Metabolism and residue depletion of albendazole and its metabolites in rainbow trout, tilapia and Atlantic salmon after oral administration

B. SHAIKH
N. RUMMEL
C. GIESEKER
S. SERFLING &
R. REIMSCHUESSEL

Food and Drug Administration, Center for Veterinary Medicine, Office of Research, Laurel, MD, USA

Shaikh, B., Rummel, N., Gieseker, C., Serfling, S., Reimschuessel, R. Metabolism and residue depletion of albendazole and its metabolites in rainbow trout, tilapia and Atlantic salmon after oral administration. *J. vet. Pharmacol. Therap.* **26**, 421–427.

Metabolic and residue depletion profiles of albendazole (ABZ) and its major metabolites in three fish species, rainbow trout, tilapia and Atlantic salmon are reported. Based on these profiles, similarities (or dissimilarities) between species will determine the potential to group fish species. ABZ at 10 mg/kg body weight was incorporated into fish food formulated in a gelatin base or in gel capsule and fed as a single dose to six fish from each species. Rainbow trout were held three each in a partitioned 600-L tank. Tilapia and Atlantic salmon were housed in separate 20-L tanks. Samples of muscle with adhering skin were collected at 8, 12, 18, 24, 48, 72, and 96 h postdose from trout kept at 12 °C, at 4, 8, 12, 24, 48, 72, 96, 120, and 144 h postdose from tilapia kept at 25 °C and at 8, 14, 24, 48, 72, and 96 h postdose from Atlantic salmon kept at 15 °C. The samples were homogenized in dry ice and subjected to extraction and cleanup procedures. The final extracts were analyzed for parent drug ABZ and its major metabolites, albendazole sulfoxide (ABZ-SO), albendazole sulfone (ABZ-SO_2) and albendazole aminosulfone using high-performance liquid chromatography with fluorescence detection. ABZ was depleted by 24 h in trout and tilapia and by 48 h in salmon; ABZ-SO, a pharmacologically active metabolite, was depleted by 48 h in tilapia, by 72 h in rainbow trout and was present until 96 h in salmon; and low levels of ABZ-SO_2 and albendazole aminosulfone, both inactive metabolites, were detectable at least till 96 h in all three fish species.

(Paper received 16 April 2003; accepted for publication 25 September 2003)

Badar Shaikh, Food and Drug Administration, Center for Veterinary Medicine, Office of Research, 8401 Muirkirk Road, Laurel, MD 20708, USA. E-mail: bshaikh@cvm.fda.gov

INTRODUCTION

Albendazole (ABZ) is a potent broad-spectrum benzimidazole anthelmintic agent widely used against intestinal helminth infections in mammals (McKellar & Scott, 1990). ABZ is metabolized reversibly to its major active metabolite albendazole sulfoxide (ABZ-SO) by liver microsomal enzymes (Galtier *et al.*, 1986). ABZ-SO is further oxidized irreversibly to an inactive metabolite albendazole sulfone (ABZ-SO_2) (Lacey, 1990; Lanusse *et al.*, 1992). The carbamate group of ABZ-SO_2 is further deacetylated to form a polar and inactive metabolite, albendazole-2-aminosulfone (ABZ-2-NH_2SO_2) (Lubega & Prichard, 1991). Figure 1 shows proposed major metabolites of ABZ. ABZ-SO, like parent ABZ, is also marketed as ricobendazole as it has significant anthelmintic activity (Formentini *et al.*, 2001).

Albendazole metabolism has been studied in a variety of animals. In sheep, the parent ABZ was not detected in plasma at any time after oral dosing; however, ABZ-SO and ABZ-SO_2 were recovered up to 60 h post-treatment (Lanusse *et al.*, 1995). In goat milk, a third metabolite, albendazole aminosulfone along with ABZ-SO and ABZ-SO_2 were all prevalent up to 48 h after oral dosing (Cinquina *et al.*, 1997). Following a single oral dose in chickens, ABZ concentration peaked at 2.5 h and was detected up to 6 h in plasma. However, two of its major metabolites, ABZ-SO and ABZ-SO_2 were detected up to 24 and 30 h, respectively (Csiko *et al.*, 1996).

In a radiolabel study, nine metabolites of ABZ were identified in the urine of cattle, sheep, rats, and mice (Gyurik *et al.*, 1981) after oral administration with ^{14}C-ABZ. Very little unchanged ABZ was excreted in urine. However, ABZ-SO,

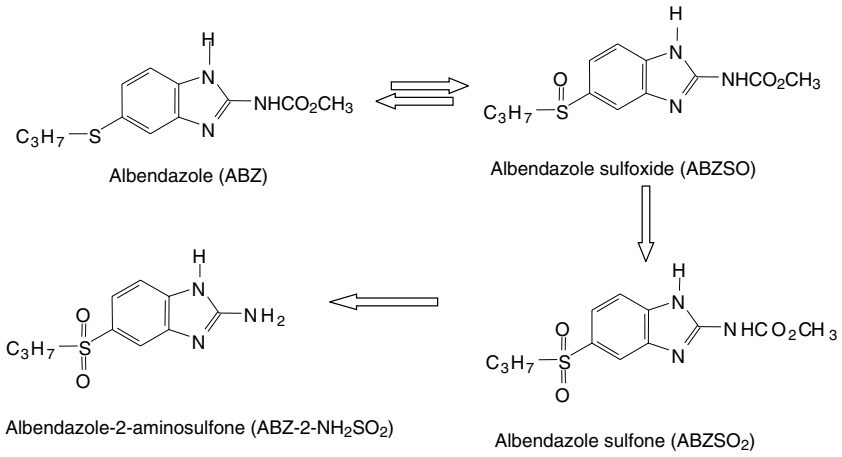

Fig. 1. Structures of albendazole and its metabolites.

$ABZ\text{-}SO_2$, $ABZ\text{-}2\text{-}NH_2SO_2$ and alkyl, and aromatic hydroxylation products were identified in the urine.

Little information is available on the metabolism and pharmacokinetic behavior of benzimidazoles in fish species. Fenbendazole (FBZ) and its metabolites, FBZ-SO, $FBZ\text{-}SO_2$ and FBZ-OH were detected in a number of tissues and fluids after oral administration to channel catfish. $FBZ\text{-}2\text{-}NH_2SO_2$ metabolite was not detected in any of the samples (Kitzman *et al.*, 1990). ABZ and FBZ at a dosage of 5 and 8 mg/kg, respectively, were administered orally to Atlantic salmon and killed over 28 days for depletion analysis (Nafstad *et al.*, 1991). ABZ could not be detected in the muscle tissue, and its metabolites ABZ-SO and $ABZ\text{-}SO_2$ could only be detected in very low concentrations on day 1 after administration. FBZ and its metabolites were detectable only up to day 2. Depletion of FBZ in muscle and skin tissues of rainbow trout over 120 h after oral treatment was investigated (Iosifidou *et al.*, 1997). Parent drug FBZ was detected in both muscle and skin up to 96 h. Its metabolite, FBZ-SO could only be detected in skin up to 48 h and $FBZ\text{-}SO_2$ could not be detected either in muscle or skin tissue samples.

Toxicological studies in both farm and laboratory animals have shown ABZ and its active metabolite ABZ-SO to be teratogenic (Delatour & Parish, 1986).

The metabolism and residue depletion of a drug obtained from single species have been used to assess human food safety. As conducting such studies on every potential fish species is quite expensive, a number of efforts are being made to identify species with similar metabolic profiles. The goal is to identify model species in each group, which will be used to predict food safety issues for the entire group. This study presents a potential approach to assess the feasibility of species (crop) grouping in farmed finfish by identifying species with similar metabolic and residue depletion profiles. Accordingly, ABZ, a member of benzimidazole group of anthelminitics was chosen as a model drug and orally administered to three different species, Atlantic salmon, rainbow trout and tilapia, held under three different environmental temperatures. The residue profiles and concentrations of parent drug and its major metabolites, ABZ-SO, $ABZ\text{-}SO_2$ and $ABZ\text{-}2\text{-}NH_2SO_2$ in muscle with adhering skin tissue for the three finfish are reported.

MATERIALS AND METHODS

Animals

Fingerlings of rainbow trout (*Oncorhynchus mykiss*), tilapia hybrid (*Oreochromis nilotica* × *O. mosambicus*) and Atlantic salmon (*Salmo salar*) were obtained from local farms. Atlantic salmon and rainbow trout were housed in a number of 2000-L and tilapia in 400-L re-circulating round fiber glass tanks containing fresh water. All fish including Atlantic salmon were cultured in freshwater, as our facilities were not equipped to handle salt water. Fish were provided *ad lib* with commercially available diet and maintained at 12 ± 2, 25 ± 2, and 15 ± 2 °C water temperature for rainbow trout, tilapia and Atlantic salmon, respectively. The pH of the water was maintained at 7.5 ± 0.5. The fish were cultured for over a year until they reached the weight range suitable for dosing experiments, totaling 49 rainbow trout, 49 tilapia and 42 Atlantic salmon. Seven fish from each species, six for dosing and one as a control at each sampling time were used. The average weights (±SD) were: rainbow trout, 285 ± 50 g; tilapia, 254 ± 54 g; Atlantic salmon, 896 ± 114 g. At a later stage, 14 additional tilapia with a mean weight of 613 ± 126 g were included in the experiment for sample collection at two additional, 120 and 144 h, withdrawal times.

The animal experiments in this study were approved by the CVM Institutional Animal Care and Use Committee.

Preparation of gel-food

Gel-food was prepared by mixing gelatin (unflavored Knox) with 50 mL cold water. To this was added about 40 g of pulverized fish chow and one ripped up sheet of Nori and mixed until homogenous. Then about 60 mL of boiling water was added. The solution was mixed well, poured into plastic molds and

refrigerated solid. Gel-food was cut into ~1 cm square pieces and stored in zip-lok bags at −20 °C until used.

Preparation of dose

A dose of 10 mg/kg was used. This dose has been recommended to be effective for removal of internal parasites in cattle (McKellar & Scott, 1990; Code of Federal Regulations, 21, 2000) and also has been used to study metabolism of ABZ in chickens (Csiko *et al.*, 1996) and sheep (Gyurik *et al.*, 1981). For rainbow trout and tilapia, the dose was prepared in gel-food squares. For Atlantic salmon, it was prepared in gel capsules. Gel-food squares were defrosted and a hole was made in the center using a cork borer. ABZ powder was weighed in a plastic boat and transferred into the hole, which was then plugged with the gel-food. Gel-food squares containing drug were inserted into glass or living streams (LS) tanks holding fish. In most cases fish jumped up and accepted these as food. In cases where fish did not accept the food, a new fish was used and dosing procedure repeated.

Oral treatment

Rainbow trout and Atlantic salmon were weighed and allocated to about 600-L LS tanks (Frigid Units, Toledo, OH, USA). Tilapia were weighed and each was transferred to a separate 80-L flow-through glass tanks to be held individually. The LS tanks were partitioned into three compartments to hold three fish, one in each compartment at a time. The fish were fed drug free gel-food squares and allowed to acclimate for about 14 days and denied food 2 days before dosing. Six fish from each species were orally dosed with ABZ at 10 mg/kg body weight. ABZ was incorporated into gel-food squares and individually fed to trout and tilapia as described above. For salmon, ABZ was weighed into gel-capsules, #4 (Torpac, Fairfield, NJ, USA), and administered to fish via stomach tube with manual restraint. The fish were killed with a sharp blow on the head followed by decapitation with a sharp knife. One control and six treated fish from each species were killed at 8, 12, 24, 48, 72, and 96 h after dosing. Fourteen additional tilapia were dosed and killed at two additional sampling time periods. Muscle fillets with adhering skin were collected and stored at −80 °C until analyzed.

Apparatus

The liquid chromatographic system consisted of Hewlett-Packard (HP) Model 1050 system (Palo Alto, CA, USA) fitted with a quaternary pump, autosampler, Agilent Series1100 fluorescence detector, set at 290 and 330 nm excitation and emission wavelengths, respectively, HP ChemStation software, HP Laser Jet 5000N printer, and a Dell Optiplex GX1 computer. The analytical (150 × 4.6 mm) and guard (4 × 3 mm) columns employed were Luna C18 (2) and ODS C18, respectively (Phenomenex, Torrence, CA, USA), with a packing of five μm particles. Both the analytical and guard columns were used at ambient temperature. All centrifugations were carried out at 4100 RCF using a swing-out rotor (M4) in a Jouan CR 422 refrigerated centrifuge (Jouan Inc., Winchester, VA, USA) set at 4 °C for 15 min. Conical bottom tubes (polypropylene, 15 mL) were used (Corning Glass Works, Corning, NY, USA). All liquid transfers were made with Eppendorf digital pipettes. Eberbach shaker (Eberbach Corp., Ann Arbor, MI, USA), Zymark Turbo Vac LV evaporator (Zymark Corp., Hopkinton, MA, USA) and Tru-Sweep Ultrasonic cleaner (Crest Ultrasonic Corp, Trenton, NJ, USA) were used during cleanup steps.

Chemicals and reagents

Glass-distilled organic solvents (Burdick & Jackson Laboratories, Muskegon, MI, USA) and water from Milli-Q plus Ultra-Pure Water System (Millipore Corporation, Bedford, MA, USA) were used. Dimethyl sulfoxide, sodium metabisulfite, ammonium acetate, and ABZ were purchased from Sigma Chemical Company (St Louis, MO, USA). Glacial acetic acid and potassium carbonate were from J.T. Baker (Phillipsburg, NJ, USA). ABZ metabolites, ABZ-SO, ABZ-SO_2 and albendazole aminosulfone were a gift from Pfizer (Groton, CT, USA). ABZ-SO_2 was also procured from Lancaster Synthesis (Pelham, NH, USA).

High-performance liquid chromatography (HPLC) mobile phase

A stock solution of 0.5 M ammonium acetate, pH 5, was prepared by adding 38.6 g of ammonium acetate to 500 mL of water in a 1-L glass volumetric flask. Ten milliliter of acetic acid was added and the flask was taken to volume with additional water and mixed. A mobile phase buffer of 0.05 M ammonium acetate was prepared by transferring 50 mL of stock 0.5 solution into a 500-mL flask; additional water was added to reach the mark and mixed. An isocratic mobile phase combination of acetonitril/methanol/0.05 M buffer in the ratio of 30:15:55 and 17:8:75 was used for ABZ and metabolites, respectively.

Sample extraction, cleanup and HPLC analysis

Muscle fillets with adhering skin, stored at −80 °C, were semi-defrosted and blended for further processing as reported previously (Shaikh *et al.*, 2003). One gram each of control, fortified, and incurred muscle tissue samples from each species was used for the assay. The sample extraction, cleanup and HPLC analysis was carried out by a procedure developed and reported by our laboratory (Shaikh *et al.*, 2003).

Typically a four-point standard curve was constructed for ABZ and three of its metabolites on each day of the assay. These curves were used to quantitate incurred muscle tissue samples. The concentration ranges for these standard curves for ABZ, ABZ-SO, ABZ-SO_2 and ABZ-2-NH_2SO_2, were 20–200, 5–100, 0.5–10 and 5–100 p.p.b., respectively, and were linear with average coefficient of determinations, $r^2 = 0.995$.

During the analysis of incurred samples of each species, quality control samples comprising a control and a fortified control muscle were run. Accordingly, control muscle tissues were fortified with ABZ and its metabolites at various concentration

ranges and carried through extraction, cleanup and analysis procedures along with the study samples for each species. Mean recoveries of ABZ (25–100 p.p.b.), ABZ-SO (15–62 p.p.b.), ABZ-SO_2 (1–10 p.p.b.), and ABZ-2-NH_2SO_2 (20–100 p.p.b.) for rainbow trout were 99, 79, 94, and 69%, respectively; for Atlantic salmon were 86, 81, 90, and 75%, respectively and for tilapia were 100, 76, 84, and 72% respectively.

RESULTS AND DISCUSSION

The median and the range of concentration levels of ABZ and its metabolites, ABZ-SO, ABZ-SO_2, and ABZ-2-NH_2SO_2 found in the muscle with adhering skin tissue of Atlantic salmon, rainbow trout and tilapia after oral administration are shown in Tables 1–3, respectively. The range of concentrations obtained at each sampling point is highly variable; therefore the values are given as median instead of mean. The limits of quantitation (LOQ) for ABZ, ABZ-SO, ABZ-SO_2, and ABZ-2-NH_2SO_2 were 20, 1.6, 0.5 and 5 μg/kg (p.p.b.), respectively.

In both tilapia and rainbow trout, ABZ was detected until 12 h post-treatment; however, it was present until 24 h in Atlantic salmon. This indicates a slower metabolism of ABZ in Atlantic salmon as compared with tilapia and rainbow trout. The absence of ABZ in five of six Atlantic salmon is also perhaps due to the slower metabolism of ABZ at 8 h postdose. The highest median concentration was obtained at the 8 h sampling time in both tilapia and rainbow trout and at 24 h in Atlantic salmon. This suggests that, like in mammals (Marriner & Bogan, 1980; Prichard *et al.*, 1985; Delatour *et al.*, 1990), ABZ is poorly absorbed and the absorbed drug is metabolized quickly by the three fish species.

Albendazole sulfoxide, a pharmacologically active metabolite of ABZ, was detected until 48 h in both tilapia and rainbow trout and until 96 h in Atlantic salmon. In tilapia and rainbow trout the median concentration was highest at 8 and 12 h post-treatment, respectively, whereas in Atlantic salmon it was at 24 h sampling time. ABZ-SO_2 and albendazole aminosulfone, the two inactive metabolites of ABZ, were also detected. Both ABZ-SO_2 and ABZ-2-NH_2SO_2 were present at least until 96 h post-treatment in the three fish species. Although in tilapia, the median/mean concentration of ABZ-2-NH_2SO_2 at 96 h sampling time was below its tolerance for cattle in muscle (Code of Federal Regulations, 21, 2000),

Table 1. Concentrations of ABZ, ABZ-SO, ABZ-SO_2, and ABZ-2-NH_2SO_2 in the muscle and skin tissue of Atlantic salmon at various withdrawal times (WD) after oral treatment with ABZ

WD (h)	ABZ (μg/kg)*	$N^{\dagger}$	ABZ-SO (μg/kg)*	$N^{\dagger}$	ABZ-SO_2 (μg/kg)*	$N^{\dagger}$	ABZ-2-NH_2SO_2 (μg/kg)*	$N^{\dagger}$
8	m^1	1	5 (12)	3	2 (3)	6	blq	0
14	20 (27)	3	21 (33)	6	5 (6)	6	blq	0
24	23 (42)	6	69 (129)	6	12 (23)	6	7 (17)	5
48	blq	0	19 (38)	6	9 (12)	6	11 (12)	5
72	blq	0	19 (178)	5	9 (65)	6	9 (32)	3
96	blq	0	16 (20)	6	7 (14)	6	9 (12)	6

*Median (highest concentration found). When there were no measurable concentrations of an analyte in up to three fish, the median was calculated using its LOQ.
†Number of fish samples with measurable concentrations at or above LOQ.
m^1, Median is not given because only one of six fish had concentrations at or above LOQ.
blq, Not detected or below the LOQ.

Table 2. Concentrations of ABZ, ABZ-SO, ABZ-SO_2, and ABZ-2-NH_2SO_2 in the muscle and skin tissue of rainbow trout after oral treatment with ABZ

WD (h)	ABZ (μg/kg)*	$N^{\dagger}$	ABZ-SO (μg/kg)*	$N^{\dagger}$	ABZ-SO_2 (μg/kg)*	$N^{\dagger}$	ABZ-2-NH_2SO_2 (μg/kg)*	$N^{\dagger}$
8	20 (237)	3	22 (27))	5	3 (8)	6	m^1 (7)	1
12	m^2 (195)	2	43 (84)	6	12 (17)	6	6 (13)	4
18	blq	0	30 (37)	6	9 (15)	6	9 (11)	6
24	blq	0	22 (28)	6	6 (13)	6	5 (15)	3
48	blq	0	6 (29)	6	7 (22)	6	5 (13)	3
72	blq	0	blq	0	4 (15)	6	m^1 (9)	1
96	blq	0	blq	0	4 (8)	5	m^2 (9)	2

*Median (concentration range). When there were no measurable concentrations of an analyte in up to three fish, median was calculated using their LOQ.
†Number of fish samples with measurable concentrations.
m^1, Median is not given because only one of six fish had concentrations at or above LOQ.
m^2, Median is not given because only two of six fish had measurable concentrations at or above LOQ.
WD, withdrawal time; blq, not detected or below the LOQ.

Table 3. Concentrations of ABZ, ABZ-SO, ABZ-SO_2, and ABZ-2-NH_2SO_2 in the muscle and skin tissue of tilapia after oral treatment with ABZ

WD (h)	ABZ (μg/kg)*	$N^{\dagger}$	ABZ-SO (μg/kg)*	$N^{\dagger}$	ABZ-SO_2 (μg/kg)*	$N^{\dagger}$	ABZ-2-NH_2SO_2 (μg/kg)*	$N^{\dagger}$
4	48 (119)	6	30 (43)	6	3 (5)	6	6 (8)	3
8	64 (158)	4	55 (88)	6	9 (13)	6	14 (18)	6
12	45 (83)	4	51 (97)	6	10 (20)	6	31 (37)	6
24	blq	0	32 (68)	6	11 (15)	6	75 (190)	7
48	blq	0	2 (2)	3	0.5 (1)	6	46 (110)	6
72	blq	0	blq	0	1 (5)	6	83 (140)	6
96	blq	0	blq	0	1 (2)	6	43 (117)	6
120	blq	0	m^1 (4)	1	m^1 (0.5)	1	43 (122)	6
144	blq	0	blq	0	m^2 (0.7)	2	12 (50)	5

*Median (concentration range). When there were no measurable concentrations of an analyte in up to three fish, median was calculated using their LOQ.

†Number of fish samples with measurable concentrations at or above LOQ.

blq, Not detected or below the LOQ.

m^1, Median is not given because only one of six fish had measurable concentrations at or above LOQ.

m^2, Median is not given because two of six fish had measurable concentrations at or above LOQ.

c.a. 50 p.p.b.; however, one of six fish contained higher concentrations (117 p.p.b.) of this metabolite. Therefore additional tilapia were dosed to collect more tissue samples at 120 and 144 h post-treatment and assayed. The results (Table 3) showed that at 144 h sampling period the concentrations of ABZ-2-NH_2SO_2 for all six tilapia were within 50 p.p.b., a tolerance for cattle and sheep muscle tissue (Code of Federal Regulations, 21, 2000). Figure 2 shows the depletion curves of the median concentration of ABZ and its metabolites in the three fish species.

The depletion data for ABZ-2-NH_2SO_2 was further evaluated by statistical analysis using ANOVA to determine potential differences in the depletion concentrations at 96 and 144 h post-treatment among the three fish. The *P*-value for 96 h was 0.016, indicating significant differences among the fish. As no ABZ-2-NH_2SO_2 was detected in Atlantic salmon and rainbow trout at 144 h withdrawal period, its LOQ value of 5 p.p.b. was used. The *P*-value was calculated to be 0.050, signifying no differences among the three fish at 144 h. The data at 144 h withdrawal period was further evaluated using Kruskal–Wallis (KW) analysis of variance (Daniel, 1990). The decision from KW test was that the three species do not have same median. The follow-up pairwise comparisons showed that salmon and trout were not significantly different; however, salmon–tilapia and tilapia–trout pairs were significantly different.

There is a report in the literature (Nafstad *et al.*, 1991) where ABZ (5 mg/kg) was administered orally to Atlantic salmon and muscle tissues were collected on days 1, 7, 14 and 28. The muscle tissue samples were assayed for ABZ, ABZ-SO and ABZ-SO_2. No ABZ was detected at any of the sampling times. These findings appear to be consistent with our results that ABZ is metabolized prior to the 24 h sampling time. The metabolites, ABZ-SO and ABZ-SO_2, were detected only at the 24 h sampling time in the above study, where as, in our study both metabolites were detected up to 96 h post-treatment. This is perhaps due to differences in the experimental design between the two studies. In the above-referenced study the second, third and fourth sampling time periods were much longer, 7, 14 and 28 days post-treatment.

Table 4 indicates that an additional unknown metabolite peak, eluting just in front of ABZ-2NH_2SO_2 peak, was detected in a number of fish samples. This unknown polar metabolite was more prominent in tilapia at all sampling times, followed by Atlantic salmon and then in a few samples of rainbow trout. This unknown metabolites is perhaps the hydroxyl metabolite of ABZ as a result of hydroxylation of the propyl group.

The literature reports that a number of hydroxyl metabolites of ABZ were identified in the urine of cattle, sheep, rats and mice after oral administration of ^{14}C-albendazole (Gyurik *et al.*, 1981). Kitzman *et al.* (1990) reported the presence of hydroxyl metabolite of FBZ in a number of tissues including muscle after oral administration of FBZ to channel catfish. Attempts will be made to isolate and characterize this metabolite.

Muscle and skin are the edible products of most fish; therefore muscle with adhering skin was used for residue analysis of ABZ and its metabolites in our depletion studies. In the literature, there are numerous examples where drug and/or metabolites persisted in the skin of the fish as compared with the muscle. Iosifidou *et al.* (1997) reported that both FBZ and its metabolite fenbendazole sulfoxide accumulated preferably in the skin after oral treatment. In another study, sulfadiazine and trimethopim were found to accumulate in the skin of rainbow trout after oral treatment (Bergsjo *et al.*, 1979). In addition, tetracycline and quinolone were found to accumulate in the skin of rainbow trout and salmon, respectively (Ingebrigtsen *et al.*, 1985; Steffenak *et al.*, 1991). These studies suggest that skin of the fish is important and as it is consumed along with muscle as human food, it should be taken into account while collecting metabolism and residue depletion data for human food safety.

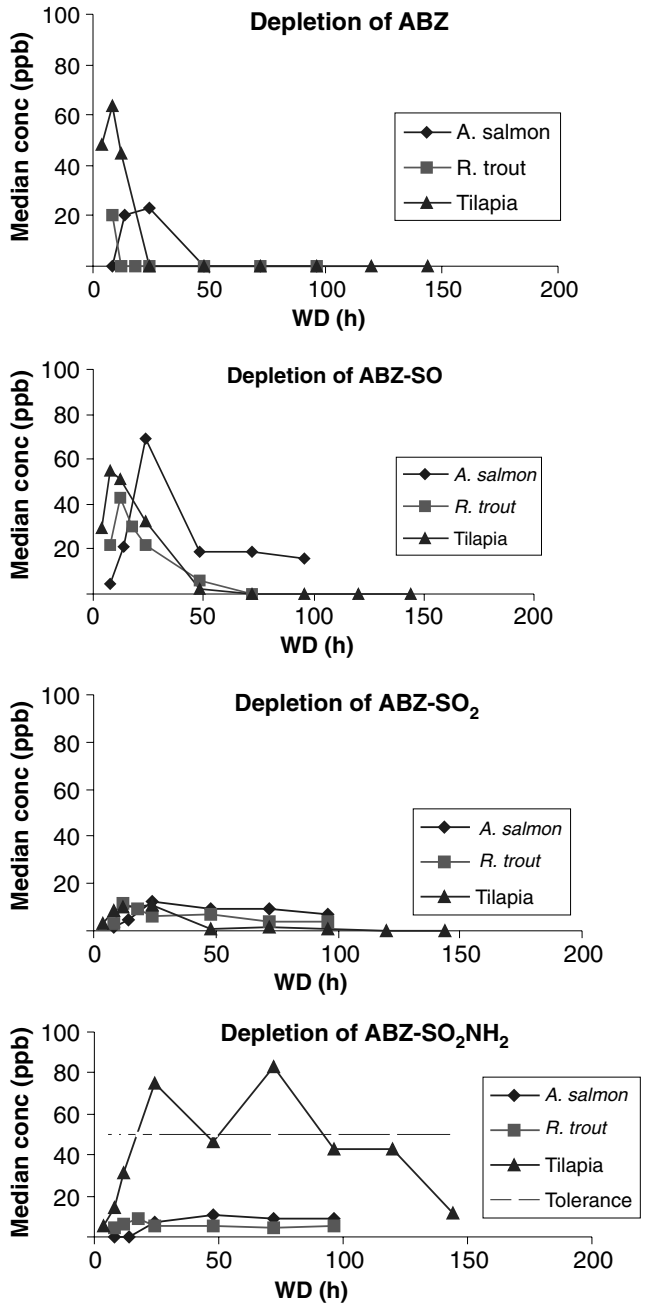

Fig. 2. Plots of the depletion of albendazole and its metabolites in Atlantic salmon, rainbow trout and tilapia, median concentration in μg/kg (p.p.b.) vs. withdrawal time (WD).

CONCLUSION

This work demonstrates that the three species, rainbow trout, tilapia and Atlantic salmon, were able to biotransform ABZ into three major metabolites, ABZ-SO, ABZ-SO_2 and ABZ-2-NH_2SO_2. While parent drug ABZ was depleted within 24 h in the three fish, its oxidation product and pharmacologically active metabolite, ABZ-SO persisted to <20 p.p.b. at 96 h in Atlantic salmon and was depleted within 48 h in rainbow trout and tilapia. One of its inactive metabolites, ABZ-SO_2, depleted to <10 p.p.b. in the three fish. However, the second inactive metabolite, ABZ-2-NH_2SO_2, persisted longer and was depleted to <10 p.p.b. in rainbow trout and Atlantic salmon, but continued to be present to <20 p.p.b. in tilapia at 144 h.

Table 4. Concentrations of metabolite X in Atlantic salmon, tilapia and rainbow trout

WD (h)	A. Salmon (Pk Ar)	*N**	Tilapia (Pk Ar)	*N**	R. Trout (Pk Ar)	*N**
4			X	5		
8			X	6		
12			X	6		
24	X	1	X	6	X	1
48	X	3	X	6		
72	X	4	X	5	X	1
96	X	6	X	2		
120			X	4		
144			X	3		

*Number of fish samples with measurable concentrations; Pk Ar, Peak Area.

The results of our study clearly demonstrate that in the three species studied, the pharmacologically active metabolite ABZ-SO depletes slowest in Atlantic salmon. The inactive metabolite ABZ-2-NH_2SO_2 depletes slowest in tilapia.

This work represents a part of our long-term effort to investigate a variety of finfish to ascertain their potential for species (crop) grouping. We intend to include more fish species of importance to aquaculture to our research effort. Furthermore, additional model drugs of different chemical class are being investigated. ABZ in this study can potentially serve as a model drug for understanding the metabolism and residue depletion profiles of other benzimidazole anthelmintics in fish species.

ACKNOWLEDGMENTS

The authors are grateful to Dr Janice Derr for statistical assistance and Christopher Middendorf and Ruth Barrett for their assistance in necropsy during their internship.

REFERENCES

Bergsjo, T., Nafstad, I. & Ingebrigtsen, K. (1979) The disposition of ^{32}S-sulfadiazine and ^{14}C-trimethoprim in rainbow trout (*Salmo gairdneri*). *Acta Veterinaria Scandinavica*, **20**, 25–37.

Cinquina, A.L., Longo, F., Raponi, A., Fagiolo, A., Bocca, A., Brambilla, G. & Cozzani, R. (1997) Pharmacokinetic of albendazole metabolites in goat milk, milk products and by-products. *Italian Journal of Food Science*, **3**, 231–237.

Code of Federal Regulations, 21 (2000) *Food and Drugs*. Parts 520.45a,b and 556.34. US Government Printing Office, Washington, DC.

Csiko, G., Banhidi, G., Semjen, G., Laczay, P., Sandor, G.V., Lehel, J. & Fekete, J. (1996) Metabolism and pharmacokinetics of albendazole after oral administration to chickens. *Journal of Veterinary Pharmacology and Therapeutics*, **19**, 322–325.

Daniel, W.W. (1990) *Kruskal-Wallis One-Way Analysis of Variance by Ranks. Applied Nonparametric Statistics*, 2nd edn. pp. 226–230. PWS-Kent Publishing Co., Boston, MA.

Delatour, P. & Parish, R.C. (1986) Benzimidazole anthelmintics and related compounds: toxicity and evaluation of residues. In *Drug Residues in Animals*. Eds Rico, A.G. pp. 175–204. Academic Press, Orlando, FL.

Delatour, P., Benoit, E., Lechenet, J. & Besse, S. (1990) Pharmacokinetics in sheep and cattle of albendazole administered by an intraruminal slow release capsule. *Research in Veterinary Science*, **48,** 271–275.

Formentini, E.A., Mestorini, O.N., Marino, E.L. & Errecalde, J.O. (2001) Pharmacokinetics of ricobendazole in calves. *Journal of Veterinary Pharmacology and Therapeutics*, **24,** 199–202.

Galtier, P., Alvinerie, M. & Delatour, P. (1986) *In vitro* sulfoxidation of albendazole by ovine liver microsomes: assay and frequency of various xenobiotics. *American Journal of Veterinary Research*, **46,** 447–450.

Gyurik, R.J., Chow, A.W., Zaber, B., Brunner, E.L., Miller, J.A., Villani, A.J., Petka, L.A. & Parish, R.C. (1981) Metabolism of albendazole in cattle, sheep, rats and mice. *Drug Metabolism and Disposition*, **6,** 503–508.

Ingebrigtsen, K., Nafstad, I. & Maritim, A. (1985) The distribution of ^{3}H-tetracycline after a single oral dose in the rainbow trout (*Salmo gairdneri*) as observed by whole body autoradiography. *Acta Veterinaria Scandinavica*, **2,** 428–430.

Iosifidou, E.G., Haagsma, N., Tanck, M.W.T., Boon, J.H. & Olling, M. (1997) Depletion study of fenbendazole in rainbow trout (*Oncorhynchus mykiss*) after oral and bath treatment. *Aquaculture*, **154,** 191–199.

Kitzman, J.V., Holley, J.H., Huber, W.G., Koritz, G.D., Davis, L.E., Neff-Davis, C.A., Bevill, R.F., Short, C.R., Barker, S.A. & Hsieh, L.C. (1990) Pharmacokinetics and metabolism of fenbendazole in channel catfish. *Veterinary Research Communications*, **14,** 217–226.

Lacey, E. (1990) Mode of action of benzimidazoles. *Parasitology Today*, **5,** 112–115.

Lanusse, C.E., Nare, B., Gason, L.H. & Prichard, R.K. (1992) Metabolism of albendazole and albendazole sulfoxide by ruminal and intestinal fluids of sheep and cattle. *Xenobiotica*, **22,** 419–426.

Lanusse, C.E., Gascon, L.H. & Prichard, R.K. (1995) Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfendazole and their metabolites in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **18,** 196–203.

Lubega, G. & Prichard, R. (1991) Interaction of benzimidazole anthelmintics with *Haemonchus tubulin*: binding affinity and anthelimintic efficacy. *Experimental Parasitology*, **73,** 203–209.

McKellar, Q.A. & Scott, E.W. (1990) The benzimidazole anthelmintics agents – a review. *Journal of Veterinary Pharmacology and Therapeutics*, **13,** 223–247.

Marriner, S.E. & Bogan, J.A. (1980) Pharmacokinetics of albendazole in sheep. *American Journal of Veterinary Research*, **41,** 1126–1129.

Nafstad, I., Ingebrigtsen, K., Langseth, W., Hektoen, H., Gross, I.L. & Bergsjo, B. (1991) Benzimidazoles for antiparasite therapy in salmon. *Acta Veterinaria Scandinavica. Supplement*, **87,** 302–304.

Prichard, R.K., Hennessy, D.R., Steel, J.W. & Lacey, E. (1985) Metabolite concentrations in plasma following treatment of cattle with five anthelmintics. *Research in Veterinary Science*, **39,** 173–178.

Shaikh, B., Rummel, N. & Reimschuessel, R. (2003) Determination of albendazole and its major metabolites in the muscle tissues of Atlantic salmon, tilapia and rainbow trout by high performance liquid chromatography with fluorometric detection. *Journal of Agricultural and Food Chemistry*, **51,** 3254–3259.

Steffenak, I., Hormazabal, V. & Yndestad, M. (1991) Reservoir quinolone residues in fish. *Food Additives and Contamination*, **8,** 777–780.