JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Tissue Distribution, Metabolism, and Residue Depletion Study in Atlantic Salmon Following Oral Administration of [^{3}H]Emamectin Benzoate

HEASOOK KIM-KANG,*,† ALICE BOVA,§ LOUIS S. CROUCH,§ PETER G. WISLOCKI,§ ROBERT A. ROBINSON,† AND JINN WU†

XenoBiotic Laboratories, Inc., 107 Morgan Lane, Plainsboro, New Jersey 08536, and Schering-Plough Corporation, 1095 Morris Avenue, Union, New Jersey 07083

Atlantic salmon (~1.3 kg) maintained in tanks of seawater at 5 ± 1 °C were dosed with [^{3}H]emamectin B_1 benzoate in feed at a nominal rate of 50 μg of emamectin benzoate/kg/day for 7 consecutive days. Tissues, blood, and bile were collected from 10 fish each at 3 and 12 h and at 1, 3, 7, 15, 30, 45, 60, and 90 days post final dose. Feces were collected daily from the tanks beginning just prior to dosing to 90 days post final dose. The total radioactive residues (TRR) of the daily feces samples during dosing were 0.25 ppm maximal, and >97% of the TRR in pooled feces covering the dosing period was emamectin B_{1a}. Feces TRR then rapidly declined to ~0.05 ppm by 1 day post final dose. The ranges of mean TRR for tissues over the 90 days post dose period were as follows: kidney, 1.4−3 ppm; liver, 1.0−2.3 ppm; skin, 0.04−0.09 ppm; muscle, 0.02−0.06 ppm; and bone, <0.01 ppm. The residue components of liver, kidney, muscle, and skin samples pooled by post dose interval were emamectin B_{1a} (81−100% TRR) and desmethylemamectin B_{1a} (0−17% TRR) with *N*-formylemamectin B_{1a} seen in trace amounts (<2%) in some muscle samples. The marker residue selected for regulatory surveillance of emamectin residues was emamectin B_{1a}. The emamectin B_{1a} level was quantified in individual samples of skin and muscle using HPLC−fluorometry and was below 85 ppb in all samples analyzed (3 h to 30 days post dose).

KEYWORDS: Emamectin benzoate; SCH 58854; emamectin B_{1a}; salmon; metabolism; residue depletion

INTRODUCTION

Avermectins are a class of natural products consisting of a disaccharide linked to a pentacyclic 16-membered ring, which are produced by the soil microorganism *Streptomyces avermilitis*. Avermectin B_1, consisting of a mixture of the natural avermectins B_{1a} and B_{1b} and also called abamectin, is a potent broad spectrum acaricide/insecticide (*1*) but does not show high innate toxicity against some specific insect species.

Emamectin benzoate [SCH 58854; 4″-deoxy-4″-(epimethylamino)avermectin B_1 benzoate salt] is a semisynthetic avermectin (*2*) registered for use to control lepidopterous pests on vegetable crops in the United States and Japan (*3*). It is also highly effective for the treatment and control of sea lice infestations on Atlantic salmon, *Salmo salar* L. (*4*−*8*), and is registered for use in the United Kingdom, Chile, Ireland, Finland, Faroe Islands, Spain, and Norway. The compound, also known as MK-244, is derived through chemical modification of abamectin; an *epi*-aminomethyl (−$NHCH_3$) group is substituted for a hydroxyl (−OH) group at the 4″-position (**Figure 1**). Similar to abamectin, it is a mixture of two homologues designated B_{1a} and B_{1b}, which differ by one methylene (CH_2) unit on the C-25 side chain, wherein B_{1a} contains a *sec*-butyl group and B_{1b} contains an isopropyl group. By specification it consists of at least 90% of B_{1a} benzoate and not more than 10% of B_{1b} benzoate. The emamectin homologues are each ~900 Da. Emamectin benzoate is formulated in a 0.2% feed mix (SLICE) and administered to fish as medicated feed.

In the present study, the tissue distribution, metabolism, and depletion of emamectin B_1 in Atlantic salmon following the dietary administration of a nominal dose of 50 μg of [^{3}H]-emamectin benzoate/kg/day for 7 consecutive days were investigated. As a result of this study emamectin B_{1a} (free base) has been established as the marker residue in edible tissues of Atlantic salmon for regulatory surveillance of the food supply. Edible tissues in Atlantic salmon are separate muscle, separate skin (Canada), and intact skin/muscle (all other countries). The maximum residue limit (MRL) for emamectin B_{1a} in salmon muscle with skin attached was set at 100 ppb by the European Commision for Veterinary Medicinal Products (CVMP; *9*).

In the present study, the muscle and skin tissue residue levels of emamectin B_{1a} were also determined by a determinative HPLC−fluorometric assay (*10*) to characterize the depletion of the marker residue in tissues of individual treated fish as well as to provide the proportionality between the marker residue and total radioactive residue.

* Author to whom correspondence should be addressed. Fax: (609) 799-7497. E-mail: hkimkang@xbl.com.
† XenoBiotic Laboratories, Inc.
§ Schering-Plough Corp.

10.1021/jf035435v CCC: $27.50
Published on Web 03/05/2004

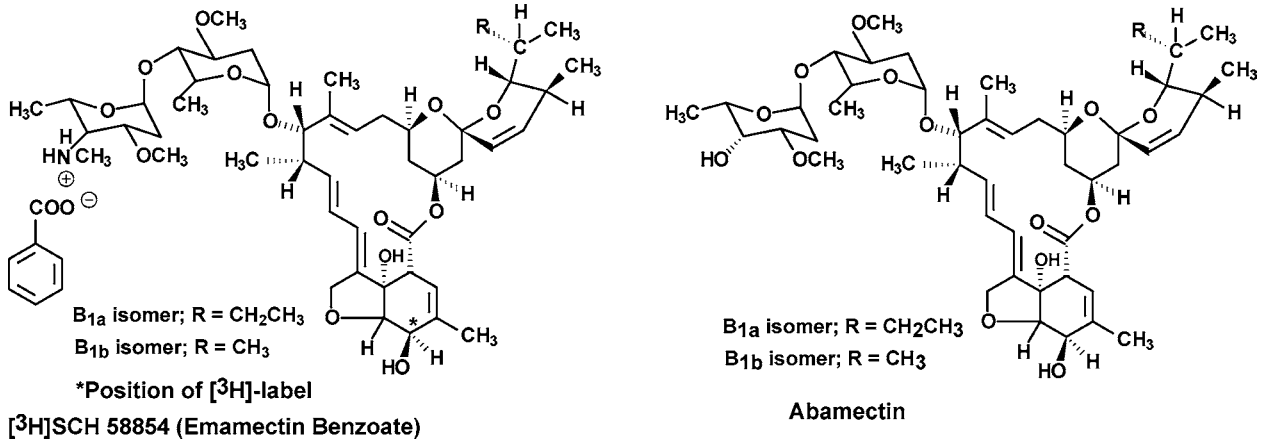
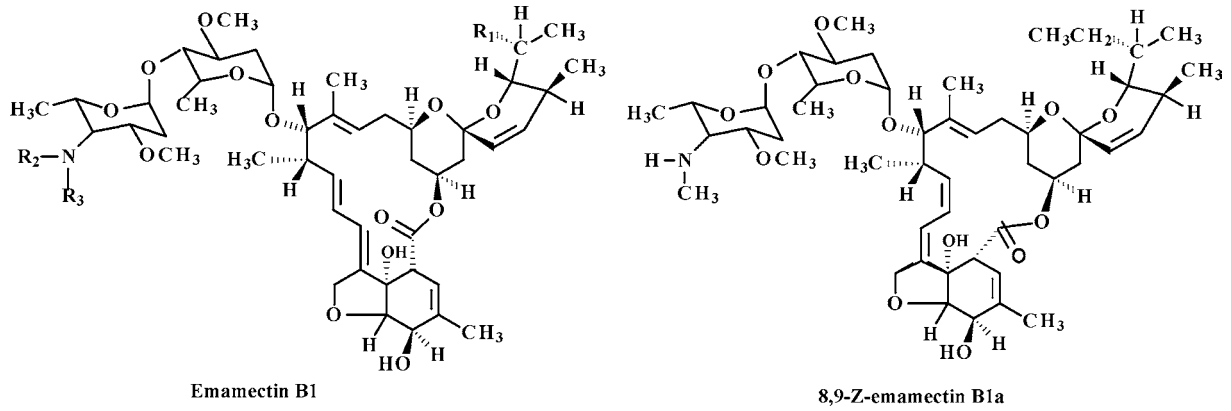


Figure 1. Structures of emamectin benzoate and abamectin.



Compound ID	R_1	R_2	R_3
8,9-Z-emamectin B1a	CH_2CH_3	H	CH_3
N-formyl emamectin B1	CH_2CH_3 for B1a,, CH_3 for B1b	CH=O	CH_3
N-desmethyl, *N*-formyl-emamectin B1	CH_2CH_3 for B1a,, CH_3 for B1b	CH=O	H
N-desmethyl-emamectin B1	CH_2CH_3 for B1a,, CH_3 for B1b	H	H

Figure 2. Structures of metabolite standards.

MATERIALS AND METHODS

Chemicals, Materials, and Solvents. Non-radiolabeled emamectin B_1 benzoate [a mixture of 4″-deoxy-4″-(epimethylamino)avermectin B_{1a} and B_{1b} benzoate salts: 95% B_{1a}, 5% B_{1b}; 96.8% total chemical purity] and [5-^{3}H]-4″-deoxy-4″-(epimethylamino)avermectin B_{1a} benzoate salt (specific activity = 16.80 mCi/mg; radiochemical purity of 98.5%) were provided by Schering-Plough, Kenilworth, NJ. The emamectin benzoate and [^{3}H]emamectin B_{1a} benzoate were mixed to give a specific activity of 587,569 dpm/μg of emamectin B_1 benzoate for the compound formulated in feed for administration to fish. The specific activity of the [^{3}H]emamectin B_{1a} resulting from the mixture of emamectin B_1 benzoate with [^{3}H]emamectin B_{1a} benzoate was not determined. The following emamectin metabolite reference standards (each consisting of a major B_{1a} and a minor B_{1b} homologue except for one compound) were also provided by Schering-Plough: 4″-deoxy-4″-epi-aminoavermectin B_1 benzoate (desmethylemamectin, purity of 90.1% B_1 benzoate), 4″-deoxy-4″-(epimethylamino)avermectin B_1 delta 8,9 isomer (8,9-*Z*-emamectin B_{1a}, purity of 91.9%, B_{1a}), 4″-deoxy-4″-epi-(*N*-formyl-*N*-methyl)aminoavermectin B_1 (*N*-formylemamectin B_1, purity of 88.0% B_1), and 4″-deoxy-4″-epi-(*N*-formyl)aminoavermectin B_1 (*N*-desmethyl-*N*-formylemamectin B_1, purity of 83.4% B_1). The structures of these compounds are illustrated in **Figure 2**. Food grade butylated hydroxyanisole (BHA, 100% purity) was provided by Schering-Plough. All solvents used were of HPLC or analytical grade. They were obtained from the following suppliers: acetonitrile (ACN), ethyl acetate (EtOAc), methanol (MeOH), and potassium hydroxide (KOH) were obtained from EM Science, Gibbstown, NJ; ammonium acetate, ammonium hydroxide (28−30%), and phosphoric acid were obtained from J. T. Baker, Phillipsburg, NJ; trifluoroacetic anhydride (99+% purity) and *N*-methylimidazole (NMIM; 99+% purity) were purchased from Aldrich Chemical Co., Milwaukee, WI; hydrochloric acid was purchased from VWR Scientific Products, West Chester, PA. Water was purified on site using a Barnstead NanoPure II water purification system. A propanesulfonic acid (PRS) cartridge was purchased from Varian, Harbor City, CA. ^{3}H-Labeled liquid scintillation cocktail for the Harvey biological oxidizer was obtained from R. J. Harvey Instrument Corp., Hillsdale, NJ, and Ready Value liquid scintillation cocktail was purchased from Beckman Instruments Inc., Fullerton, CA.

Salmon and Treatment. The in-life phase of the study was conducted by Atlantic Fish Health, Inc., University of Prince Edward Island, Charlottetown, PE, Canada. One hundred and twenty-two unsexed Atlantic salmon, averaging ~1.3 kg, were used in the study. The fish were maintained at ~5 °C in two identical 1800 L tanks (A and B) of recirculating seawater connected to a single reservoir. Tank water was filtered through activated charcoal prior to being recirculated through the system. After an acclimation period of 14 days, 100 fish were given [^{3}H]emamectin benzoate formulated in feed to provide a

nominal dose of 50 μg/kg of live weight/day for 7 consecutive days based on a feeding rate of 0.4% body weight per day and the total biomass in each tank. The entire daily medicated feed ration was offered to all fish in each tank over a 30 min period and any uneaten feed was removed later the same day. The remaining 22 fish served as unmedicated controls, 4 being removed before dosing and 18 being placed in the tanks with the dosed fish after dosing was completed. The treated feed was prepared by mixing a feed premix containing the [^{3}H]emamectin benzoate, sieved Atlantic Salmon pellet feed (Moore Clark, Canada), and fish oil. The feed premix used for the treated feed preparation was of similar composition to commercial SLICE. The actual daily dose received was ~33 μg/kg. Analysis of the treated feed before and after dosing gave a [^{3}H]emamectin B_{1a} radiopurity of ~98%.

Specimen Collection. Groups of 10 dosed fish were euthanized at 3, 12, 24, and 72 h and at 7, 15, 30, 45, 60, and 90 days post dose. Control fish ($n = 4$) were removed ~96 h prior to initial dosing. After weighing, mucus was collected from both flanks. A blood sample was collected from the caudal vein, and the fish were exsanguinated by severing the gill arches. The end of the intestine was clamped closed (via a small incision) prior to descaling to prevent fecal contamination of the skin. Samples of liver (entire), kidney (entire), bile (when available), gut contents, skin (from two filets), muscle (from two filets), and bone (from the spinal column) were collected for analysis. Samples of tank water, liquid effluent (tank flush), semisolid effluent (feces), filtering charcoal, uneaten treated feed pellets, and other particulate waste were also analyzed for total ^{3}H content. Tank water, liquid effluent (tank flush), and semisolid effluent (feces) were removed daily during dosing and until 1 week post dose and then were collected once per week thereafter until 90 days post final dose. Each salmon was scaled with a knife and divided into two fillets. From one fillet, the skin was peeled from the muscle and any remaining muscle tissue was removed from the skin. Collected fish samples were stored frozen until further processing. Muscle, skin, and intact skin/muscle tissues containing incurred residues from the 3-, 12-, 24-, and 72-h and 7-, 15-, and 30-day withdrawal times were used for HPLC−fluorometric analysis. Additional control muscle and control skin samples were obtained from a commercial source and were used to monitor assay performance.

Specimen Preparation and Assay for Total Radioactivity. Muscle samples were partially thawed and diced into smaller pieces. Diced samples were blended in a food processor. Separate skin samples were also partially thawed and minced into small pieces by hand using scissors. Intact muscle/skin samples were processed as described for muscle. Liver and kidney samples were minced with scissors and homogenized in water (1:1, w/w) using a Tekmar Tissumizer. Liver, kidney, muscle, intact muscle/skin, and skin samples were analyzed by combustion/LSC using biological sample oxidizers (models OX-300 and OX-500; R. J. Harvey Instrument Corp). Bone samples from each fish were cut into small pieces with scissors and placed into a glass centrifuge tube. To each tube was added ~100−125 mL of 2 N KOH, and the tube was sealed and shaken for 4 h on a Burrell wrist action shaker to remove extraneous cartilage and muscle. Vertebrae were isolated by filtration, rinsed with water, and air-dried. The vertebrae were weighed into a glass centrifuge tube and dissolved in 6 N HCl (25 mL). The content was then heated at 100 °C with constant stirring on a Reacti-Therm heater/stirrer for 12 h, and the bone digest was analyzed by direct LSC. Whole mucus samples were mixed with an equivalent amount of water (w/w) and vortexed. Bile and plasma samples were vortexed and directly analyzed by LSC. Tank water and liquid water effluent samples were directly analyzed by LSC. Gut contents were homogenized with an equivalent amount of water (w/w) and analyzed by combustion/LSC. Semi-solid effluent samples (feces) were homogenized using Tekmar Tissumizer and analyzed by combustion/LSC. Charcoal samples were mixed by hand and analyzed by combustion/LSC. Uneaten feed pellets were homogenized in water (1:1, w/w) and analyzed by combustion/LSC.

Extraction of Residues for Metabolite Profiling. After total radioactive residue (TRR) determination, samples ($n = 5$) of liver, kidney, gut contents, muscle, skin, and intact muscle/skin from the following intervals were pooled by matrix and post dose interval for metabolite profiling: 2 h, day 7, day 15, day 45, and day 90. The entire samples of liver, kidney, gut contents, and skin from five fish per interval were used for pooling, whereas muscle and intact muscle/skin samples were subsampled for pooling and reprocessed prior to further analyses. Each pooled subsample (~5−50 g) was extracted by blending with methanol (~4 or 8 times, v/w), three times using a Tekmar Tissumizer; supernatants were isolated by centrifugation (IEC Centra 7R, 20 min × 2500 rpm, 10 °C) and analyzed by direct LSC. After air-drying, the post-extraction solid pellet containing bound radioactive residues (PES) was analyzed by combustion/LSC. Each supernatant was concentrated using a rotary evaporator, and subsamples of the concentrates were further evaporated to dryness under a stream of nitrogen and reconstituted as final extract in a mixture of 0.1% H_3PO_4/CH_3OH (1:1, v/v) prior to HPLC analysis.

HPLC Conditions Used for Metabolite Profiling (HPLC Condition I). The HPLC systems used for the study consisted of the following instruments: Waters 600E (Milford, MA) solvent delivery system, Waters 484 UV detector at the wavelength of 246 nm, Ramona 5-LS with a 600-μL liquid cell using Ready Flow III at 2.5 mL/min, Waters 717 autosampler, Foxy 200 fraction collector (ISCO), and Waters Millennium 2020 Chromatography Manager. A Primesphere 5 C18-HC 110A (4.6 mm i.d. × 150 mm, 5 μm particle size) from Phenomenex, Torrence, CA, was used as an analytical column preceded by a Primesphere guard column (4.6 mm i.d. × 30 mm, 5 μm) at a flow rate of 1 mL/min. The column temperature was maintained at 35 °C. The mobile phases used were 0.1% H_3PO_4 in H_2O (A) and acetonitrile (B). The following gradient program was used for metabolite profiling: a linear gradient program from 60% A to 70% B in 25 min, followed by a 1-min linear gradient from 70% B to 100% B and a 10-min hold before returning to the initial condition. Due to low levels of radioactivity in most of the sample extracts (except liver and kidney), the detection of radioactivity in HPLC eluates using a liquid cell radioactivity monitoring (RAM) system was not sufficiently sensitive to allow detection of minor components. Therefore, radioactivity in those samples was monitored by a combined technique consisting of a liquid cell RAM system in tandem with fraction collection followed by LSC. In certain analyses, the eluate was bypassed directly into a fraction collector for direct LSC analysis. The HPLC eluate for each sample analysis was collected every 30 s; the entire eluate was counted by LSC, and a reconstructed HPLC chromatogram was prepared using the data. Reference standards were cochromatographed with a sample extract using HPLC condition I to obtain a more accurate assessment of retention times in the presence of extracted matrix components.

Preparation of Standards for Residue Depletion Assay. Emamectin standard solutions for use in the determinative assay were prepared in acetonitrile and stored at or below −10 °C, at which they are known to be stable for at least 6 months. Working standards were prepared by serial dilution of a stock solution of emamectin benzoate adjusted to a concentration of ~100 μg of emamectin B_{1a}/mL (the emamectin benzoate used was ~80% emamectin B_{1a} free base). The standard curve was prepared by using an aliquot of each working standard solution.

Preparation of Fortified Samples for Residue Depletion Sample Assay. Fortified tissues were prepared by adding working standards to control tissues at emamectin B_{1a} free base concentrations of 40 and 200 ppb. Fortified tissues were extracted along with incurred tissues as described below. Although the validation of the emamectin B_{1a} determinative assay (*10*) was in the range of 50−800 ppb for fortified tissues, the 40 ppb fortification was used for the quality control (QC) samples in the present study because the residue levels in some incurred tissues were expected to be <50 ppb. In addition, the results from the emamectin determinative method validation (*10*) indicated that the theoretical level of quantitation (LOQ) is considerably lower than 40 ppb, and the lowest standard used corresponded to 20 ppb in tissues.

Preparation of Untreated Control Samples for Residue Depletion Assay. For each analysis, unfortified tissue samples from a control (nontreated Atlantic salmon) served as blanks and were processed and analyzed to check for interference.

Assay Procedure for Residue Depletion Assay. The determinative HPLC−fluorometric assay, which has been previously reported in detail (*10*), was used for the present study without modification. The derivatization of emamectin residues in the assay is based on the original procedure reported by Tway et al. (*11*) involving conversion of

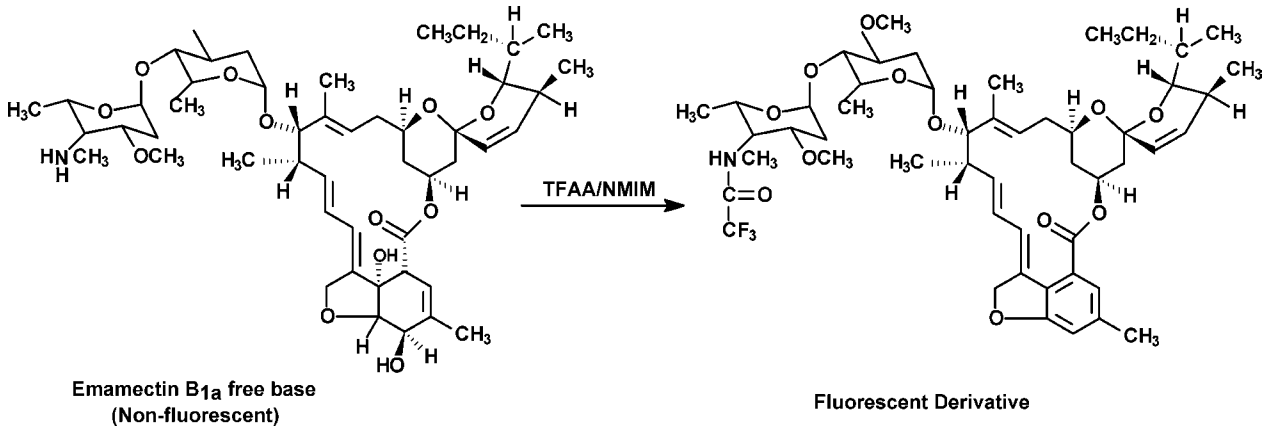


Figure 3. Structures of emamectin B_{1a} and the fluorescent derivative of the marker residue.

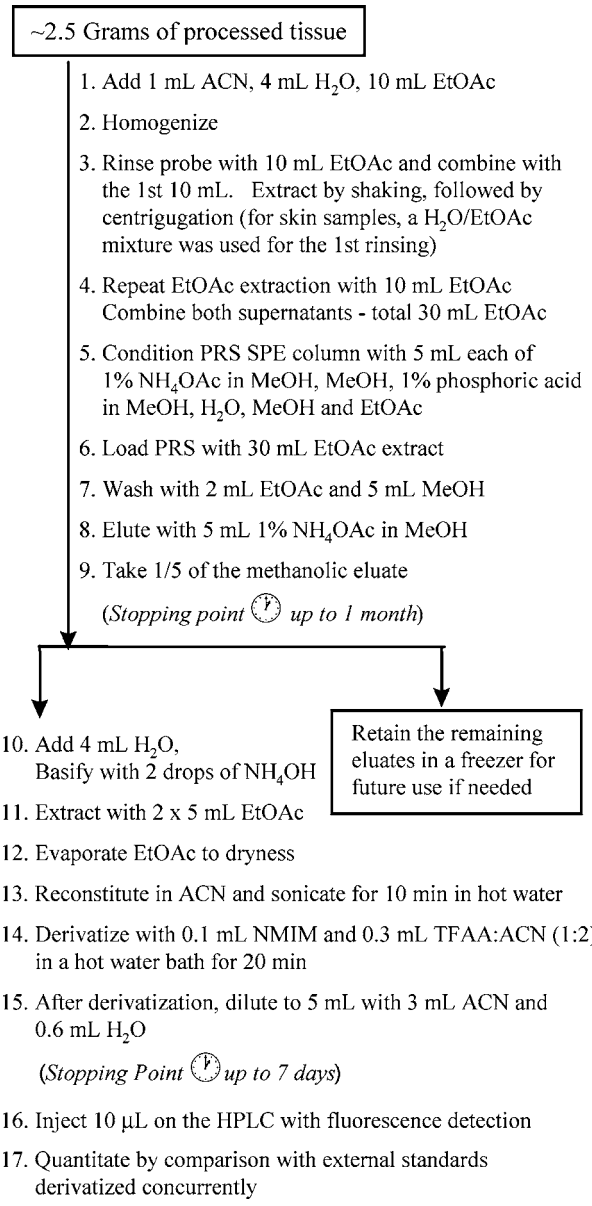


Figure 4. Flow diagram of the procedure to determine emamectin benzoate marker residue in fish tissues.

ivermectin to an intensively fluorescent aromatic derivative. The structure of the derivative of the marker residue, emamectin B_{1a}, is shown in **Figure 3**. A flow diagram of the procedure is presented in **Figure 4**.

Preparation of Standard Curve (Absolute). An absolute standard curve was prepared containing six points in the concentration range of 2 and 100 ng/mL emamectin B_{1a} free base (equivalent to 20 and 1000 ppb for the 2.5-g tissue sample used in the assay). The calibration standard solutions were prepared by volumetrically transferring 1 mL each of the working standard solutions to separate 15-mL tubes followed by the addition of 0.1 mL of NMIM. The mixture was then subjected to the same reaction conditions as used for the sample extracts.

HPLC Conditions for Residue Depletion Assay (HPLC Condition II). The HPLC systems used for the determinative assay consisted of the following instruments: Waters 600E (Milford, MA) solvent delivery system, Waters 474 fluorescence detector, Waters 717 autosampler, and Waters Millennium 2020 Chromatography Manager. Zorbax RX-C8 (4.6 mm i.d. × 250 mm, 5-μm particle size) from MacMod, Chadds Ford, PA, was used as an analytical column preceded by a Brownlee RP-18 Newguard guard column (3.2 mm i.d. × 15 mm). The mobile phase used was Nanopure water/methanol (5:95) at a flow rate of 1 mL/min. The fluorescence detector parameters were set as follows: excitation wavelength at 365 nm and emission wavelength at 470 nm, gain at 100×, bandwidth at 18 nm, response time at standard, filter at RC filter, and flow cell size of16 μL. An injection volume of 10 μL and a total run time of 15 min were maintained for all analyses.

Quantitation of Marker Residue. A linear regression standard curve was constructed using an external standard calibration [emamectin B_{1a} free base peak response as the *y* coordinate and emamectin B_{1a} free base (derivative) standard concentration (ng/mL) as the *x* coordinate]. Six external standards were injected before and after the samples to ensure the system suitability and sample stability. A typical analysis set contained 30 tissue samples including one control and four fortified quality control samples. A single injection was made from each sample extract, and the system stability was maintained throughout the 30 injections made between the analyses of six external standards. Using the slope (*S*) and intercept (*I*) from the linear regression equation, the unknown sample concentration of emamectin B_{1a} free base (derivative) in the final sample extract was calculated as follows:

$$\text{concentration in ng/mL in the final sample extract } (x) = [\text{peak response } (y) - I]/S$$

The resulting sample extract concentration was converted to parts per billion (nanograms of emamectin B_{1a} free base per gram of tissue) by multiplying the final milliliters of take-up volume by 5 to take into account that only one-fifth of the methanolic elute was used for the further workup and dividing by the sample weight in grams. The formula is expressed as

$$\text{concentration in tissue (ppb)} = \frac{X \times V \times 5}{W}$$

where X = concentration in the final sample extract (ng/mL), V = total volume of the final sample extract = 5 mL, 5 = correction for the subsampling of one-fifth of the methanolic eluate, and W = total tissue weight (g) = 2.5 g.

Table 1. Mean Total Radioactive Residues as [^{3}H]Emamectin Benzoate Equivalents in Tissues and Bodily Fluids from Treated Salmon (n = 10 per Interval)

		av ppm									
group	interval	muscle	skin	muscle with skin	liver	kidney	bone	mucus	plasma	bile	gut contents
III-2	3 h	0.0612	0.0843	0.0635	1.9588	2.0261	0.0049	0.0054	0.1107	6.2862	0.6694
III-3	12 h	0.0590	0.0836	0.0619	2.0114	2.0671	0.0035	0.0049	0.1171	5.0495	0.6607
III-4	24 h	0.0533	0.0693	0.0554	1.8858	1.9695	0.0029	0.0070	0.0932	5.0967	0.5806
III-5	72 h	0.0648	0.0927	0.0643	2.2096	2.6719	0.0069	0.0106	0.1185	6.3770	0.5307
III-6	day 7	0.0485	0.0742	0.0487	1.9372	2.6897	0.0038	0.0057	0.0890	3.6149	0.5706
III-7	day 15	0.0511	0.0906	0.0549	2.2605	3.0647	0.0040	0.0077	0.0847	4.8685	0.3099
III-8	day 30	0.0366	0.0600	0.0368	1.5406	2.2301	0.0031	0.0107	0.0576	3.2778	0.2802
III-9	day 45	0.0308	0.0534	0.0345	1.6746	2.2678	0.0037	0.0125	0.0561	2.5925	0.2308
III-10	day 60	0.0248	0.0484	0.0273	1.4373	1.9029	0.0045	0.0091	0.0414	2.1820	0.1783
III-11	day 90	0.0185	0.0356	0.0196	1.0827	1.4355	0.0017	0.0106	0.0298	1.4925	0.1564

Table 2. Distribution of Radioactivity in Tissue and Bodily Fluids from Treated Salmon (n = 10; Results Expressed as Percent of Total Fish Residue)

		av % distribution										
group	interval	muscle	skin	muscle with skin	liver	kidney	bone	mucus	plasma	bile	gut contents	total
III-2	3 h	19.98	1.20	22.19	28.12	21.70	0.02	0.01	0.19	1.85	5.11	100.00
III-3	12 h	19.08	1.03	21.08	30.17	22.26	0.01	0.01	0.21	3.61	4.54	100.00
III-4	24 h	18.72	1.01	20.78	29.66	22.93	0.01	0.01	0.18	3.78	3.69	100.00
III-5	72 h	18.53	1.07	19.62	30.09	26.99	0.02	0.01	0.21	1.20	2.75	100.00
III-6	day 7	17.18	0.96	18.61	29.18	29.58	0.02	0.01	0.16	2.12	2.83	100.00
III-7	day 15	15.84	1.12	18.26	31.01	30.77	0.01	0.01	0.18	1.27	1.67	100.00
III-8	day 30	16.01	0.97	16.48	28.37	34.64	0.01	0.01	0.16	1.32	2.70	100.01
III-9	day 45	13.99	0.95	16.47	32.11	33.72	0.01	0.01	0.13	2.23	1.28	100.00
III-10	day 60	13.33	1.00	15.86	34.44	32.62	0.02	0.01	0.12	1.63	1.14	100.00
III-11	day 90	14.01	1.04	15.42	31.52	33.35	0.02	0.01	0.10	2.36	2.18	100.00

Recovery was determined by dividing the calculated parts per billion in the fortified samples by the theoretical concentration (nanograms of emamectin B_{1a} free base added per gram of control tissue) and expressing the result as a percentage (×100%).

Notes to Analysts and Safety Considerations. Care should be taken to wear proper eye and hand protection, and work should be done in a properly vented laboratory hood when TFAA is handled.

RESULTS

Total Radioactive Residues. TRR (expressed as [^{3}H]-emamectin benzoate equivalents) in tissues, bodily fluids, and gut contents generally declined with time post dose, although many tissues exhibited peaks at 3 h, 72 h and 15 days **(Table 1**). The highest TRR level in tissues was found in kidney (3.06 ± 0.73 ppm on day 15, declining to 1.44 ± 0.44 ppm by day 90). Muscle TRR levels varied from 0.053 ± 0.19 to 0.065 ± 0.019 ppm through 72 h, declining to 0.019 ± 0.005 ppm by day 90, whereas TRR levels in skin ranged from 0.069 ± 0.027 to 0.093 ± 0.034 ppm through 72 h, declining to 0.036 ± 0.010 ppm by day 90. Muscle-with-skin TRR levels varied from 0.055 ± 0.019 to 0.064 ± 0.02 ppm through 72 h, decreasing to 0.020 ± 0.006 ppm by day 90. TRR levels in plasma were modest, ranging from 0.119 ± 0.04 ppm at 72 h to 0.030 ± 0.01 ppm by day 90, whereas those in mucus amounted to ∼0.01 ± 0.007 ppm through the entire testing interval. Residues in bone were also low, ranging from 0.007 ± 0.002 ppm at 72 h to 0.002 ± 0.001 ppm by day 90. The TRR levels in bile and gut contents ranged from 6.38 ± 1.21 ppm (bile, 72 h) to 1.49 ± 0.53 ppm and from 0.669 ± 0.140 ppm (gut content, 3 h) to 0.156 ± 0.062 ppm by day 90.

The highest distribution of total fish residue occurred in liver and kidney, with 28−34 and 22−35% of the total fish residue found in these two organs, respectively. Muscle (13−20%) and intact muscle/skin (15−22%) also contained a sizable portion of the total fish residue. Bile and gut contents contained a small proportion of the total fish residue (∼1−5%), whereas skin, bone, mucus, and plasma each contained only ∼1% or less (**Table 2**). The total radioactive residue depletion results are depicted graphically in **Figure 5** for muscle, skin, and intact muscle/skin tissues (edible tissues) and in **Figure 6** for liver and kidney tissues.

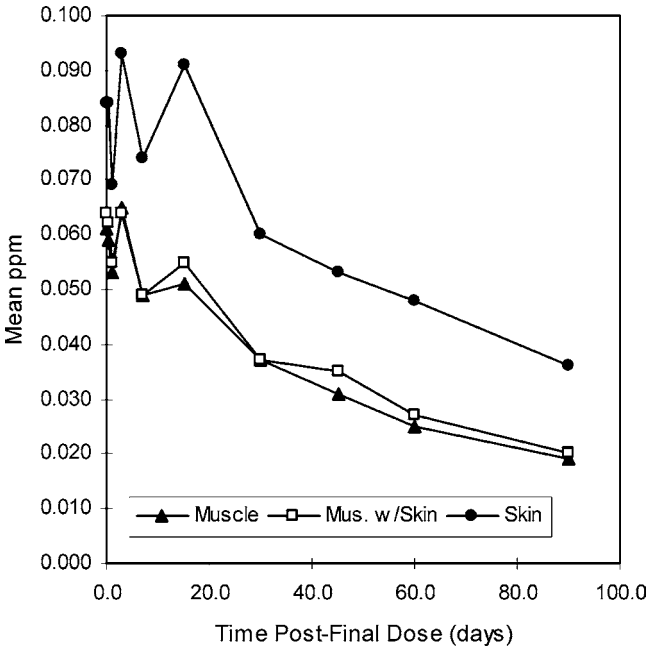


Figure 5. Total ^{3}H residue depletion curves for muscle, skin, and intact muscle with skin tissues.

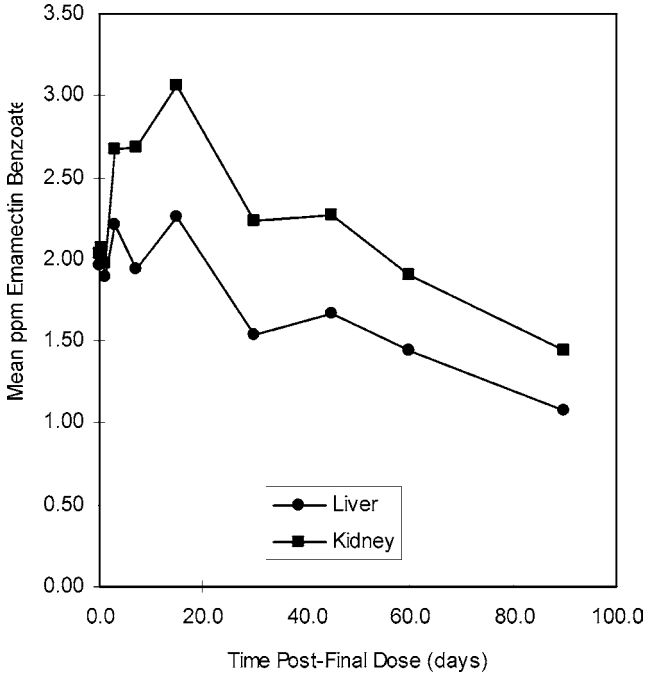


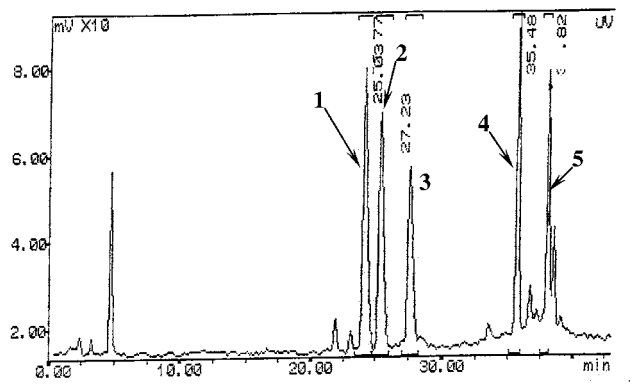
Figure 6. Total ^{3}H residue depletion curves for liver and kidney.

TRR levels in tank water samples were extremely low (<0.0001 ppm) at all intervals, demonstrating that the recirculating amount of the active component due to the recirculation of the tank water was considered to be minimal. Feces TRR values during dosing reached a maximum of ~0.25 ppm on the fifth or sixth day of the 7 day dosing period, declining to ~0.015 ppm by 7 days after dosing, although not steadily, after which the TRR was variable but remained below 0.017 ppm (data not shown). This indicated that the excretion in feces was largely complete by 1 day post dose. Insignificant radioactivity was detected in the tank flushes and charcoal filters relative to the dose administered (data not shown). Control (untreated) tissue sample analyses were used to determine LOQ for each tissue matrix, specifically estimated at 2 times control tissue ppm values. The actual control ppm values ranged from 0 to 1.8×10^{-4} ppm, yielding LOQ levels ranging from 0.00 to 3.6×10^{-4} ppm.

Extraction of Residues from Tissues, Gut Contents, and Feces. Methanolic extractions of pooled tissues (liver, kidney, muscle, skin, and intact muscle with skin), as well as pooled gut contents and feces samples for selected intervals were conducted as described under Extraction of Residues for Metabolite Profiling to prepare samples for HPLC analysis. The extractability of ^{3}H residues in each matrix at all time points was excellent: >99% (liver), >98% (kidney, intact muscle/skin), >97% (muscle, gut contents), >94% (skin and feces). Recovery of radioactivity for the method was excellent, ranging from 98.31% for feces to >100% (104−115%) for other matrices.

Metabolic Profiles of Emamectin Residues in Tissues, Gut Contents, and Feces. Using the HPLC condition I outlined under HPLC Condition Used for Metabolite Profiling, the retention times were determined for emamectin B_{1a} and the four metabolite standards: ~24−25 min for desmethylemamectin B_{1a}, ~25−27 min for emamectin B_{1a}, ~27−29 min for 8,9-*Z*-emamectin B_{1a}, ~35−36 min for *N*-desmethyl-*N*-formylemamectin B_{1a}, and ~37.5−38 min for *N*-formylemamectin B_{1a} (**Figure 7**). The HPLC conditions were sufficient to afford good baseline separation of these five components.

The final extracts of pooled samples from each time point (12 h through day 90) were analyzed by HPLC using condition I. Results are provided in **Table 3** (liver, kidney, muscle, skin, andmuscle with skin) and **Table 4** (gut contents and feces). The major ^{3}H component observed in all tissues was the parent, [^{3}H]-emamectin B_{1a}. The proportion of parent in all tissues generally decreased from 98 to 100% of TRR at 12 h post final dose to 81−89% by day 90. Lesser amounts of ^{3}H component **7**, which cochromatographed with desmethylemamectin B_{1a}, were observed in nearly all tissue extracts, increasing from 0 to 1%



1. *N*-desmethyl emamectin B1a; 2. emamectin B1a
3. 8,9-*Z*-emamectin B1a 4.: *N*-desmethyl, *N*-formayl emamectin B1a
5. *N*-formyl emamectin B1a

Figure 7. Representative HPLC profiles of reference standards.

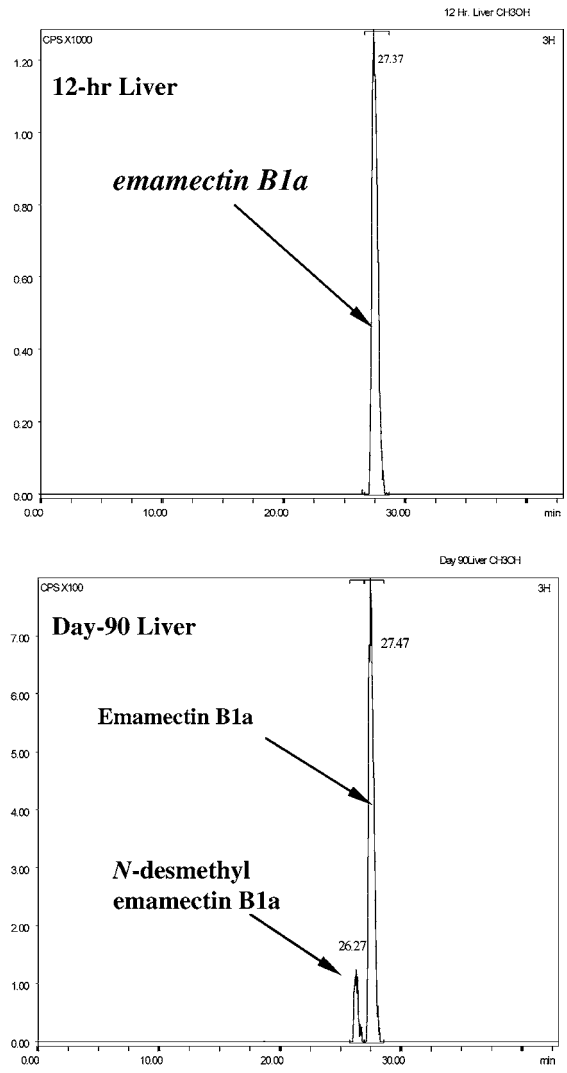


Figure 8. HPLC ^{3}H profiles of the methanolic extracts of 12-h and day-90 liver.

Table 3. Distribution of [^{3}H]Emamectin B_{1a} and Metabolites[a] in Liver, Kidney, Muscle, Skin, and Intact Muscle with Skin As Determined by ^{3}H Radiochromatography (HPLC)

matrix	interval post final dose	**6** (t_R = 24.0–25.2 min)		**7** (t_R = 25.0–26.9 min)		emamectin B_{1a} (t_R = 26.5–28.5 min)		**11** (t_R = 38.0 –39.5 min)	
		% TRR[b]	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
liver	12 h	ND[c]	ND	ND	ND	99.81	1.9413	ND	ND
	day 7	ND	ND	2.54	0.0562	97.29	2.1574	ND	ND
	day 15	ND	ND	3.50	0.0854	96.32	2.3470	ND	ND
	day 45	ND	ND	5.51	0.0958	94.31	1.6397	ND	ND
	day 90	ND	ND	11.00	0.1421	88.69	1.1458	ND	ND
kidney	12 h	ND	ND	0.61	0.0139	98.34	2.2215	ND	ND
	day 7	0.55	0.0143	2.91	0.0749	94.29	2.4260	ND	ND
	day 15	ND	ND	0.54	0.0170	97.99	3.0773	ND	ND
	day 45	0.34	0.0086	5.53	0.1378	92.35	2.3010	ND	ND
	day 90	ND	ND	14.64	0.2560	84.00	1.4691	ND	ND
muscle	12 h	ND	ND	ND	ND	97.62	0.0926	1.56	0.0015
	day 7	ND	ND	3.49	0.0016	95.55	0.0441	ND	ND
	day 15	ND	ND	6.11	0.0031	86.54	0.0440	ND	ND
	day 45	ND	ND	10.95	0.0041	84.96	0.0318	1.50	0.0006
	day 90	ND	ND	16.30	0.0037	81.38	0.0186	ND	ND
skin	12 h	ND	ND	ND	ND	97.83	0.0930	ND	ND
	day 7	ND	ND	3.19	0.0034	91.75	0.0965	ND	ND
	day 15	ND	ND	1.58	0.0015	97.20	0.0929	ND	ND
	day 45	ND	ND	10.00	0.0060	88.01	0.0526	ND	ND
	day 90	ND	ND	14.58	0.0066	81.32	0.0365	ND	ND
muscle with skin	12 h	ND	ND	ND	ND	97.56	0.0761	1.30	0.0010
	day 7	ND	ND	5.88	0.0035	91.20	0.0535	1.81	0.0011
	day 15	ND	ND	6.06	0.0035	93.16	0.0531	ND	ND
	day 45	ND	ND	14.04	0.0056	84.49	0.0337	ND	ND
	day 90	ND	ND	15.25	0.0036	82.94	0.0193	ND	ND

[a] **7**, *N*-desmethylemamectin B_{1a}; 11, *N*-formylemamectin B_{1a}. [b] % TRR, % distribution of the metabolite in the extract. [c] ND, not detected.

TRR at 12 h post final dose to 11−17% TRR by day 90. Another component (^{3}H component **11**, *N*-formylemamectin B_{1a}) was inconsistently observed at low levels (<2% TRR) in several extracts. No other significant residues were observed in tissues. **Tables 3** and **4** also include concentration estimates for each characterized component.

Representative ^{3}H radiochromatograms of liver extracts (12 h and 90 day) are presented in **Figure 8**. HPLC analysis of 12-h and 90-day gut contents indicated that emamectin B_{1a} and its desmethyl metabolite were the primary residues, although several minor residues were also present. HPLC analysis of feces collected during dosing showed emamectin B_{1a} as essentially the only component, whereas analysis of pooled feces collected from 0 to 7 days post final dose indicated the presence of several minor components of 2−10% TRR each in addition to emamectin B_{1a} at ∼57% TRR (**Table 4**).

Method Performance for the Determinative Residue Assay. One unfortified control and two SCH 58854 fortified control samples (at 40 and 200 ppb of emamectin B_{1a}) were analyzed for each full set of muscle and skin tissue samples. The procedural recoveries for the emamectin B_{1a} muscle control samples fortified at 40 ppb (LLOQ) ranged from 87.9 to 96.1% with a mean and standard deviation of 90.9 ± 2.9% (N = 6). The procedural recoveries for the emamectin B_{1a} skin control samples fortified at 40 ppb ranged from 78.4 to 88.7% with a mean and standard deviation of 86.5 ± 5.6% (N = 4). A summary of the procedural (i.e., QC) recoveries is presented in **Table 5**. Emamectin B_{1a} was not detected in any of the control samples. Standard curve linearity was excellent for all assay intervals (r^2 > 0.99). A system suitability was determined for each set of analyses as described in the determinative assay (*10*), and the data demonstrated the stability of the analysis system throughout the analysis. The retention time for emamectin B_{1a} was established to be ∼10.29 min. The levels of detection (LODs) have been determined as 2.6 and 3.3 ppb for muscle and skin, respectively (*10*).

Tissue Levels of Marker Residue. The tissues assayed for the marker residue, emamectin B_{1a}, obtained from the [^{3}H]SCH 58854 metabolism study, were considered to be appropriate to demonstrate the decline of the marker residue. This was so because the fish were dosed in the same manner as proposed for commercial use and the [^{3}H]SCH 58854 residues were found to be stable (*10*). Although 10 fish were sampled at each post dose interval, only 5 individual skin samples remained from the metabolism study for some intervals—the other 5 skin samples were used for the metabolite profiling (see Extraction of Residues for Metabolite Profiling). Emamectin B_{1a} in muscle ranged from 32 to 67 ppb at 3 h, from 2.6 to 58 ppb for 12 h, from 18 to 60 ppb at 24 h, from 24 to 64 ppb at 72 h, from 12 to 55 ppb at 7 days, from 19 to 48 ppb at 15 days, and from 13 to 39 ppb at 30 days. The fish containing the residue level of 2.6 ppb at a 12 h withdrawal time was observed as premature fish during the in-life phase of the study. The mean ratio of the marker to total radioactive residue in muscle was rather constant, ranging from 0.66 to 0.73. The individual results, including marker residue to total radioactive residue ratios as well as mean and standard deviations, are reported in **Table 6**. Emamectin B_{1a} in skin ranged from 37 to 84 ppb at 3 h, from 3.3 to 68 ppb at 12 h, from 24 to 74 ppb at 24 h, from 23 to 84 ppb at 72 h, from 15 to 48 ppb at 7 days, from 31 to 61 ppb at 15 days, and from 16 to 59 ppb at 30 days. The mean ratio of the marker to total residue in skin was also rather constant, ranging from 0.56 to 0.66. The individual results for skin, including marker residue to total residue ratios as well as mean and standard deviations, are reported in **Table 7**. Representative HPLC chromatograms are presented in **Figure 9**. The ratio of emamectin B_{1a} as

Table 4. Distribution of [^{3}H]Emamectin B_{1a} and Metabolites[a] in Gut Contents and Feces As Determined by ^{3}H Radiochromatography (HPLC)

matrix	interval post final dose	**2** ($t_R \sim$ 12–13.7 min)		**3** ($t_R =$ 16.5–17.8 min)		**4** ($t_R =$ 17.5 –18.8 min)		**5** ($t_R =$ 20.5 –21.5 min)		**7** ($t_R =$ 25.0–26.9 min)	
		% TRR[b]	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
gut contents	12 h	ND[c]	ND	5.76	0.0323	4.81	0.0269	1.98	0.0111	5.25	0.0294
	day 7	ND	ND	3.58	0.0253	1.90	0.0134	ND	ND	8.16	0.0577
	day 15	ND	ND	6.20	0.0227	4.28	0.0156	ND	ND	9.16	0.0335
	day 45	ND	ND	6.63	0.0193	4.07	0.0119	ND	ND	15.08	0.0440
	day 90	ND	ND	5.57	0.0108	2.63	0.0051	ND	ND	23.52	0.0456

matrix	interval post final dose	emamectin B_{1a} ($t_R =$ 26.5–28.5 min)		**8** ($t_R =$ 29.0–30.0 min)		**9** ($t_R =$ 34.0 min)		**10** ($t_R =$ 36.5 min)		**11** ($t_R =$ 38.0–39.5 min)	
		% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
gut contents	12 h	66.83	0.3742	3.16	0.0177	2.83	0.0158	3.10	0.0174	ND	ND
	day 7	82.94	0.5862	ND	ND	ND	ND	ND	ND	ND	ND
	day 15	71.57	0.2617	2.71	0.0099	ND	ND	ND	ND	ND	ND
	day 45	65.55	0.1912	2.50	0.0073	ND	ND	ND	ND	ND	ND
	day 90	59.41	0.1152	3.41	0.0066	ND	ND	ND	ND	ND	ND

matrix	extract. a/b	**1** ($t_R =$ 3.5 min)		**2** ($t_R =$ 12–13.7 min)		**3** ($t_R =$ 16.5 –17.8 min)		**4** ($t_R =$ 17.5 –18.8 min)		**5** ($t_R =$ 20.5 –21.5 min)	
		% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
feces during dosing	a	ND	ND	ND	ND	0.55	0.0006	1.15	0.0013	ND	ND
	b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	av	ND	ND	ND	ND	0.28	0.0003	0.58	0.0007	ND	ND
feces post dose	a	ND	ND	4.66	0.0017	11.44	0.0042	5.22	0.0019	1.51	0.0006
	b	7.34	0.0027	14.44	0.0053	ND	ND	ND	ND	ND	ND
	av	3.67	0.0014	9.55	0.0035	5.72	0.0021	2.61	0.0010	0.76	0.0003

matrix	extract. a/b	**7** ($t_R =$ 25.0–26.9 min)		emamectin B_{1a} ($t_R =$ 26.5–28.5 min)		**8** ($t_R =$ 29.0–30.0 min)		**10** ($t_R =$ 36.5 min)		**11** ($t_R =$ 38.0–39.5 min)	
		% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
feces during dosing	a	ND	ND	96.64	0.1054	ND	ND	ND	ND	ND	ND
	b	0.29	0.0003	98.04	0.1070	ND	ND	ND	ND	ND	ND
	av	0.15	0.0002	97.34	0.1062	ND	ND	ND	ND	ND	ND
feces post dose	a	6.88	0.0025	54.90	0.0201	4.21	0.0015	2.74	0.0010	2.97	0.0011
	b	5.30	0.0019	58.67	0.0215	3.12	0.0011	2.14	0.0008	4.04	0.0015
	av	6.09	0.0022	56.79	0.0208	3.67	0.0013	2.44	0.0009	3.51	0.0013

[a] **7**, *N*-desmethylemamectin B_{1a}; **8**, 8,9-*Z*-emamectin B_{1a}; **10**, *N*-desmethyl-*N*-formylemamectin B_{1a}; **11**, *N*-formylemamectin B_{1a}. [b] % TRR = % distribution of TRR in the extract. [c] ND, not detected.

Table 5. Procedural (Quality Control) Recovery Summary for Analysis of Residues by HPLC–Fluorometry

	muscle fortified at						skin fortified at					
	40 ppb			200 ppb			40 ppb			200 ppb		
analysis set	1	2	av[a]	1	2	av[a]	1	2	av[a]	1	2	av[a]
1	90.1	87.9	89.0	92.4	88.0	90.2						
2	89.8	96.1	93.0	93.4	89.1	91.3						
3	89.5	91.8	90.7	87.8	84.6	86.2						
4							87.9	88.7	88.3	80.0	81.1	80.6
5							78.4	91.0	84.7	83.3	86.6	85.0
mean			90.9			89.2			86.5			82.8
SD ($n-1$)[b]			2.85			3.24			5.56			2.91
CV%			3.14			3.63			6.43			3.51
% bias			–9.1			–10.8			–13.5			–17.2

[a] Average of replicate data per each assay day. [b] $n = 6$ for muscle and $n = 4$ for skin.

determined by HPLC−fluorometry in the determinative assay to the TRR is significantly less than 1 despite the high extractability of ^{3}H residues and the high proportion of [^{3}H]-emamectin B_{1a} as determined by HPLC−radiometry (**Tables 6** and **7**). This difference is accounted for by the expression of TRR in the study as emamectin B_1 benzoate equivalents, whereas emamectin B_1 benzoate is only ~80% emamectin B_{1a} free base and recoveries of 83−91% were obtained in the determinative assay (**Table 5**).

DISCUSSION

Emamectin B_{1a}, the major homologue of emamectin B_1 benzoate, when administered in SLICE-medicated feed to Atlantic salmon, is readily absorbed and distributed to tissues

Table 6. Emamectin B_{1a} (MAB_{1A}) Concentrations in Muscle

fish	collection time interval[a]	TRR[b] ng/g (ppb)	marker residue[c] (ppb)	mean SD *n*	ratio marker vs total	mean SD *n*
1	3 h	96.4	67		0.70	
2	3 h	60.2	44		0.73	
3	3 h	68.2	49		0.72	
4	3 h	54.5	39*		0.72	
5	3 h	59.9	49		0.82	
6	3 h	65.2	47		0.72	
7	3 h	55.1	40		0.73	
8	3 h	42.8	32*	*45*	0.75	*0.73*
9	3 h	53.1	40	*10*	0.75	*0.04*
10	3 h	56.8	38*	*10*	0.67	*10*
11	12 h	51.5	36*		0.70	
12	12 h	76.9	39*		0.51	
13	12 h	60.9	56		0.92	
14	12 h	62.7	47		0.75	
15[d]	12 h	1.5	2.6**		NC[e]	
16	12 h	32.6	23*		0.71	
17	12 h	45.6	37*		0.81	
18	12 h	66.5	38*	*37*	0.57	*0.71*
19	12 h	51.7	38*	*16*	0.74	*0.12*
20	12 h	82.6	57	*10*	0.69	*9*
21	24 h	76.1	57		0.75	
22	24 h	59.4	42		0.71	
23	24 h	67.4	38*		0.56	
24	24 h	25.5	18*		0.71	
25	24 h	31.5	22*		0.70	
26	24 h	41.1	26*		0.63	
27	24 h	44.8	35*		0.78	
28	24 h	82.1	60	*37*	0.73	*0.70*
29	24 h	55.6	40	*14*	0.72	*0.06*
30	24 h	49.8	35*	*10*	0.70	*10*
31	72 h	70.4	57		0.81	
32	72 h	82.2	64		0.78	
33	72 h	62.0	46		0.74	
34	72 h	81.6	62		0.76	
35	72 h	33.7	24*		0.71	
36	72 h	54.0	35*		0.65	
37	72 h	57.7	41		0.71	
38	72 h	93.9	58	*46*	0.62	*0.71*
39	72 h	70.3	47	*14*	0.67	*0.06*
40	72 h	42.2	28*	*10*	0.66	*10*
41	7 days	60.3	46		0.76	
42	7 days	59.4	40		0.67	
43	7 days	64.4	43		0.67	
44	7 days	53.2	39*		0.73	
45	7 days	78.8	55		0.70	
46	7 days	25.0	15*		0.60	
47	7 days	52.2	30*		0.57	
48	7 days	18.1	12*	*33*	0.66	*0.68*
49	7 days	43.4	32*	*14*	0.74	*0.06*
50	7 days	29.9	20*	*10*	0.67	*10*
51	15 days	67.2	45		0.67	
52	15 days	65.3	38*		0.58	
53	15 day	58.0	38		0.66	
54	15 days	30.2	19*		0.63	
55	15 days	46.2	32*		0.69	
56	15 days	46.6	33*		0.71	
57	15 days	35.8	25*		0.70	
58	15 days	36.8	32*	*35*	0.87	*0.68*
59	15 days	52.0	35*	*9*	0.67	*0.08*
60	15 days	72.5	48	*10*	0.66	*10*
61	30 days	51.9	36*		0.69	
62	30 days	30.4	21*		0.69	
63	30 days	47.2	32*		0.68	
64	30 days	30.7	17*		0.55	
65	30 days	56.5	39*		0.69	
66	30 days	20.5	13*		0.63	
67	30 days	19.7	14*		0.71	
68	30 days	33.9	22*	*24*	0.65	*0.66*
69	30 days	42.4	29*	*9*	0.68	*0.05*
70	30 days	32.5	21*	*10*	0.65	*10*

[a] Interval post final dose. [b] Total radioactive residue (TRR) value obtained from the metabolism study. [c] Concentration of emamectin B_{1a} free base determined using the determinative HPLC–fluorometric method (*10*). *, Number is less than the lower limit of quantitation (LLOQ) of 40 ppb. **, LOD = 2.6 ppb (*10*). [d] Premature fish. Marker residue value assigned as LOD for calculation of mean. [e] NC, not calculated.

Table 7. Emamectin B_{1a} (MAB_{1A}) Concentrations in Skin

fish	collection time interval[a]	TRR[b] ng/g (ppb)	marker residue[c] (ppb)	mean SD *n*	ratio marker vs total	mean SD *n*
1	3 h	140.3	84		0.60	
2	3 h	83.1	57		0.69	
3	3 h	92.6	62		0.67	
4	3 h	67.0	43		0.64	
5	3 h	76.6	58		0.76	
6	3 h	89.5	53		0.59	
7	3 h	85.4	49		0.57	
8	3 h	61.6	37*	*54*	0.60	*0.64*
9	3 h	73.7	46	*13*	0.62	*0.06*
10	3 h	73.2	49	*10*	0.67	*10*
11	12 h	67.0	43		0.64	
15[d]	12 h	0.8	3.3**		NC[e]	
16	12 h	41.1	28*	*35*	0.68	*0.65*
17	12 h	55.2	31*	*24*	0.56	*0.07*
18	12 h	96.1	68	*5*	0.71	*4*
21	24 h	124.8	74		0.59	
22	24 h	81.6	45		0.55	
23	24 h	61.8	60		0.97	
24	24 h	34.7	24*		0.69	
25	24 h	35.5	24*		0.68	
26	24 h	57.8	38*		0.66	
27	24 h	56.8	37*		0.65	
28	24 h	87.3	59	*45*	0.68	*0.66*
29	24 h	68.2	41	*16*	0.60	*0.13*
30	24 h	84.0	43	*10*	0.51	*10*
31	72 h	94.5	67		0.71	
32	72 h	127.2	80		0.63	
33	72 h	103.9	66		0.64	
34	72 h	132.0	83		0.63	
35	72 h	49.2	27*		0.55	
36	72 h	82.2	45		0.55	
37	72 h	69.6	47		0.68	
38	72 h	132.3	84	*59*	0.63	*0.63*
39	72 h	99.6	66	*22*	0.66	*0.05*
40	72 h	36.4	23*	*10*	0.63	*10*
42	7 days	84.0	48		0.57	
44	7 days	74.1	45		0.61	
46	7 days	29.6	16*	*29*	0.54	*0.56*
48	7 days	27.1	15*	*16*	0.55	*0.04*
50	7 days	45.4	23*	*5*	0.51	*5*
41	7 days	60.3	46		0.76	
42	7 days	59.4	40		0.67	
43	7 days	64.4	43		0.67	
44	7 days	53.2	39*		0.73	
45	7 days	78.8	55		0.70	
46	7 days	25.0	15*		0.60	
47	7 days	52.2	30*		0.57	
48	7 days	18.1	12*	*33*	0.66	*0.68*
49	7 days	43.4	32*	*14*	0.74	*0.06*
50	7 days	29.9	20*	*10*	0.67	*10*
51	15 days	67.2	45		0.67	
52	15 days	65.3	38*		0.58	
53	15 days	58.0	38		0.66	
54	15 days	30.2	19*		0.63	
55	15 days	46.2	32*		0.69	
56	15 days	46.6	33*		0.71	
57	15 days	35.8	25*		0.70	
58	15 days	36.8	32*	*35*	0.87	*0.68*
59	15 days	52.0	35*	*9*	0.67	*0.08*
60	15 days	72.5	48	*10*	0.66	*10*
61	30 days	51.9	36*		0.69	
62	30 days	30.4	21*		0.69	
63	30 days	47.2	32*		0.68	
64	30 days	30.7	17*		0.55	
65	30 days	56.5	39*		0.69	
66	30 days	20.5	13*		0.63	
67	30 days	19.7	14*		0.71	
68	30 days	33.9	22*	*24*	0.65	*0.66*
69	30 days	42.4	29*	*9*	0.68	*0.05*
70	30 days	32.5	21*	*10*	0.65	*10*

[a] Interval post final dose. [b] Total radioactive residue (TRR) value obtained from the metabolism study. [c] Concentration of emamectin B_{1a} free base determined using the determinative HPLC–fluorometric method (*10*). [d] Premature fish. Marker residue value assigned as LOD for calulation of mean. *, number is less than the lower limit of quantitation (LLOQ) of 40 ppb. **, LOD = 3.3 ppb (*10*). [e] NC, not calculated.

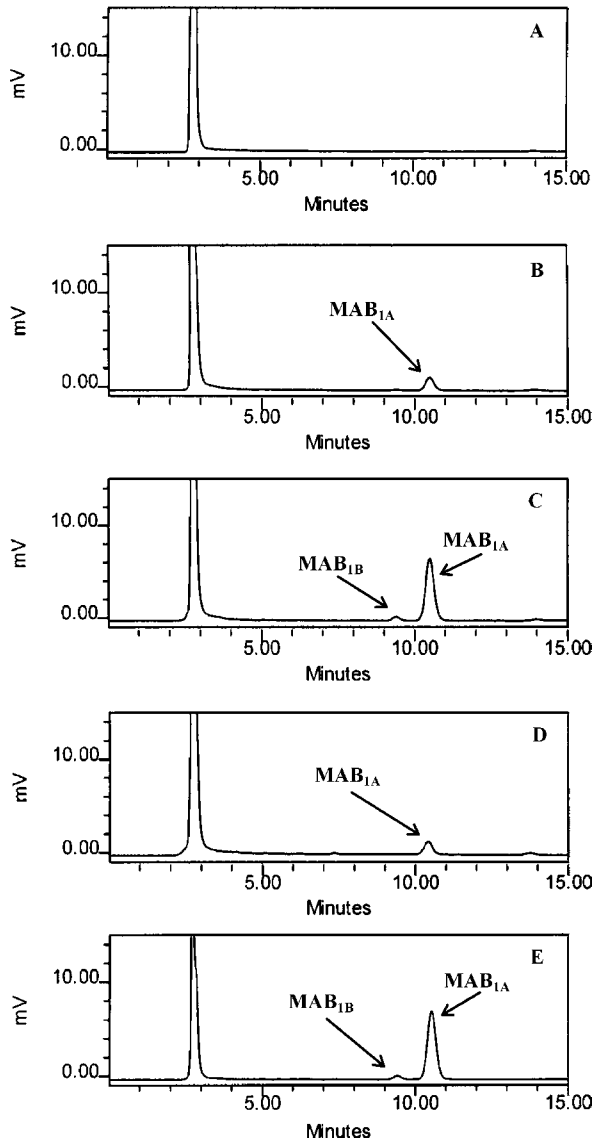


Figure 9. Representative chromatograms for HPLC–fluorometric assay: (**A**) control muscle; (**B**) fortified muscle at 40 ppb; (**C**) fortified muscle at 200 ppb; (**D**) incurred residue muscle at 36 ppb (fish 61); (**E**) calibration standard at 20 ng/mL (equivalent to 200 ppb).

with excretion in feces largely complete by 1 day post dose and essentially all of the excreted residues being emamectin B_{1a}. Tissue residues decline rather slowly, apparently due to sustained enterohepatic recirculation as evidenced by several peaks in the tissue residue levels and the presence of residues in bile and gut contents long after dosing. The relatively slow depletion of a similar compound, ivermectin, was also observed from the tissues of Atlantic salmon treated with [^{3}H]ivermectin (*12*). Emamectin B_{1a} levels in skin and muscle are known to follow a similar depletion pattern suggestive of enterohepatic recirculation in SLICE-treated rainbow trout (*Onchorynchus mykiss*), a closely related species (Roy, unpublished results). Metabolism of emamectin B_{1a} is rather limited, with the major and essentially only metabolite, desmethylemamectin B_{1a}, accounting for <20% of the total radioactive residue in tissues out to 90 days post final dose and mostly considerably <20%. The reported prolonged efficacy of SLICE against sea lice (*4*−*8*) is consistent with the sustained residue level and high proportion of emamectin B_{1a} in the major tissues of attack, skin and muscle, seen in this study.

The metabolism of emamectin B_{1a} in Atlantic salmon was very similar to that reported for the rat (*13*) in which emamectin B_{1a} and its desmethyl metabolite constituted essentially all of the residues in tissues and feces after oral administration, although the desmethyl metabolite was at a somewhat higher proportion in the rat at earlier intervals post dose. Therefore, the rat serves as a good toxicological model for evaluation of dietary exposure to residues of emamectin B_{1a} from the consumption of Atlantic salmon. Although only [^{3}H]emamectin B_{1a} was used to study metabolism in Atlantic salmon, metabolism of both [^{3}H]ememectin B_{1a} and [^{14}C]emamectin B_{1a} in the rat indicated that the ^{3}H label was metabolically stable. The metabolism of emamectin B_{1a} has also been reported for bluegill sunfish exposed to [^{3}H]emamectin B_{1a} in water for 28 days (*14*). TRR levels in bluegill sunfish fillets after 28 days of exposure (0.04 ppm) were comparable to those observed for muscle and skin of Atlantic salmon. The TRR in bluegill sunfish was completely extractable after 28 days exposure, and the major residues were emamectin B_{1a} (63%) and the desmethyl metabolite (14%) with the remainder accounted for by multiple minor metabolites of <5% each. Therefore, the metabolism of emamectin B_{1a} in bluegill sunfish (*Leopmis machrochirus*, order Perciformes) is quite similar to that in Atlantic salmon (*Salmo salar*, order Salmoniformes) despite their being unrelated fish species.

The marker residue, emamectin B_{1a}, varied over an ~3-fold concentration range in muscle and skin at each post dose interval, perhaps due at least in part if not mostly to variable medicated feed consumption. The MRL of 100 ppb for emamectin B_{1a} in muscle (with skin attached) of Atlantic salmon is the maximum level considered to be safe for consumption of emamectin benzoate-treated Atlantic salmon by the European Union. The mean level of emamectin B_{1a} in separate muscle (which very closely approximates that of muscle with skin attached) did not exceed 46 ppb, nor did any individual muscle specimen exceed 64 ppb at any time from 1 day to 90 days post final dose. Therefore, even after dosing at ~33 μg of emamectin benzoate/kg/day for 7 days rather than the therapeutic 50 μg/kg and the recovery of the method (~90%; *10*) is accounted for, the residue levels in the edible tissue of Atlantic salmon maintained at 5 ± 1 °C resulting from use of SLICE are safe. Note that the residue levels obtained by radioactive assay are expressed in parts per million, whereas the levels obtained by the determinative assay are expressed in parts per billion.

ACKNOWLEDGMENT

We express our sincere gratitude to Matthew J. Wisocky and John Ryan for their dedicated and excellent technical support in the successful completion of the project.

LITERATURE CITED

(1) Campbell, W. C.; Fisher, M. H.; Stapley, E. O.; Albers-Schönberg, G.; Jacob, T. A. Ivermectin: A Potent New Antiparasitic Agent. *Science* **1983**, *221*, 823−830.

(2) Mrozik, H.; Eskola, P.; Arison, B. H.; Linn, B. O.; Lusi, A.; Matzuk, A.; Shih, T. L.; Tischler, M.; Waksmanski, F. S.; Wyvratt, M. J.; Blizzard, T. J.; Margiatto, G. M.; Fisher, M. H.; Shoop, W. L.; Egerton, J. R. 4″-Deoxy-4″-aminoavermectins with potent broad spectrum antiparasitic activities. *Bioorg. Med. Chem.* **1995**, *5*, 2435−2440.

(3) Lasota, J. A.; Dybas, R. A. Avermectins, a Novel Class of Compounds: Implications for Use in Anthropod Pest Control. *Annu. Rev. Entomol.* **1991**, *36*, 91−117.

(4) Stone, J.; Sutherland, I. H.; Sommerville, C.; Richards, R. H.; Varma, K. J. The Efficacy of Emamectin Benzoate as an Oral Treatment of Sea Lice, *Lepeophtheirus salmonis* (Kroyer), Infestations in Atlantic Salmon, *Salmo Salar* L. *J. Fish Dis.* **1999**, *22*, 261−270.

(5) Stone, J.; Sutherland, I. H.; Sommerville, C.; Richards, R. H.; Varma, K. J. Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus Nordmann*, infestations in Atlantic salmon, *Salmo salar* L. *Aquaculture* **2000**, *186*, 205−219.

(6) Stone, J.; Sutherland, I. H.; Sommerville, C.; Richards, R. H.; Varma, K. J. Commercial trials using emamectin benzoate to control sea lice *Lepeophtheirus salmonis* infestations in Atlantic salmon, *Salmo salar*. *Dis. Aquat. Org.* **2000**, *41*, 141−149.

(7) Stone, J.; Sutherland, I. H.; Sommerville, C.; Richards, R. H.; Endris, R. G. The duration of efficacy following oral treatment with emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis* (Krøyer) in Atlantic salmon, *Salmo Salar* L. *J Fish Dis.* **2000**, *23*, 185−192.

(8) Armstrong, R.; MacPhee, D.; Katz, T.; Endris, R. A field efficacy evaluation of emamectin benzoate for the control of sea lice on Atlantic salmon. *Can. Vet. J.* **2000**, *41*, 607−612.

(9) Anonymous. Committee for Veterinary Medicinal Products, Emamectin Summary Report. *EMEA, The European Agency of the Evaluation of Medicinal Products; Veterinary Medicines Evaluation Unit*; Jan 1999.

(10) Kim-Kang, H.; Crouch, L. S.; Bova, A.; Robinson, R. A.; Wu, J. Determination of Emamectin Residues in the Tissues of Atlantic Salmon (*Salmo salar* L.) Using HPLC with Fluorescence Detection. *J. Agric. Food Chem.* **2001**, *49*, 5294−302.

(11) Tway, P. C.; Wood, J. S.; Downing, G. V. Determination of Ivermectins in Cattle and Sheep Tisues Using High-Performance Liquid Chromatography with Fluorescence Detection. *J. Agric. Food Chem*, **1981**, *29*, 1059−1063.

(12) Hoy, T.; Horseberg, T. E.; Nafstad, I. The Disposition of Ivermectin in Atlantic salmon (*Salmo salar*). Pharmacol. Toxicol. **1990**, *67*, 307−312.

(13) Mushtaq, M.; Syintsakos, L. R.; Krieter, P. A.; Colletti, A.; Arison, B.; Crouch, L. S.; Wislocki, P. G. Absorption, Tissue Distribution, Excretion, and Metabolism of ^{3}H- and ^{14}C-Labeled Emamectin Benzoate in Rats. *J. Agric. Food Chem.* **1996**, *44*, 3342−3349.

(14) Chukwudebe, A. C.; Andrew, N.; Drottar, K.; Swigert, J.; Wislocki, P. G. Bioaccumulation Potential of 4″-epi-(Methylamino)-4″-deoxyavermectin B1a Benzoate (Emamectin Benzoate) in Bluegill Sunfish. *J. Agric. Food Chem*, **1996**, *44*, 2894−2899.

Received for review December 5, 2003. Revised manuscript received January 29, 2004. Accepted February 2, 2004. Presentation at the 12th North American International Society of Study of Xenobiotics (ISSX), October 2003. The project was funded by Schering-Plough Animal Health.

JF035435V