

## Laboratory Preparation of a Protein-Xanthophyll Concentrate from Sweet Potato Leaves

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Utilization of sweet potato leaves as a source of vegetable protein and xanthophyll pigment was evaluated for 2 years. Protein-xanthophyll concentrates were prepared on a laboratory scale from leaves of Jewel and Centennial cultivars during the normal growing season of 130–145 days. The leaves were pulped and blended with water, and the resulting juices were expressed in a hydraulic press. The juice was heated to 83 °C, cooled, and centrifuged. Depending upon the age of the plant, from 32–49% of the total crude leaf protein ( $N \times 6.25$ ) was removed and recovered in the pellet. Dried leaf protein concentrate contained 35–45% protein and 0.12–0.15% xanthophyll. The amino acid pattern of the protein compared favorably with the FAO reference, although slightly deficient, possibly, in total sulfur amino acids. The amino acid composition did not change significantly with the age of the plant. There was no evidence of a xanthophyll-destroying enzyme system in the leaves.

Sweet potatoes produce a lush foliage in addition to the commercially valuable roots. After the roots are harvested, the above-ground portion of the plant is returned to the soil. This practice results in the underutilization of a potential source of high-quality protein and xanthophyll pigments. Studies with dehydrated sweet potato vine meal (Garlich et al., 1974) have shown that this material can be used in poultry rations both as a source of protein and as a pigmentation agent for egg yolks and broiler skins. However, high fiber content and low xanthophyll levels of the crop at harvest (ca. 130–140 days after planting) limit its usefulness. Preparation of a protein-xanthophyll concentrate (PXC) from the leaves should result in a product containing low fiber, high protein, and high xanthophyll pigment levels.

During the last two decades, a great deal of research activity has occurred in the area of extraction of leaf protein from various sources. Pirie and co-workers (1971) published results of their work on agronomy, nutritional value, and extraction methods for leaf protein concentrate and described large-scale extraction and processing methods. The primary purpose of the concentrate is to supplement human foods. Workers at the U.S. Department of Agriculture, Western Regional Research Laboratory (Kohler et al., 1968; Spencer et al., 1970; and Edwards et al., 1975) have developed two processes for preparation of LPC. In the first process, the expressed plant juice is heated to coagulate the protein, thus providing a concentrate suitable for feeding nonruminants. In the second process, the plant juices are separated prior to heat coagulation. As a result, a green concentrate suitable for supplementing poultry rations and a white protein concentrate which could be incorporated into human food are produced. Byers (1961) reported on the extraction of protein from 60 tropical plant species growing in Ghana. Sweet potato leaves were included in the study, but very little information was presented.

Many of the states in the southeast import dehydrated alfalfa for use in poultry rations. About 80 000 acres of sweet potatoes are grown in this region, and, if properly handled, the foliage could possibly be used instead of alfalfa. This study was conducted to provide information on the preparation and properties of leaf protein extract from the two major sweet potato cultivars produced in the

southeastern United States.

### EXPERIMENTAL SECTION

Sweet potatoes (Centennial cultivar) grown on a commercial farm in northern Wake County, North Carolina, were used for the first year of study; and Jewel cultivar from the North Carolina Agricultural Experiment Station farm (Johnston County) was used for the second year. Recommended cultural practices were followed both years. Six replicate samples (~500 g each) of leaves (only) from different locations within the plots were hand harvested around 8:00 a.m. at intervals throughout the growing season and then transported directly to the laboratory for analysis.

Yields of the Jewel cultivar were measured on six replicates of plant material at 120 and 135 days after planting. For these determinations, leaves, stems, and petioles were harvested separately by hand, weighed, and analyzed.

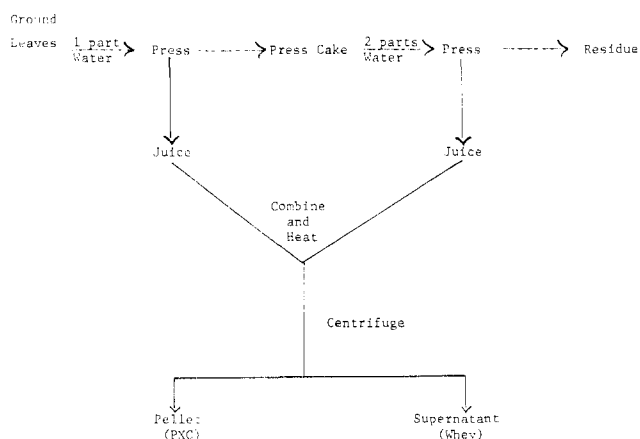
Two 75-g samples of leaves from each replicate were removed. One sample was held for 24 h at 98 °C in a forced draft oven and dry matter was determined from the moisture loss. Crude protein content was then determined on the dried, ground leaves (Kjeldahl nitrogen  $\times 6.25$ ).

The other 75-g sample was ground with an Oster Food Grinder, equipped with a meat grinder attachment, and mixed with 75 g of H<sub>2</sub>O. The mixture was placed in cheesecloth and pressed for 5 min at 1000 psi in a hydraulic press to express the juice. The mat was removed, mixed with 150 g of H<sub>2</sub>O and again pressed. The juice samples were combined, heated to 83 °C, cooled to room temperature, and centrifuged at 16 300g for 10 min. The supernatant and pellet (PXC) were weighed. Aliquots of the supernatant were removed, and the crude protein content was measured (Kjeldahl nitrogen  $\times 6.25$ ). Portions of the PXC were removed for dry matter and xanthophyll determinations. The remainder was freeze-dried.

Freeze-dried PXC from several harvest dates were examined for protein, fat, ash, and fiber levels (proximate analysis) according to the AOAC methods (1970). In addition, PXC was hydrolyzed with 6 N hydrochloric acid (Walter, et al., 1978), and amino acid levels were determined.

For measurement of xanthophyll concentration, 3.00 g of freshly prepared PXC coagulum was mixed with 5 mL of methanol and exhaustively extracted with hexane-acetone (1:1). The extract was evaporated in vacuo, and the residue was mixed with 10 mL each of methanol and saturated alcoholic sodium hydroxide. The flask with the residue was flushed with nitrogen gas, capped, and stored

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**Figure 1.** Schematic diagram for preparation of sweet potato leaf protein-xanthophyll concentrates (PXC).

in the dark for 1.5 h. The mixture was transferred to a separatory funnel with peroxide-free ethyl ether, followed by water. After phase separation had occurred, the lower, aqueous layer was discarded and the upper layer washed with equal volumes of water until it was no longer basic. The upper layer was retained and evaporated *in vacuo*. The pigments were then dissolved in hexane, diluted to 100 mL with hexane, and dried over anhydrous sodium sulfate. The total carotenoid concentration was determined spectrophotometrically (Purcell and Walter, 1968). An aliquot of the total carotene solution was then separated on a silica gel-methanol partition column (Purcell, 1958), and the relative amounts of carotenoids and xanthophylls were determined spectrophotometrically. The xanthophyll concentration was obtained by multiplying the total carotenoid concentration by the xanthophyll percent composition. All of the above operations were performed in subdued light.

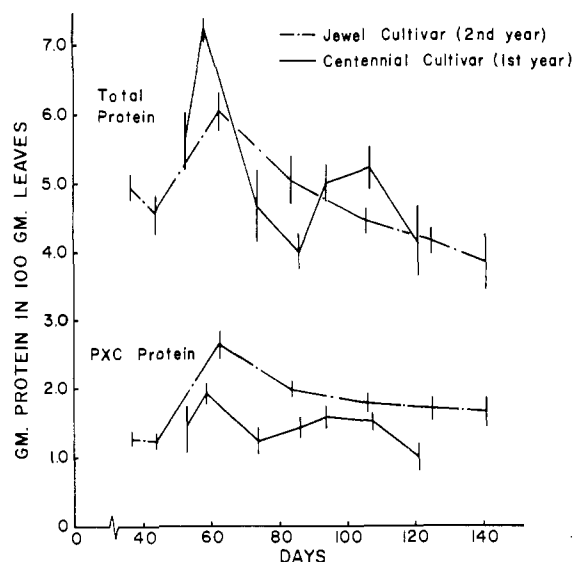
In order to evaluate the effect of holding the extracted juice for extended periods, samples of juice (150 mL) freshly expressed from leaves were held in a covered water bath at 30, 40, and 50 °C for 6 h. Portions of each sample were removed at 1, 2, 4, and 6 h, heated to 83 °C, cooled, and centrifuged. The PXC and supernatant were analyzed for crude protein (Kjeldahl nitrogen  $\times$  6.25). The xanthophyll content of the pellet was also measured. The protein and xanthophyll concentrations of the PXC from each storage time were compared to those of juice which was heated to 83 °C and analyzed immediately after it was prepared (zero time).

#### RESULTS AND DISCUSSION

Sweet potato leaves contain a sticky, mucilaginous substance which very efficiently binds water and prevents the direct extraction of juice from the ground leaves. However, it was found that protein and xanthophyll could be efficiently extracted by grinding the leaves, mixing with an equal weight of water, pressing out the juice, and then extracting the press-cake with two parts water (Figure 1).

The juice pH was 5.9–6.1 depending upon the sample. A series of extractions were run using pH values of 2.0–11.0. It was found that more protein was extracted at the higher pH but that the differences were not great enough to warrant the additional operational steps which would be required to extract at the higher pH and then readjust the extract to a pH optimum for precipitation. Consequently, for this report, leaves were extracted without pH adjustment.

Since the cultivars used in this study were grown at different locations in different years, it is not proper to draw conclusions concerning similarities and differences



**Figure 2.** Total and protein-xanthophyll concentrate (PXC) leaf protein levels during the growing season for two sweet potato cultivars. Each data point represents the mean and standard deviation from six replicate samples.

**Table I.** Proximate Analyses of PXC from Jewel Leaves

component	time after planting (days)	
	77 <sup>a</sup>	133 <sup>a</sup>
protein	51.73	36.57
fiber	5.96	9.59
ash	5.50	5.58
fat	6.86	6.42
"carbohydrate" <sup>b</sup>	29.95	41.84

<sup>a</sup> Mean of two analyses; percent composition. <sup>b</sup> By difference.

in the extracts. However, if this fact is kept in mind, it is appropriate to compare the properties of the leaves and extracts of both cultivars.

Mean leaf dry matter content for Centennial and Jewel cultivars for the entire growing season was 18.28 and 16.11 g/100 g of leaves (fresh weight), respectively. The standard deviations were 5.96% for Centennial and 9.93% for Jewel. There was no statistically significant interaction between dry matter content and harvest date. The protein levels in the leaves of both Centennial and Jewel cultivars reached a maximum at about 60 days after planting (Figure 2) and then decreased during the remainder of the growing season. However, the trend in Centennial was much more erratic than that in Jewel. Protein content of the leaves was calculated from Kjeldahl nitrogen ( $\times$  6.25) and thus included all nitrogenous material. Nitrogen extracted into the juice was found to be 20–25% non-heat precipitable (NHP) for both cultivars. Depending upon the age of the plant, from 32–49% of the total leaf crude protein was recovered in the heat precipitated pellet (PXC protein). The amount of protein isolated in the PXC for both cultivars decreased after the maximum was reached at around 60 days. Generally, PXC protein varied directly with the total protein of the leaves. Since protein levels in many leaf species have been shown to decrease as the plant ages (Pirie, 1971), this finding for sweet potato leaves was not surprising.

Protein levels in dried PXC were 47–51% for the first 80 days of the growing season (Figure 3), then decreased to values between 34 and 37%. Proximate analyses of PXC (Table I) showed that after 133 days, as compared to 77 days, the fiber values had almost doubled while the

Table II. Xanthophyll and Dry Matter of PXC<sup>a</sup> from Jewel Leaves

time <sup>b</sup>	PXC weight <sup>c</sup>	% xanthophyll <sup>d</sup>
37	2.78	0.13
63	5.73	0.13
84	4.59	0.14
106	4.60	0.14
126	4.82	0.15
141	4.84	0.12

<sup>a</sup> Protein xanthophyll concentrate from 100 g of leaves. <sup>b</sup> Time in days after planting. <sup>c</sup> Dry weight (g) of PXC from 100 g of leaves. <sup>d</sup> In PXC (dry basis); six replicate analyses.

Table III. Yield Data<sup>a</sup> from the Above-Ground Portion of Jewel Sweet Potato

plant part	time (days) <sup>b</sup>	
	113	151
leaves	4474 (34.0) <sup>c</sup>	5172 (30.9) <sup>c</sup>
petioles	5019 (38.1)	5893 (35.3)
stems	3680 (27.9)	5652 (33.8)

<sup>a</sup> Yield in pounds per acre (six replications). <sup>b</sup> Time after planting. <sup>c</sup> Percent of total weight.

protein content had decreased. These results may indicate that less protein was available for extraction in the later stages of growth; but certainly less protein was present in the leaves (Figure 2). Ash and fat contents were similar at both sampling periods; consequently, the level of non-assayed components—carbohydrate and possibly other undefined substances—was greater for the later assay.

If the concentrate is to be used to supplement broiler rations, its xanthophyll content would be of considerable importance. The xanthophyll (XAN) extracted (Figure 4) increased during the first part of the growing season and then, like protein content, decreased with maturity of the leaves. XAN content varied considerably, particularly toward the end of the season. For example, XAN levels ranged from 4.51 to 8.07 mg extracted from 100 g of leaves on day 141. The variations were attributed to the wide range of leaf maturities at each harvest date. Nevertheless, the XAN content of the dry PXC (Table II) was greater than 0.1% regardless of harvest date; thus, the extraction process removed sufficient pigment to maintain high levels. One of the main objections to using dehydrated sweet potato vine meal was due to the fact that XAN had decreased significantly by the normal harvest date of 135 days. Preparation of the PXC makes it possible to obtain a product with a high level of pigment.

We have yield data on sweet potato leaves for 1 year only (Table III); however, our total yield of green matter is similar to that reported by Covington and Chamblee (1971) (12000 lbs/acre). Using our value of 5172 lbs of leaves/acre and a dry PXE yield of 0.0471 lbs/lb of fresh weight of leaves (Table II), we calculated the yield of PXC/acre of leaves at harvest to be 244 lbs and that it contained 89.5 lbs of protein and 0.34 lbs of xanthophyll.

Table IV. Dry Matter and Protein Content<sup>a</sup> of the Above-Ground Portion of Jewel Sweet Potato

plant part	harvest date <sup>b</sup>			
	113		151	
	dry matter <sup>c</sup>	crude protein <sup>c,d</sup>	dry matter <sup>c</sup>	crude protein <sup>c,d</sup>
leaves	16.08 (±1.02)	3.56 (±0.17)	16.90 (±1.71)	3.63 (±0.44)
petioles	7.83 (±0.90)	0.60 (±0.13)	7.57 (±0.61)	0.43 (±0.06)
stems	13.68 (±1.06)	1.07 (±0.16)	12.51 (±1.92)	1.22 (±0.39)

<sup>a</sup> Grams in 100 g of fresh material. Six replicate samples. <sup>b</sup> Days after planting. <sup>c</sup> Numbers in parentheses are standard deviations. <sup>d</sup> Kjeldahl nitrogen × 6.25.

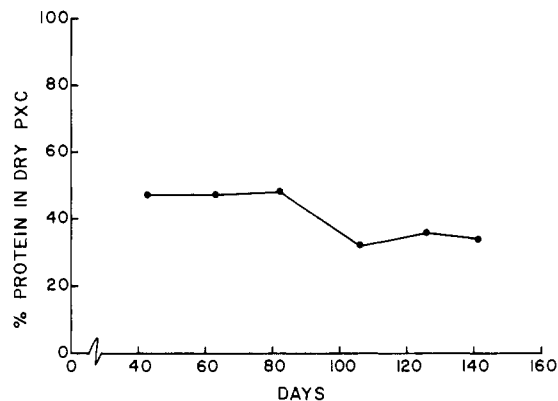


Figure 3. The percent protein present in dry protein-xanthophyll concentrate (PXC) from the leaves of Jewel cultivar sweet potato.

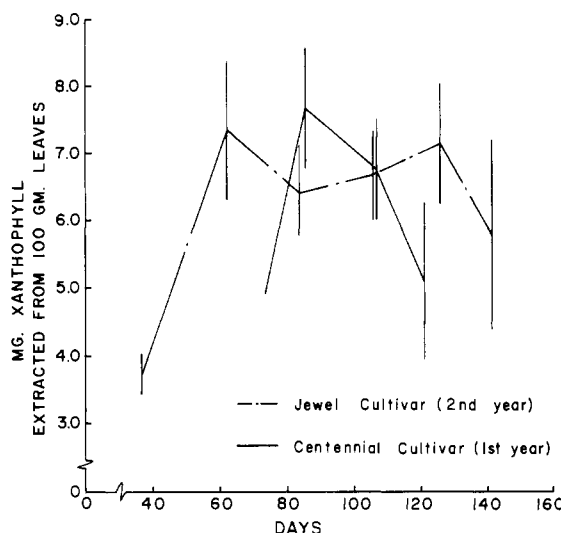
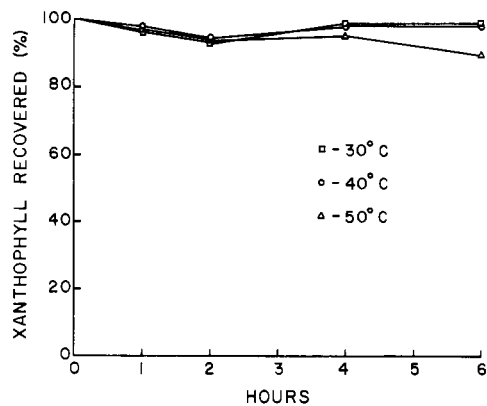


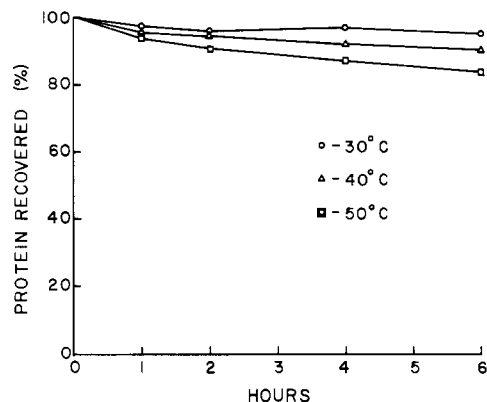
Figure 4. Xanthophyll extracted during the growing season from the leaves of two sweet potato cultivars. Each data point with a vertical line represents the mean and standard deviation from six replicate samples.

The purpose of this study was to examine the leaves of sweet potato with the purpose of preparation of a protein-xanthophyll concentrate. Consequently, the major portion of this research utilized leaves only. If PXC is to be produced commercially, petioles and/or stems would probably be harvested and processed with the leaves. Analysis of the three plant sections indicated (Table IV) that for Jewel, the leaves contained about three times as much crude protein as did the stems. The petioles contained only 12–17% of the protein of the leaves and about 50% as much dry matter.

These data, although admittedly meager, indicate one possible approach would be harvesting of the leaves and petioles and preparation of the PXC from this material. This scheme is predicated upon the fact that the stems would contribute a large amount of extraneous fibrous material containing relatively little protein. The petioles,



**Figure 5.** The effect of storage at elevated temperatures on the xanthophyll content of an aqueous extract prepared from Jewel sweet potato leaves.



**Figure 6.** The effect of storage at elevated temperatures on the heat-precipitable protein content of an aqueous extract prepared from Jewel sweet potato leaves.

of course, contain the least protein of the three portions, but because of the configuration of the sweet potato plant, it would be feasible to harvest the protein-rich leaves and a portion of the petioles and yet leave the fibrous stems in the field. Additional work needs to be done in this area.

In large-scale preparation of PXC, the extracted juice may have to be held for a fairly long time before enough material is obtained for efficient processing. It has been found (de Fremery et al., 1972) that unless the pH of alfalfa juice was raised above 7, XAN and protein were rapidly destroyed. Garlich et al. (1974) reported that sweet potato green matter does not contain a system for XAN destruction. We, therefore, evaluated the effects of juice storage at elevated temperatures on the XAN and protein levels of sweet potato PXC.

The XAN levels (Figure 5) were not affected by storage up to 40 °C for 6 h. At 50 °C there was a 10% loss after 6 h. In both the XAN and protein studies, the juice was used at the unadjusted pH of 6.1. Protein content was affected a little more than XAN levels (Figure 6). About 5, 10, and 15% of the protein was hydrolyzed when held at 30, 40, and 50 °C, respectively. Our results supported the finding of Garlich et al. (1974) that sweet potato leaves contained no XAN-destroying system. We concluded that extended storage of the juice at temperatures up to 50 °C had very little effect on XAN and a slight effect on protein content.

Leaf protein from many sources has been found to have a well-balanced amino acid pattern with most preparations being only marginally deficient in the sulfur amino acids. The amino acid patterns of alfalfa and sweet potato leaf protein extracts (Table V) were remarkably similar. Without exception, the essential amino acid compositions

**Table V.** Amino Acid Content<sup>a</sup> of Alfalfa and Sweet Potato Leaf Protein Concentrates

amino acids	sweet potato	alfalfa <sup>c</sup>	FAO <sup>d</sup> (1973)
isoleucine	4.91	5.50	4.0
leucine	9.75	9.61	7.0
lysine	6.22	6.56	5.5
methionine	1.86	1.90	
total sulfur		2.93	3.5
phenylalanine + tyrosine	6.21	5.99	
threonine	10.58	10.72	6.0
tryptophan	5.30	5.14	4.0
valine	<sup>b</sup>	2.15	1.0
alanine	6.24	6.27	5.0
arginine	6.46	6.20	
aspartic acid	6.70	6.60	
cysteine	9.08	10.09	
glutamic acid	Trace <sup>b</sup>	1.03	
glycine	11.29	11.50	
histidine	5.41	5.46	
proline	1.61	2.32	
serine	5.28	4.63	
tyrosine	4.28	4.56	
	4.37	4.73	

<sup>a</sup> Grams of amino acid/16 g of nitrogen. Means from two replicate analyses. <sup>b</sup> Tryptophan and cysteine destroyed during hydrolysis. <sup>c</sup> From Wang and Kinsella (1975). <sup>d</sup> FAO/WHO reference (1973).

were almost identical. We could not compare tryptophan and cysteine because acidic hydrolysis destroys them. Among the non-essential amino acids, only the level of aspartic acid, histidine, and proline differed in the two extracts.

The amino acid patterns of protein from sweet potato leaf protein extract were determined at 94, 107, and 121 days after planting. Our results showed little if any differences in the patterns at any of these harvest dates.

We conclude that sweet potato leaves are a possible source of a useful protein-xanthophyll concentrate. At the usual harvest date of 130-140 days after planting, the concentrate would contain about 36% protein, about 9% fiber, and 0.10% xanthophyll pigment. Based on the amino acid analyses, the protein appears to be of high nutritional quality and may be a valuable supplement either as a poultry ration or as food for other nonruminants.

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## Uptake, Distribution, and Elimination of the Lampricide 2',5-Dichloro-4'-nitro[<sup>14</sup>C]salicylanilide (Bayer 2353) and Its 2-Aminoethanol Salt (Bayer 73) by Largemouth Bass

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In two groups of largemouth bass exposed to a 0.05- $\mu$ g/mL mixture of the lampricide 2',5-dichloro-4'-nitro[<sup>14</sup>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 <sup>14</sup>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 <sup>14</sup>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- $\mu$ g/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 <sup>14</sup>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 [<sup>14</sup>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 [<sup>14</sup>C]Bayer 2353 and Bayer 73 in polyethylene tanks containing 75 L of water (pH 7.3, temperature 13.5  $\pm$  1.0  $^{\circ}$ 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 [<sup>14</sup>C]Bayer 2353 (uniformly 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- $\mu$ g/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  $\pm$  1.0  $^{\circ}$ 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  $^{\circ}$ 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<sub>2</sub>SO<sub>4</sub> to pH 1.5-2.0 and

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