein from *Azolla* and casein; determine the effect of the fiber and mineral components in *Azolla* on its utilization by rats; determine the effect of supplementation of *Azolla* with lysine, methionine, and histidine in rat rations; and

Department of Nutrition (K.W.B.), Division of Environmental Studies (S.W.E., C.R.G.), and the Department of Animal Science (J.G.M.), University of California, Davis, California 95616.

[1]Present address: The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

0021-8561/78/1426-1230$01.00/0