JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Residue Depletion of Nitrofuran Drugs and Their Tissue-Bound Metabolites in Channel Catfish (*Ictalurus punctatus*) after Oral Dosing

PAK-SIN CHU,*,† MAYDA I. LOPEZ,† ANN ABRAHAM,‡ KATHLEEN R. EL SAID,‡ AND STEVEN M. PLAKAS‡

Center for Veterinary Medicine, U.S. Food and Drug Administration, 8401 Muirkirk Road, Laurel, Maryland 20708, and Gulf Coast Seafood Laboratory, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, P.O. Box 158, 1 Iberville Drive, Dauphin Island, Alabama 36528

The depletion of the nitrofuran drugs furazolidone, nitrofurazone, furaltadone, and nitrofurantoin and their tissue-bound metabolites [3-amino-2-oxazolidinone (AOZ), semicarbazide (SC), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), and 1-aminohydantoin (AH), respectively] were examined in the muscle of channel catfish following oral dosing (1 mg/kg body weight). Parent drugs were measurable in muscle within 2 h. Peak levels were found at 4 h for furazolidone (30.4 ng/g) and at 12 h for nitrofurazone, furaltadone, and nitrofurantoin (104, 35.2, and 9.8 ng/g respectively). Parent drugs were rapidly eliminated from muscle, and tissue concentrations fell below the limit of detection (1 ng/g) at 96 h. Peak levels of tissue-bound AMOZ and AOZ (46.8 and 33.7 ng/g respectively) were measured at 12 h, and of SC and AH (31.1 and 9.1 ng/g, respectively) at 24 h. Tissue-bound metabolites were measurable for up to 56 days postdose. These results support the use of tissue-bound metabolites as target analytes for monitoring nitrofuran drugs in channel catfish.

KEYWORDS: Nitrofurans; channel catfish; LC/MS-MS; depletion; bound residues

INTRODUCTION

Nitrofuran drugs (NFs, **Figure 1**) have been used since the 1940s in human and veterinary medicine, principally for treatment of bacterial and protozoan infections (*1*). Due to mutagenicity and carcinogenicity concerns, many countries, including the United States (*2*), have banned the use of NFs in food-producing animals. However NFs continue to be a regulatory concern, as violative residues are still being found in animal food products.

Previous studies have demonstrated that parent NFs deplete rapidly in animals and that NFs are extensively metabolized to tissue-bound metabolites (*3–5*). As such, newer analytical methods have been directed to the determination of the intact side-chains of tissue-bound residues, instead of the parent NFs. To date, methods have been described for animal tissues (*6–9*), honey (*10, 11*), shrimp (*12*), eggs (*13*), and milk (*14*). Despite the availability of analytical methods, few studies have addressed the residue depletion of NFs and of their tissue-bound metabolites in aquatic animal species. Although Stehly et al. (*15*) examined the pharmacokinetics, tissue distribution, and metabolism of ^{14}C-nitrofurantoin in channel catfish after intravenous and oral dosing, and Plakas et al. (*16*) of ^{14}C-furazolidone in channel catfish, these studies were conducted with the use of radiotracer techniques over relatively short withdrawal times. Studies investigating the persistence of bound residues over prolonged withdrawal periods and the usefulness of using bound residues as marker residues of NFs for regulatory purposes are needed.

The present study examines the depletion of furazolidone, nitrofurazone, furaltadone, and nitrofurantoin, and of their tissue-bound metabolites [3-amino-2-oxazolidinone (AOZ), semicarbazide (SC), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), and 1-aminohydantoin (AH), respectively], from muscle of channel catfish following oral dosing. Tissue-bound metabolites were monitored for up to eight weeks after dosing by using LC-MS/MS. Residues resulting from waterborne exposures to NFs were also examined.

MATERIALS AND METHODS

Reagents. LC grade water was purified in-house with a Milli-Q Plus water system for use in preparation of all solutions. ACS-grade ammonium acetate (NH_4OAc), anhydrous potassium phosphate dibasic (K_2HPO_4), *N*,*N*-dimethylformamide (DMF), polyethylene glycol (PEG, mol. wt. 200), and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich (St. Louis, MO), and glacial acetic acid (HOAc) from J. T. Baker (Phillipsburg, PA). Methanol (MeOH), hexane, acetonitrile (CH_3CN), and ethyl acetate (EtOAc) were high purity solvents obtained from Burdick & Jackson (Muskegon, MI). Dimethyl sulfoxide (DMSO) and reagent alcohol (containing 95% 200 proof ethanol and 5% isopropyl alcohol) were obtained from EM Science (Gibbstown, NJ).

* To whom correspondence should be addressed. Phone: 301-210-4583. Fax: 301-210-4653. E-mail: pak.chu@fda.hhs.gov.
† Center for Veterinary Medicine.
‡ Gulf Coast Seafood Laboratory.

10.1021/jf801398p
Published on Web 08/13/2008

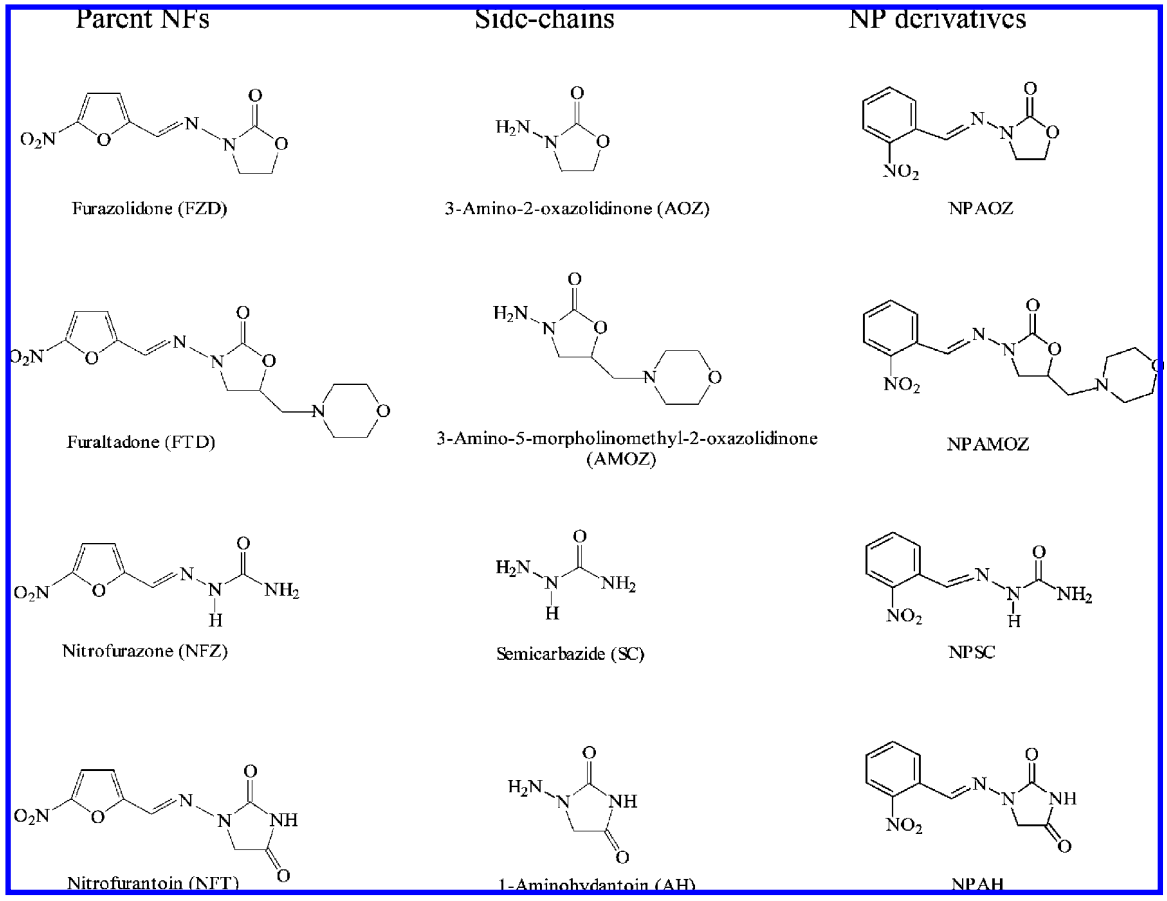

Figure 1. Structures of parent nitrofurans (NFs), their side-chains, and nitrophenyl derivatives.

Nitrofurazone, furazolidone, furaltadone, nitrofurantoin, 1-aminohydantoin hydrochloride, semicarbazide hydrochloride, and 2-nitrobenzaldehyde (2-NBA) were purchased from Sigma-Aldrich. AOZ and AMOZ were obtained from WITEGA (Berlin, Germany). The internal standards semicarbazide-$^{13}C,^{15}N_2$ (SC+3) hydrochloride, and 3-amino-2-oxazolidinone-d_4 (AOZ-d_4) were obtained from Sigma-Aldrich while 3-amino-5-morpholinomethyl-2-oxazolidinone-d_5 (AMOZ-d_5) was obtained from WITEGA. In this paper, the letters NP are added to the abbreviated notation for the side chains when referring to their nitrophenyl derivatives (e.g., SC vs NPSC).

Standard Solutions for Tissue-Bound Metabolites. Standard solutions (100 μg/mL) of AOZ, SC, AH, and AMOZ were prepared by dissolving appropriate weights of each solid standard in methanol. All standards are expressed as the un-ionized moiety equivalent. Each standard was weighed to the nearest 0.1 mg into respective 100 mL volumetric flask and brought to the mark with MeOH. These solutions were immediately used to prepare an intermediate mixed standard solution consisting of 2 μg/mL of each analyte by dilution with methanol. Working standard solutions (0.2 μg/mL and 0.04 μg/mL) were prepared by diluting the intermediate mixed standard solution with methanol. Internal standard solutions (AOZ-d_4, AMOZ-d_5, and SC+3) were prepared in a similar manner, except that smaller quantities were weighed. All standard solutions were stored at −20 °C until use.

Standard Solutions for Parent NFs. Solid standards (5.0 mg) of nitrofurazone, furazolidone, furaltadone, and nitrofurantoin were weighed, dissolved in 10 mL DMF, and diluted to 50 mL with CH_3CN to give 100 μg/mL stock solutions. Mixed-standard intermediate solutions (0.125−4 μg/mL) were prepared by diluting in CH_3CN, and working standards (5−160 ng/mL) by dilution with water. All standard solutions were protected from light, and stored at −20 °C until use.

Liquid Chromatograph (for Parent NFs). The LC system consisted of Shimadzu Corp. (Kyoto, Japan) SCL-10A system controller, LC-10AS pumps, SCL-10A autoinjector, CTO-10A column oven, and SPD-10AV UV−vis spectrophotometric detector, with EZChrom chromatography data system. The LC column was an Inertsil ODS-3 5 μm, 4.6 × 150 mm, with a guard column (MetaGuard) of the same packing (Varian Inc., Walnut Creek, CA).

Liquid Chromatograph-Tandem Mass Spectrometer (for Bound Residues). The liquid chromatography system consisted of two Perkin-Elmer (Norwalk, CT) Series 200 micro pumps and a Perkin-Elmer Series 200 autosampler equipped with a 100 μL loop. The LC column was an Inertsil ODS-3 5 μm, 150 × 2.1 mm with a guard column of the same packing (Varian Inc., Walnut Creek, CA). A precolumn filter (Upchurch, Oak Harbor, WA) was installed between the autosampler and the guard column.

An Applied Biosystems (Foster City, CA) Sciex API 2000 triple quadrupole mass spectrometer with an atmospheric pressure chemical ionization (APCI) source in the positive ion mode was used. The protonated molecules, $[M+H]^+$ at m/z 249 (NPAH), 209 (NPSC), 236 (NPAOZ), and 335 (NPAMOZ) were selected as the precursor ions for collision induced dissociation (CID). The following product ions were identified for selected reaction monitoring (SRM) LC/MS-MS analysis: NPAH: m/z 249 → 134, 104, 178; NPSC: m/z 209 → 166, 192, 134; NPAOZ: m/z 236 → 134, 104, 149; NPAMOZ: m/z 335 → 291, 262, 128. For the internal standards, the following transitions were selected for SRM: NPSC+3, m/z 212 → 168; NPAOZ-d_4, m/z 240 → 134; NPAMOZ-d_5, m/z 340 → 296. The dwell time for each monitored transition was 150 ms. The source temperature and ion spray voltage were set at 350 °C and 5.5 kV, respectively. Quantitation was performed using internal standard ratio method. Peak areas for quantitation were computed by summing the areas of the product ions of the respective analytes upon integration using PE-Sciex Analyst version 1.3.2 software. The corresponding isotopically labeled compounds were used as internal standards for AOZ, SC, and AMOZ. SC+3 was used as the internal standard for AH, as an isotopically labeled analogue was not available. A weighting of $1/x$ for area responses of standards provided the best linear fit of data. A negative control and a fortified control at 2 ng/g were processed together with each sample set.

Dosing and Sampling. *Oral Dosing.* Drug-free channel catfish (*Ictalurus punctatus*), mean body weight 0.56 kg, were obtained from a local fish farm (Brewton, AL), and acclimated for a minimum of two weeks at the Gulf Coast Seafood Laboratory in 350-gal holding tanks. On the day before dosing, fish were randomly selected and placed in 75-gal Living Stream tanks (Frigid Units Inc., Toledo, OH) equipped with activated carbon filtration system, five fish per tank. Mean water temperature was 19 °C and pH 7.8. Dosing solutions (light protected) for each drug were prepared by solubilizing 100 mg in 10 mL of DMF, and diluting to 50 mL with PEG, to give 2 mg/mL active drug. Fish were dosed orally at 1 mg/kg body weight by delivering a gelatin capsule (size No. 00, Eli Lilly and Co., Indianapolis, IN) containing the dose solution (0.5 mL/kg body weight) and 0.25 g ground catfish feed (Purina Mills Inc., St. Louis, MO) directly into the stomachs of anesthetized (tricaine methanesulfonate, MS 222, Sigma-Aldrich) animals. Fish ($n = 5$) were sampled at each of the following time points per drug: 2, 4, 8, and 12 h; and 1, 4, 7, 10, 14, 28, and 56 days after dosing. Each Living Stream tank was dedicated to a single withdrawal period per given drug and reused as necessary to accommodate all sampling time points. Fish were killed by cervical dislocation while under anesthesia. Muscle tissues (skinless fillets) were collected, homogenized, and frozen at −80 °C in 50-mL polypropylene centrifuge tubes.

Bath Treatment. To examine tissue-bound residues after waterborne exposure, groups of five channel catfish were exposed in a bath treatment containing 10 mg/L of the individual NFs for 8 h. After treatment at zero-hour withdrawal time, fish were rinsed briefly and killed under anesthesia. The muscle tissue was harvested, homogenized, and stored for analysis as above.

Extraction and Analysis of Parent NFs Using LC-UV. Extraction of parent NFs from muscle tissues followed the method of Rupp et al. (*17*). Briefly, parent NFs were extracted from the tissue with CH_3CN, and the tissue lipids removed from the extracts with hexane. Extracts were evaporated by rotary evaporation, and the dried residues were redissolved in 90:10 water/CH_3CN. The mixture was sonicated, centrifuged, and filtered. Extracts were analyzed for parent NFs using the following isocratic LC conditions: mobile phase, 10 mM NH_4OAc in 0.02% HOAc/CH_3CN (90/10); flow rate, 1 mL/min; column temperature, 40 °C; UV detector, 368 nm; and injection volume, 50 μL. The limit of detection and the limit of quantitation of parent NFs in muscle were estimated to be 1 and 2.5 ng/g, respectively.

Extraction and Analysis of Tissue-Bound Metabolites Using LC-MS/MS. The extraction procedure was a modification of that described for shrimp (*12*). In brief, channel catfish tissues (2.0 ± 0.2 g) were weighed into 50 mL polypropylene centrifuge tubes. Five mL of 70% aqueous MeOH were added, and the tubes vortex-mixed for 15 s or until the pellets were dispersed. After centrifugation at 15 °C for 5 min at 4000 rpm (∼3300*g*), the supernatants were decanted and discarded. The remaining pellets were sequentially washed with 5 mL each of ethyl acetate and reagent alcohol (200 proof ethanol) by vortex-mixing and centrifugation. The supernatants were decanted and discarded. Fortification of tissue samples and preparation of calibration standards were performed at this step. A calibration standard curve bracketing the unknown concentrations was prepared by adding the appropriate volume of the fortification solution to 50 mL centrifuge tubes. One hundred μL of the mixed internal standard (equivalent to 2 ng/g of AMOZ-d_5, 5.9 ng/g of AOZ-d_4 and 8 ng/g SC+3 in tissue) was added to all samples, including the control and the standards. To the tissue samples and calibration standards were added 10 mL of 0.125 M HCl and 400 μL of freshly prepared 2-NBA solution (50 mM in DMSO). The samples were vortex-mixed for 15 s and placed in a 37 °C water bath overnight (∼14−16 h) with gentle shaking. One milliliter of 0.1 M K_2HPO_4 was added to the samples, and the pH of each adjusted to 7.1−7.5 by adding 0.8 M aqueous NaOH. The tissue samples (but not the calibration standards) were centrifuged at 4000 rpm for 5 min at 4 °C. The supernatants were decanted into respective 20 mL reservoirs equipped with filtering frits and attached to an SPE manifold. A slight vacuum was needed to start the flow. The filtrates were collected into 50 mL polypropylene tubes. To avoid analyte loss, the remaining pellets were washed twice with 3 mL of water. The tubes were vigorously vortex-mixed to break up the pellets and centrifuged at 4000 rpm for 5 min at 4 °C. The supernatants were decanted into their respective reservoirs and filtered. All samples (including the calibration standards) were adjusted to 20−22 mL by adding Milli-Q water. The actual volume could vary from set to set; however, all samples within the set were made to the same volume. Approximately 1.5 g of NaCl, 3 mL of reagent alcohol, and 10 mL of hexane were added. The samples were gently hand-shaken and centrifuged at 4 °C for 10 min at 4000 rpm for phase separation. The top hexane layers were removed and discarded. The remaining aqueous layers were partitioned three times with 4 mL of EtOAc by hand-shaking. After centrifugation for 5 min at 4 °C and 4000 rpm, the EtOAc layers were transferred into 15 mL polypropylene centrifuge tubes. The combined EtOAc layers were washed twice with 1 mL of Milli-Q water. The bottom aqueous layers were discarded. The remaining EtOAc layers were evaporated to dryness using a Zymark evaporator at 40 °C. To prevent analyte loss on the tube wall, EtOAc or MeOH (2 mL) was added to the samples followed by vortex-mixing and evaporation to dryness. The residues were dissolved in 200 μL of the LC mobile phase. After vortex-mixing, sonication for 2 min, and vortex-mixing again, the final extracts were filtered through 0.2 μm Gelman nylon filters into autosampler vials with 300 μL inserts.

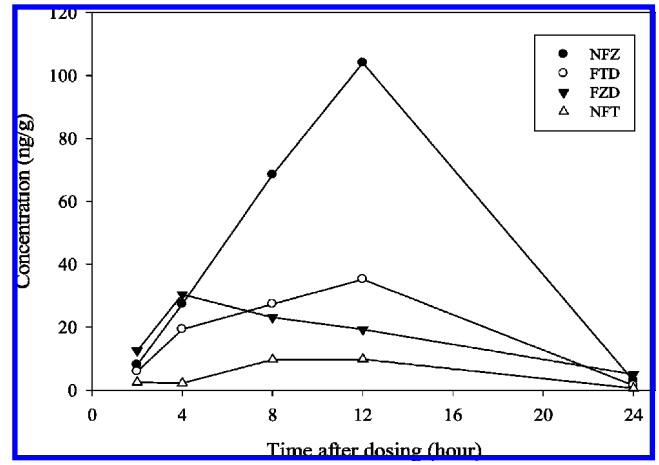

Figure 2. Depletion of parent NFs in muscle of channel catfish after oral dosing (1 mg/kg body weight) with furazolidone, nitrofurazone, furaltadone, and nitrofurantoin.

Extracts were analyzed for bound residues of NFs using the following isocratic LC conditions: mobile phase, 55% MeOH/45% 20 mM NH_4OAc and flow rate, 200 μL/min. Typical injection sequence was as follows: a water blank to equilibrate the LC system, 40 μL of each calibration standard, a sample set, and the standards again. At the end of each day, the analytical and guard columns were flushed with water-MeOH (10 + 90, v/v).

The limits of detection were calculated as defined in the U.S. Pharmacopeia-National Formulary (*18*) and estimated to be 0.1, 0.1, 0.2, and 0.1 ng/g for AOZ, SC, AH, and AMOZ, respectively. The limits of quantitation were estimated to be 0.1, 0.2, 0.3, and 0.1 ng/g for AOZ, SC, AH, and AMOZ, respectively.

RESULTS AND DISCUSSION

Parent NFs were found in the muscle of channel catfish within 2 h after oral dosing (**Figure 2**). Peak concentrations were found at 4 h for furazolidone (30.4 ng/g), and at 12 h for nitrofurazone, furaltadone, and nitrofurantoin (104, 35.2, and 9.8 ng/g, respectively). Thereafter, parent NFs were rapidly eliminated from muscle, with concentrations ≤5 ng/g at 24 h. At 96 h, parent NFs were not detectable (<1 ng/g). These results are consistent with those of Plakas et al. (*16*) and Stehly et al. (*15*), where parent furazolidone and nitrofurantoin were not detectable in channel catfish muscle at 24 h after dosing.

Tissue-bound metabolites of NFs were rapidly formed in the muscle of channel catfish after oral dosing, with peak levels at

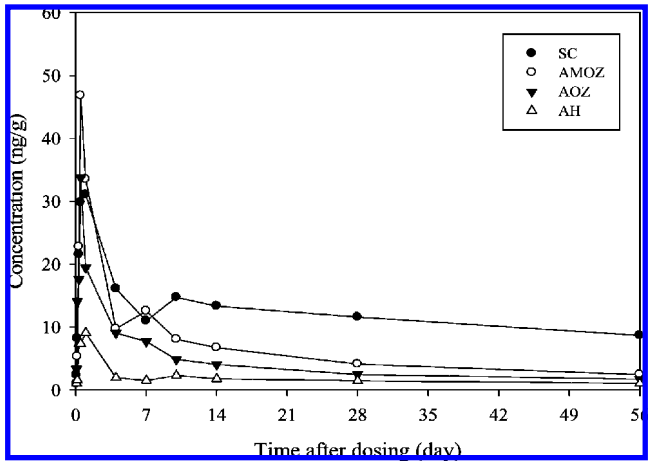

Figure 3. Depletion of tissue-bound residues of NFs in muscle of channel catfish after oral dosing (1 mg/kg body weight) with furazolidone, nitrofurazone, furaltadone, and nitrofurantoin.

Table 1. Mean Levels of Parent NFs and Their Tissue-Bound Residues in Muscle of Channel Catfish after Waterborne Exposure (10 mg/L for 8 h) to Furazolidone, Nitrofurazone, Furaltadone, and Nitrofurantoin

parent drug	parent drug conc (ng/g) ± SD	metabolite	metabolite conc (ng/g) ± SD
FZD	401 ± 127	AOZ	203 ± 41
NFZ	61 ± 31	SC	18 ± 4
FTD	46 ± 13	AMOZ	36 ± 9
NFT	3 ± 2	AH	2 ± 2

12 or 24 h (**Figure 3**). Highest mean concentrations of AMOZ, AOZ, SC, and AH were 46.8, 33.7, 31.1, and 9.1 ng/g, respectively. Elimination of bound residues was biphasic, with residues measurable throughout the 56-day study. In the terminal phase of elimination, SC levels were highest, followed by AMOZ, AOZ, and AH. The terminal phase half-lives, calculated from the semilogarithm plots of tissue concentration versus time using least-squares regression, were 63, 27, 32, and 45 days for SC, AMOZ, AOZ, and AH, respectively. These values were estimates, as accurate measurement of the terminal half-lives required an extended sampling period of 3 to 5 half-lives. Determination of tissue concentrations at such prolonged withdrawal times was not feasible because of our method sensitivity.

In the earlier work of Plakas et al. (*16*), tissue-bound residues of furazolidone in channel catfish muscle were highest at 8 h after oral dosing, compared with 12 h in the present study. In the previous study, bound residues were measured by using radiolabeled drug (i.e., as total bound radioactivity), over a relatively short time frame. The half-life of tissue-bound radioactive residues was 97 h between 8 and 168 h after dosing. The half-life of tissue-bound AOZ, as measured by LC-MS/MS, was 79 h over the comparable time frame (12 to 168 h), representing the initial phase of biphasic elimination curve (**Figure 3**).

We examined tissue-bound metabolites in muscle of catfish following waterborne exposure to NFs. Significant amounts of parent NFs and their tissue-bound metabolites were found, reflecting absorption of NFs by the branchial route (**Table 1**). Parent drug levels of furazolidone were highest, followed by nitrofurazone, furaltadone, and nitrofurantoin. Bound residue levels of AOZ were highest, followed by AMOZ, SC, and AH.

Residue depletion of NFs in muscle of channel catfish is characterized by rapid elimination of parent drugs and slow elimination of tissue-bound metabolites, a pattern similar to that in mammalian species. In mammals, NFs are known to undergo nitro-reduction to reactive intermediates, which are capable of binding covalently to amino acids, peptides, and proteins, resulting in the formation of bound residues in muscle tissue. The highly reactive intermediates are purportedly hydroxyaminofurans or nitroso derivatives; however, their true identities have not been characterized due to extreme lability (*3, 19*). The detection of bound residues and their persistence for extended periods as found in the present study suggest similar metabolic pathways for NFs in channel catfish. This view is further supported by Washburn and Di Giulio's findings of nitroreductase enzyme activity in channel catfish, and of superoxide production upon exposure to nitrofurantoin, as in mammalian systems (*20*).

Of the four NF drugs studied, parent nitrofurantoin and its tissue-bound metabolite AH in catfish muscle were consistently in the lowest concentrations after oral dosing. Similar findings have been reported for shrimp (*12*), bovine milk (*14*), chicken muscle (*21*), and chicken eggs (*22*). Low absorption of nitrofurantoin is a plausible explanation. However, Buzard et al. (*23*) demonstrated that nitrofurantoin is well absorbed through the small intestine of rat, and that the lower oral bioavailability was due to the gastric degradation in the stomach. Differences in metabolism and renal clearance may also be involved. In a study with rats, Paul et al. (*1*) found 52% of the administered dose of nitrofurantion was excreted unchanged in the urine, compared with 4.6% of nitrofurazone, 3.4% of furaltadone, and trace amounts of furazolidone. In previous studies with channel catfish, similar excretion patterns were found, where nitrofurantion (*15*) was excreted largely unchanged in the urine, compared with little or no unchanged furazolidone (*16*). These studies indicate that the disposition of nitrofurantoin differs considerably from that of the other NFs, perhaps attributable to differences in their physiochemical properties. Nitrofurantoin is anionic, whereas furaltadone is cationic, and furazolidone and nitrofurazone relatively neutral (*1*). Possibly, because of its anionic characteristic, nitrofurantoin is more readily excreted in the urine, leading to lower tissue concentrations compared with the other NFs. In channel catfish exposed to NFs as a bath treatment, where drug absorption may differ from that of the oral route, concentrations of parent nitrofurantoin and its bound metabolite in muscle were also lowest among the four NFs.

The persistence of tissue-bound residues in edible tissues of channel catfish is of regulatory concern. Previous studies conducted by Vroomen et al. (*3*) and by Gottschall et al. (*4*) have shown that when swine muscle tissue containing nonextractable radioactive residues of furazolidone were fed to rat, the nonextractable radioactive residues became bioavailable and tissue-bound. These studies underscore the importance of characterizing the identity and understanding the toxicity of the tissue-bound metabolites.

In summary, the residue depletion of NFs and their bound residues in muscle of channel catfish is similar to those of terrestrial animals. Bound residues are highly persistent, detectable for up to 56 days after oral dosing, while parent drugs are rapidly eliminated. Among the four NFs examined, nitrofurantoin exhibits the lowest levels of parent drug and tissue-bound metabolites.

ACKNOWLEDGMENT

We thank Cristina Nochetto for conducting the data audit for this study.

LITERATURE CITED

(1) Paul, M. F.; Paul, H. E.; Bender, R. C.; Kopko, F.; Harrington, C. M.; Ells, V. R.; Buzard, J. A. Studies on the distribution and excretion of certain nitrofurans. *Antibiot. Chemother.* **1960**, *10*, 287–302.

(2) *Federal Register*. Topical Nitrofurans; Extralabel Animal Drug Use; Order of Prohibition. *Fed. Regist.* **2002**, *67*, 5470−5471.

(3) Vroomen, L. H.; Berghmans, M. C.; van Bladeren, P. J.; Groten, J. P.; Wissink, C. J.; Kuiper, H. A. *In vivo* and *in vitro* metabolic studies of furazolidone: a risk evaluation. *Drug Metab Rev.* **1990**, *22*, 663–676.

(4) Gottschall, D. W.; Wang, R. Depletion and bioavailability of [14C]furazolidone residues in swine tissues. *J. Agric. Food Chem.* **1995**, *43*, 2520–2525.

(5) McCracken, R. J.; McCoy, M. A.; Kennedy, D. G. Furazolidone residues in pigs: criteria to distinguish between treatment and contamination. *Food Addit. Contam.* **2000**, *17*, 75–82.

(6) Leitner, A.; Zöllner, P.; Lindner, W. Determination of the metabolites of nitrofuran antibiotics in animal tissue by high-performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr., A* **2001**, *939*, 49–58.

(7) Conneely, A.; Nugent, A.; O'Keeffe, M.; Mulder, P. P. J.; van Rhijn, J. A.; Kovacsics, L.; Fodor, A.; McCracken, R. J.; Kennedy, D. G. Isolation of bound residues of nitrofuran drugs from tissue by solid-phase extraction with determination by liquid chromatography with UV and tandem mass spectrometric detection. *Anal. Chim. Acta* **2003**, *483*, 91–98.

(8) McCracken, R. J.; Blanchflower, W. J.; Rowan, C.; McCoy, M. A.; Kennedy, D. G. Determination of furazolidone in porcine tissue using thermospray liquid chromatography-mass spectrometry and a study of the pharmacokinetics and stability of its residues. *Analyst* **1995**, *120*, 2347–2351.

(9) Hoogenboom, L. A. P.; Polman, Th. H. G. Simultaneous Detection of Protein Bound Residues of the Nitrofuran Drugs Furazolidone, Furaltadone, Nitrofurazone and Nitrofurantoin. In *Proceedings of EuroResidue II Conference*; Haagsma, N.; Ruiter, A.; Czedik-Eysenberg, P. B., Eds.; EuroResidue II Conference: Veldhoven, The Netherlands, 1993; pp 376−381.

(10) Khong, S. P.; Gremaud, E.; Richoz, J.; Delatour, T.; Guy, P. A.; Stadler, R. H.; Mottier, P. Analysis of matrix-bound nitrofuran residues in worldwide-originated honeys by isotope dilution high-performance liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 5309–5315.

(11) Lopez, M. I.; Feldlaufer, M. F.; Williams, A. D.; Chu, P. S. Determination and Confirmation of Nitrofuran Residues in Honey Using LC-MS/MS. *J. Agric. Food Chem.* **2007**, *55*, 1103–1108.

(12) Chu, P. S.; Lopez, M. I. Liquid chromatography-tandem mass spectrometry for the determination of protein-bound residues in shrimp dosed with nitrofurans. *J. Agric. Food Chem.* **2005**, *53*, 8934–8939.

(13) McCracken, R. J.; Spence, D. E.; Floyd, S. D.; Kennedy, D. G. Evaluation of the residues of furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in eggs. *Food Addit. Contam.* **2001**, *18*, 954–959.

(14) Chu, P. S.; Lopez, M. I. Determination of nitrofuran residues in milk of dairy cows using liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 2129–2135.

(15) Stehly, G. R.; Plakas, S. M. Pharmacokinetics, tissue distribution, and metabolism of nitrofurantoin in the channel catfish (*Ictalurus punctatus*). *Aquaculture* **1993**, *113*, 1–10.

(16) Plakas, S. M.; El Said, K. R.; Stehly, G. R. Furazolidone disposition after intravascular and oral dosing in the channel catfish. *Xenobiotica* **1994**, *24*, 1095–1105.

(17) Rupp, H. S.; Munns, R. K.; Long, A. R.; Plakas, S. M. Simultaneous determination of nitrofurazone, nitrofurantoin, and furazolidone in channel catfish (*Ictalurus punctatus*) muscle tissue by liquid chromatography. *J. AOAC Int.* **1994**, *77*, 344–350.

(18) *U.S. Pharmacopeia-National Formulary 1990*, U.S. Pharmacopeia Convention: Rockville, MD, 1989; p 1711.

(19) Boyd, M. R.; Witschi, H. P. Biochemical mechanisms in chemical-induced lung injury: roles of metabolic activation. *Crit. Rev. Toxicol.* **1980**, *7*, 103–176.

(20) Washburn, P. C.; Di Giulio, R. T. Nitrofurantoin-stimulated superoxide production by channel catfish (*Ictalurus punctatus*) hepatic microsomal and soluble fractions. *Toxicol. Appl. Pharmacol.* **1988**, *95*, 363–377.

(21) McCracken, R. J.; van Rhijn, J. A.; Kennedy, D. G. Transfer of nitrofuran residues from parent broiler breeder chickens to broiler progeny. *Br. Poult. Sci.* **2005**, *46*, 287–292.

(22) McCracken, R. J.; Kennedy, D. G. Detection, accumulation and distribution of nitrofuran residues in egg yolk, albumen and shell. *Food Addit. Contam.* **2007**, *24*, 26–33.

(23) Buzard, J. A.; Conklin, J. D.; O'Keefe, E.; Paul, M. F. Studies on the absorption, distribution and elimination of nitrofurantoin in the rat. *J. Pharmacol. Exp. Ther.* **1961**, *131*, 38–43.

Received for review May 5, 2008. Revised manuscript received June 24, 2008. Accepted June 25, 2008.

JF801398P