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Disposition of ^{14}C-sarafloxacin in Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, cod *Gadus morhua* and turbot *Scophthalmus maximus*, as demonstrated by means of whole-body autoradiography and liquid scintillation counting

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ABSTRACT: The absorption, distribution and elimination of ^{14}C-labelled sarafloxacin hydrochloride were studied by means of whole-body autoradiography and liquid scintillation counting. The drug was administered to Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, cod *Gadus morhua* and turbot *Scophthalmus maximus*, either intravenously or orally in a single dose of 9.6 (3.57) and 9.7 (3.44) mg kg^{-1} (MBq kg^{-1}) respectively. The Atlantic salmon, rainbow trout and cod were held in seawater at a temperature of 7.9 ± 0.2 °C, and the turbot at 12.1 ± 1.1 °C. In the intravenously dosed groups, the drug was rapidly distributed to all major tissues and organs except the central nervous system. After the distribution phase, the levels of radioactivity were higher in most organs and tissues than in blood. The most noticeable differences between the species were the lower levels of radioactivity in the liver, and the higher levels in the muscle tissue, of cod compared to the salmonids. Incomplete absorption was observed following oral administration of sarafloxacin to Atlantic salmon, rainbow trout and turbot. Most of the radioactivity remained in the gastrointestinal tract, high levels of radioactivity otherwise being observed only in the bile and to a certain extent in the liver. The maximum sarafloxacin concentrations (C_{max}) in blood were calculated to be 0.60, 0.16 and 0.22 µg ml^{-1} for Atlantic salmon, rainbow trout and turbot respectively. In cod a significant degree of absorption took place, with high levels of radioactivity being detected in all major organs and tissues for the first 4 d after administration. The C_{max} in blood was calculated to be 2.18 µg ml^{-1}. In all 4 species tested, traces of radioactivity remained in the kidney, and also to some extent in the skin, during the excretion phase.

KEY WORDS: Quinolones · Oxolinic acid · Antimicrobial · Pharmacokinetics · Absorption · Distribution · Elimination · Autoradiography

INTRODUCTION

Sarafloxacin hydrochloride is a new fluoroquinolone, which is currently being studied for potential use as an antimicrobial drug for fish (Stamm 1989, Plumb & Vinitnantharat 1990, Martinsen et al. 1991, 1993, Johnson et al. 1992, Thune & Johnson 1992). The chemical formula of sarafloxacin is shown in Fig. 1.

Information concerning the pharmacokinetic properties and clinical effects of sarafloxacin in Atlantic salmon *Salmo salar* is inadequate. However, Martinsen et al. (1993) reported incomplete absorption of sarafloxacin and an average peak plasma concentration of 0.70 µg ml^{-1} in Atlantic salmon following a single oral dose of 10 mg kg^{-1} using corn oil as the drug vehicle.

As regards sarafloxacin in other fish species, including other salmonids, the drug has been reported to be

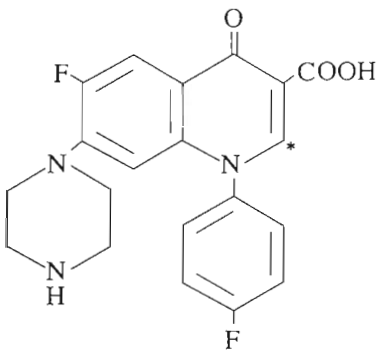

Fig. 1. Chemical formula of sarafloxacin (* indicates the ^{14}C-labelling of the molecule)

effective in eliminating asymptomatic carriers of *Aeromonas salmonicida* in juvenile spring chinook salmon *Oncorhynchus tshawytscha* (Markwardt & Klontz 1989). Sarafloxacin has also been shown to be effective in treating *Edwardsiella ictaluri* infection of channel catfish *Ictalurus punctatus* (Wilson & MacMillan 1989, Johnson et al. 1992, Thune & Johnson 1992).

Pharmacokinetic information on sarafloxacin is not available for fish species other than Atlantic salmon (Martinsen et al. 1993). As the pharmacokinetic properties of drugs may vary significantly between species (Guarino et al. 1988, Nouws et al. 1988, Grondel et al. 1989), the disposition of a drug should be investigated in the particular species in which it is intended to be used.

Whole-body autoradiography is a suitable technique for studies of drug disposition in whole animal specimens (Ullberg 1954, 1977). The technique is especially valuable for the detection of drug residues in minute anatomical structures (Ullberg et al. 1970).

The purpose of the present study was to examine the disposition of sarafloxacin following intravenous or oral administration in 4 different fish species, Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, cod *Gadus morhua* and turbot *Scophthalmus maximus*, by means of whole-body autoradiography and liquid scintillation counting.

MATERIALS AND METHODS

Test substance. ^{14}C-labelled sarafloxacin hydrochloride (2.21 MBq mg^{-1}) was obtained from Chemsyn Science Laboratories, Lenexa, KS, USA, while unlabelled sarafloxacin hydrochloride was obtained from Abbott Laboratories, North Chicago, IL, USA. The radiochemical purity of the ^{14}C-labelled sarafloxacin (Fig. 1) was ≥ 98 %.

The test substance for intravenous administration was a mixture of ^{14}C-labelled and unlabelled sarafloxacin (21:104) dissolved in 0.1 M NaOH and titrated to a pH of 10.5 using 6 M HCl. The solution contained 9.6 mg sarafloxacin ml^{-1} with a specific activity of 3.57 MBq ml^{-1}.

The test substance for oral administration was a mixture of ^{14}C-labelled and unlabelled sarafloxacin (109:541) in corn oil. The corn oil was added to the sarafloxacin mixture after 0.2 ml ethanol had first been added to ensure disintegration of the sarafloxacin crystals. The suspension was then thoroughly mixed for 15 min on a whirlimixer, ultrasonicated for 5 min and again mixed for 10 min. The ultrasonication and mixing procedures were repeated 3 times. The suspension was easily resuspended after storage by mixing for 2 min on a whirlimixer. The suspension contained 9.7 mg sarafloxacin ml^{-1} with a specific activity of 3.44 MBq ml^{-1}.

Experimental design. The study was conducted at NIVA Marine Research Station, Solbergstrand, Drøbak, Norway. Four fish species, Atlantic salmon, rainbow trout, cod and turbot, were included in the study. Atlantic salmon, rainbow trout and cod were given sarafloxacin intravenously and orally, while turbot was given sarafloxacin orally only. All fish were held in 500 l fibreglass tanks, supplied with running seawater (26.0 to 34.5‰, flow rate 10 l min^{-1}), the turbot being held at a temperature of 12.1 ± 1.1 °C, and the other fish species at 7.9 ± 0.2 °C. The mean weights of the fish in the different groups are given in Table 1. During the adaptation period, all groups were fed a commercial pelleted fish diet ad libitum using automatic feeders. Prior to drug administration, the fish were starved for 1 d, and during the sampling period, they were fed at a reduced rate (feed comprising about 0.5 % of body mass per day).

In the groups given sarafloxacin intravenously, each fish was netted from the acclimation tank and weighed (Mettler PC 16 balance) in a small tank of water. After weighing, each fish was anaesthetized with benzocaine (50 mg l^{-1} water). The intravenous injection of sarafloxacin was accomplished by placing the fish dorsally on damp paper in a V-formed tray, the sarafloxacin solution being slowly injected into the caudal vein using a 1 ml disposable syringe and a 0.6 × 25 mm needle (Terumo, Leuven, Belgium). The injection volume was 1.0 ml kg^{-1} body weight, corresponding to 9.6 mg kg^{-1} (3.57 MBq kg^{-1}).

Table 1. Mean weight and standard deviation (SD) of the test fish following intravenous or oral administration of ^{14}C-sarafloxacin (10 mg kg^{-1}), depending on fish species

Fish species	Intravenous		Oral	
	Mean (g)	SD	Mean (g)	SD
Atlantic salmon	110.8	25.8	109.2	25.5
Rainbow trout	203.0	13.7	123.9	26.5
Cod	109.0	18.6	96.1	24.7
Turbot			109.7	17.7

In the groups given sarafloxacin orally, each fish was netted from the tank and weighed. The drug suspension was administered through a stomach tube (catheter no. 12, Rush, Germany), using an attached 1 ml disposable syringe. Each fish was manually restrained without anaesthesia, and given a dose of 1.0 ml kg^{-1} corresponding to 9.7 mg kg^{-1} (3.44 MBq kg^{-1}). The test suspension was thoroughly mixed for 15 s between each fish. After drug administration, all orally dosed fish were transferred to individual tanks for observation of regurgitation before being transferred to the experimental tanks. Fish which regurgitated (indicated by oil droplets on the water surface) during the 5 min in the observation tanks were excluded from the study.

Table 2. Number of fish sampled in the different groups at each time point

Time point	Intravenous administration			Oral administration			
	Atlantic salmon	Rainbow trout	Cod	Atlantic salmon	Rainbow trout	Cod	Turbot
1 h	1	1	1	–	–	–	–
4 h	1	1	1	2	2	2	–
8 h	1	1	1	2	2	2	–
12 h	1	1	1	2	2	2	1
24 h	1	1	1	2	2	2	–
48 h	1	–	1	2	2	2	2
4 d	–	–	–	1	1	1	–
7 d	–	–	–	1	1	1	2
14 d	–	–	–	1	1	1	–
28 d	–	–	–	1	1	1	2
56 d	–	–	–	–	–	–	2

The sampling regimen and the number of individuals of the different fish species sampled are given in Table 2. Each fish was killed by an overdose of

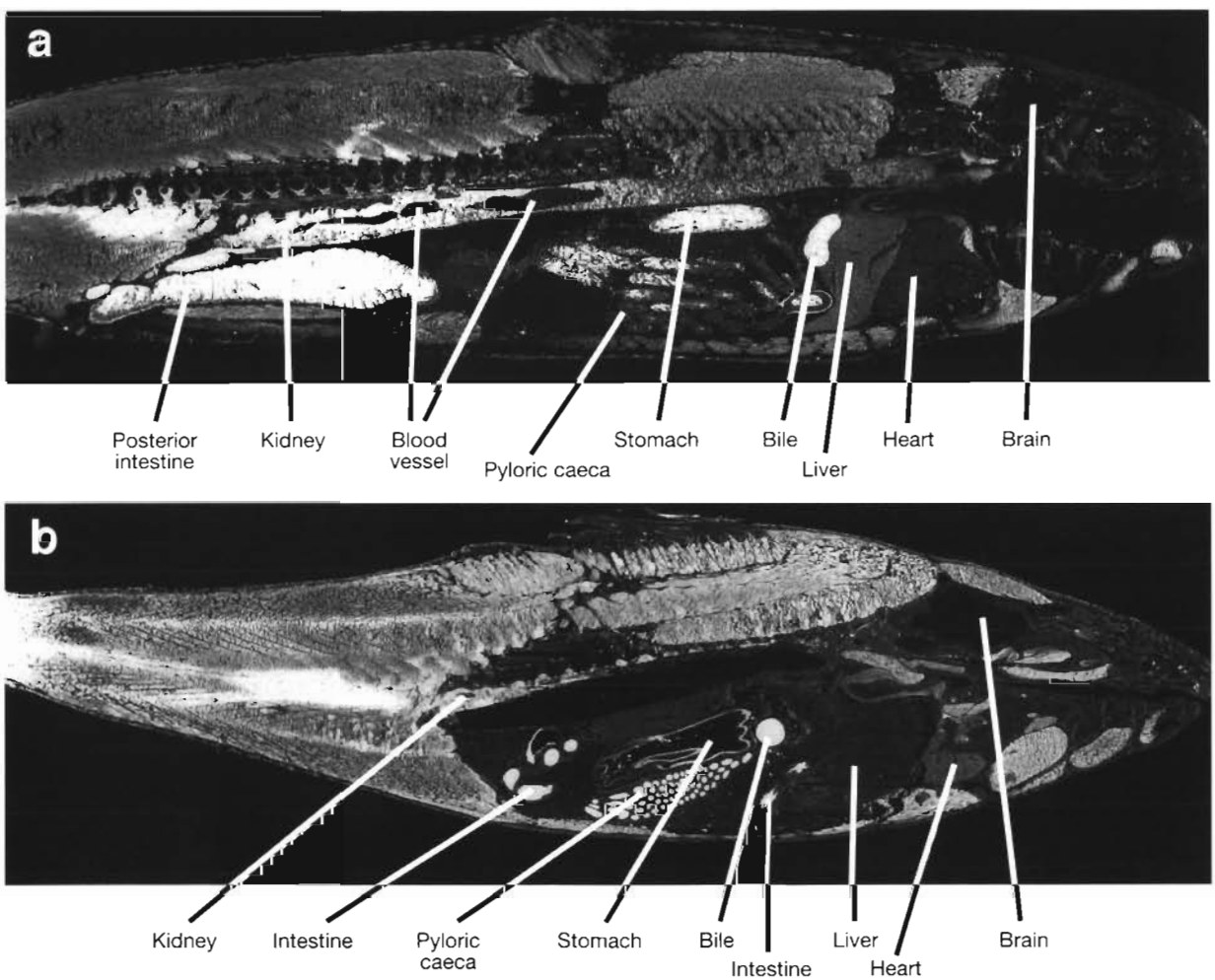

Fig. 2. *Salmo salar* and *Gadus morhua*. Distribution pattern of sarafloxacin and its metabolites in (a) Atlantic salmon and (b) cod 24 h after intravenous administration. Note the high levels of radioactivity in the bile, the intestinal tract and the kidney, and, in cod, also in the muscle

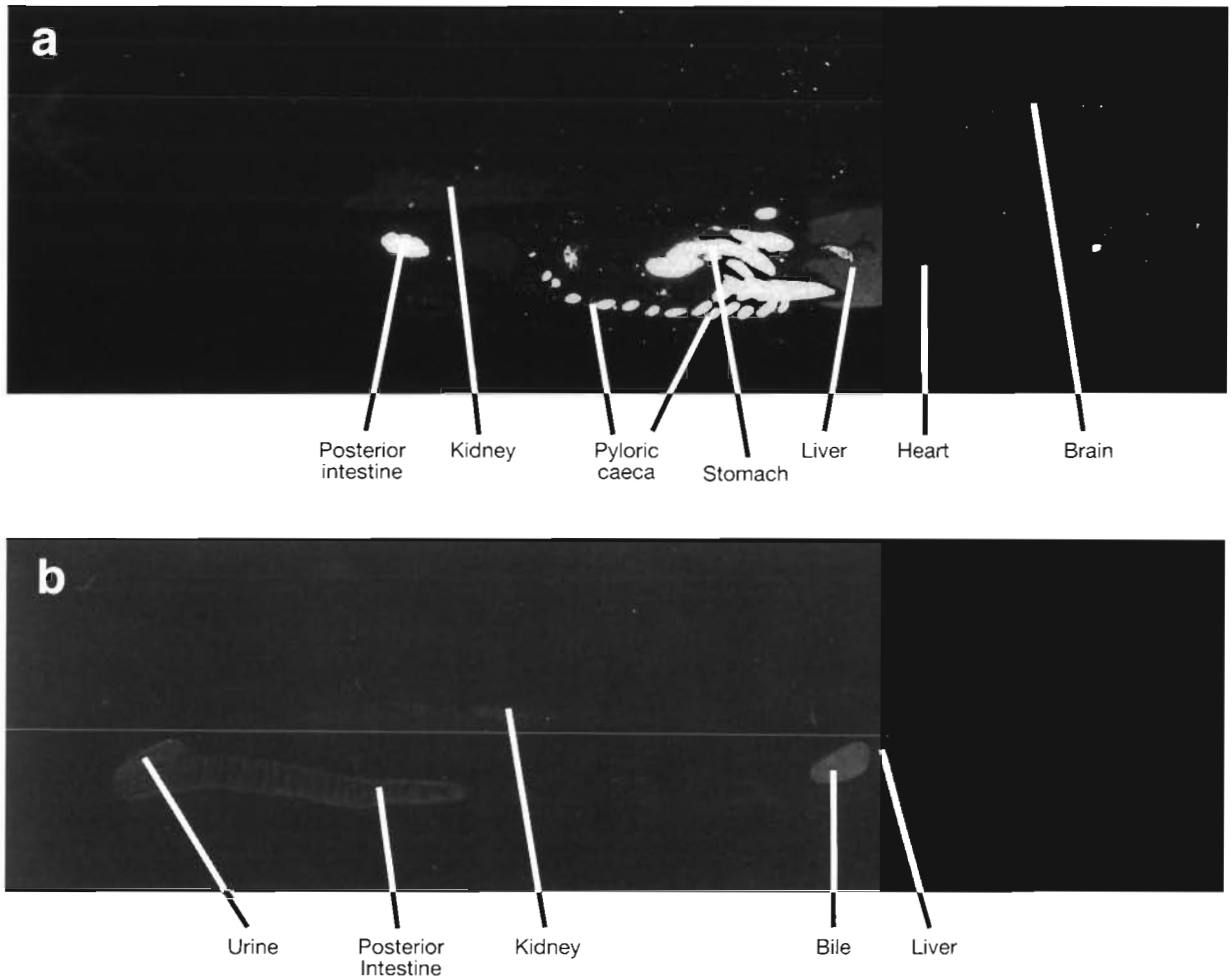

Fig. 3. *Salmo salar*. Distribution pattern of sarafloxacin and its metabolites in Atlantic salmon (a) 4 h and (b) 7 d after oral administration. Note the radioactivity in the kidney, the liver, the bile and the intestinal content

benzocaine (200 mg l^{-1} for 10 min), and frozen in liquid nitrogen (–196 °C). They were then embedded in individual blocks of cooled 1% solution of sodium carboxymethyl cellulose in water (0 °C) followed by immediate freezing with dry ice in *n*-hexane (–75 °C).

Each sample was analyzed using a standard technique for whole-body autoradiography and related studies on radioactive isotopes (Ullberg 1954, 1977). Sagittal sections (20 to 40 µm) from the whole fish were obtained on tape (No. 821, 3M Co., St. Paul, MN, USA) at –20 °C in a PMV cryomicrotome (PMV 450 MP, Palmstierna Mekaniska Verkstad, Stockholm, Sweden). The sections were freeze-dried overnight, before application on Hyperfilm-βmax (Amersham, UK) or Structurix D7 (Agfa, Antwerp, Belgium) for autoradiography. The films were exposed at –20 °C for approximately 3 mo before development.

From the material remaining in the frozen blocks, samples weighing 10 to 100 mg were obtained from brain, blood, muscle, skin, cartilage, kidney, liver and bile. The samples were digested with 1 ml soluene (Packard, Groningen, Holland) and 200 µl 96% ethanol at 37 °C overnight. The samples were then decoloured with 400 µl perhydrol (Merck, Darmstadt, Germany), before the addition of 10 ml of liquid scintillation cocktail (Hionic Fluor, Packard) to each vial. The radioactivity in the samples was counted in a Packard Tri-Carb 1900CA liquid scintillation analyzer, using a Packard automatic ^{14}C quenching standard to control the accuracy of counting.

RESULTS

Following intravenous administration, sarafloxacin was generally well distributed to all major tissues and organs, except for the central nervous system. The highest levels of radioactivity after intravenous ad-

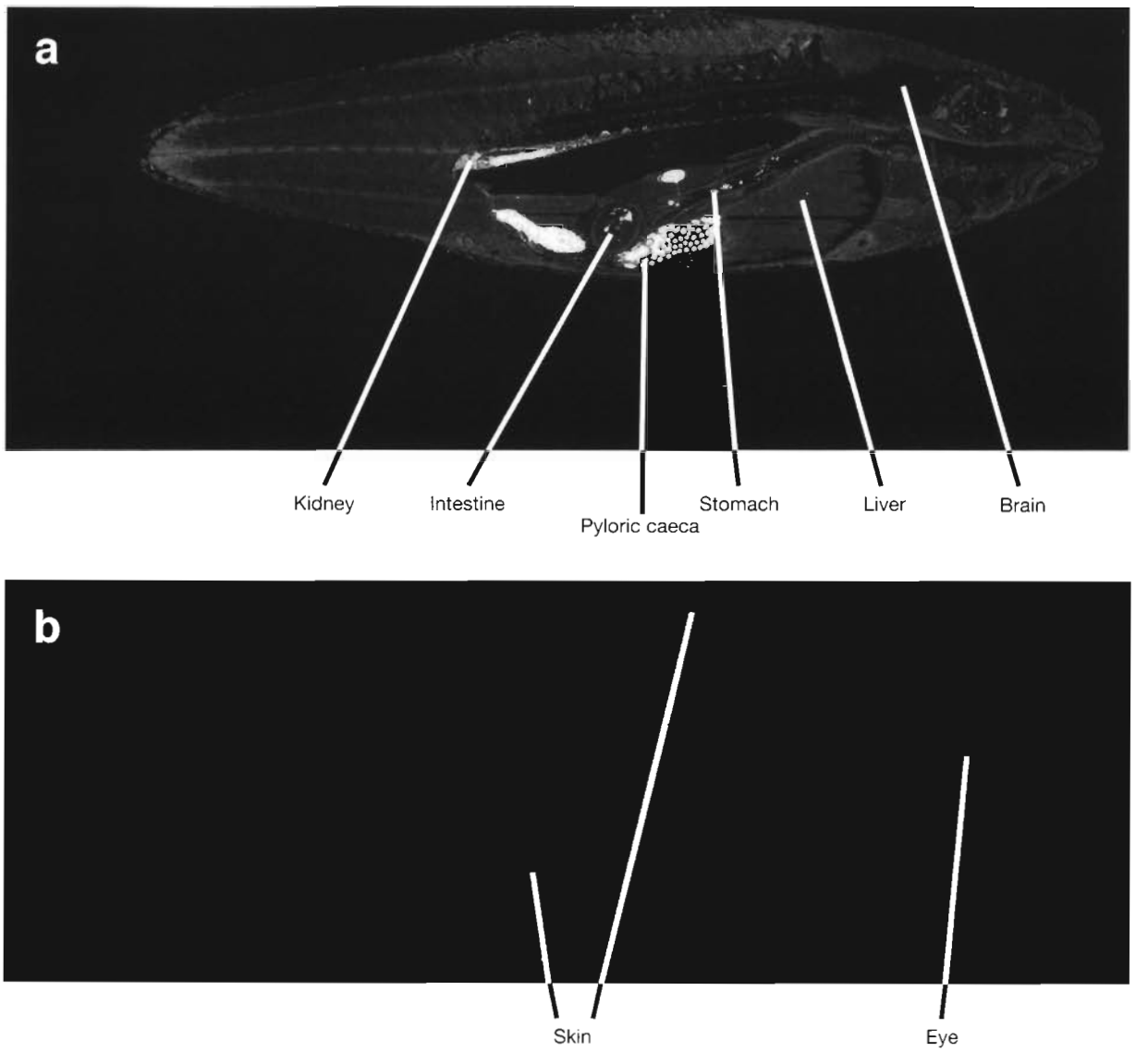

Fig. 4. *Gadus morhua*. Distribution pattern of sarafloxacin and its metabolites in cod (a) 8 h and (b) 28 d after oral administration. Note the higher tissue concentrations of sarafloxacin compared to Atlantic salmon (Fig. 3), and the radioactivity in the skin 28 d after administration

ministration were obtained in bile, liver, kidney and the intestinal tract. The most noticeable differences between the species following intravenous administration were the lower levels of radioactivity in the liver, and the higher levels in the muscle tissue, in cod compared to the salmonids. Autoradiograms of Atlantic salmon and cod 24 h after administration of the drug are shown in Fig. 2.

In the orally administered groups, drug absorption was poor in the salmonids and turbot. Most of the radioactivity in these species remained in the gastrointestinal tract. However, high levels of radioactivity were also observed in the bile and to a certain extent in the liver. During the excretion phase, traces of radioactivity persisted in the kidney and also to some extent in the skin. In cod, a significant degree of absorption was evident with high levels of radioactivity in all major organs and tissues during the first 4 d after administration. In the excretion phase, a significant amount of radioactivity was still observed in skin 28 d after administration. Autoradiograms of Atlantic salmon 4 h and 7 d after administration, and of cod 8 h and 28 d after administration, are shown in Figs. 3 & 4 respectively. One autoradiogram of turbot and one of rainbow trout 12 h after administration are shown in Fig. 5.

The concentration-time profiles of sarafloxacin determined on the basis of liquid scintillation counting in blood, muscle and skin in Atlantic salmon and cod

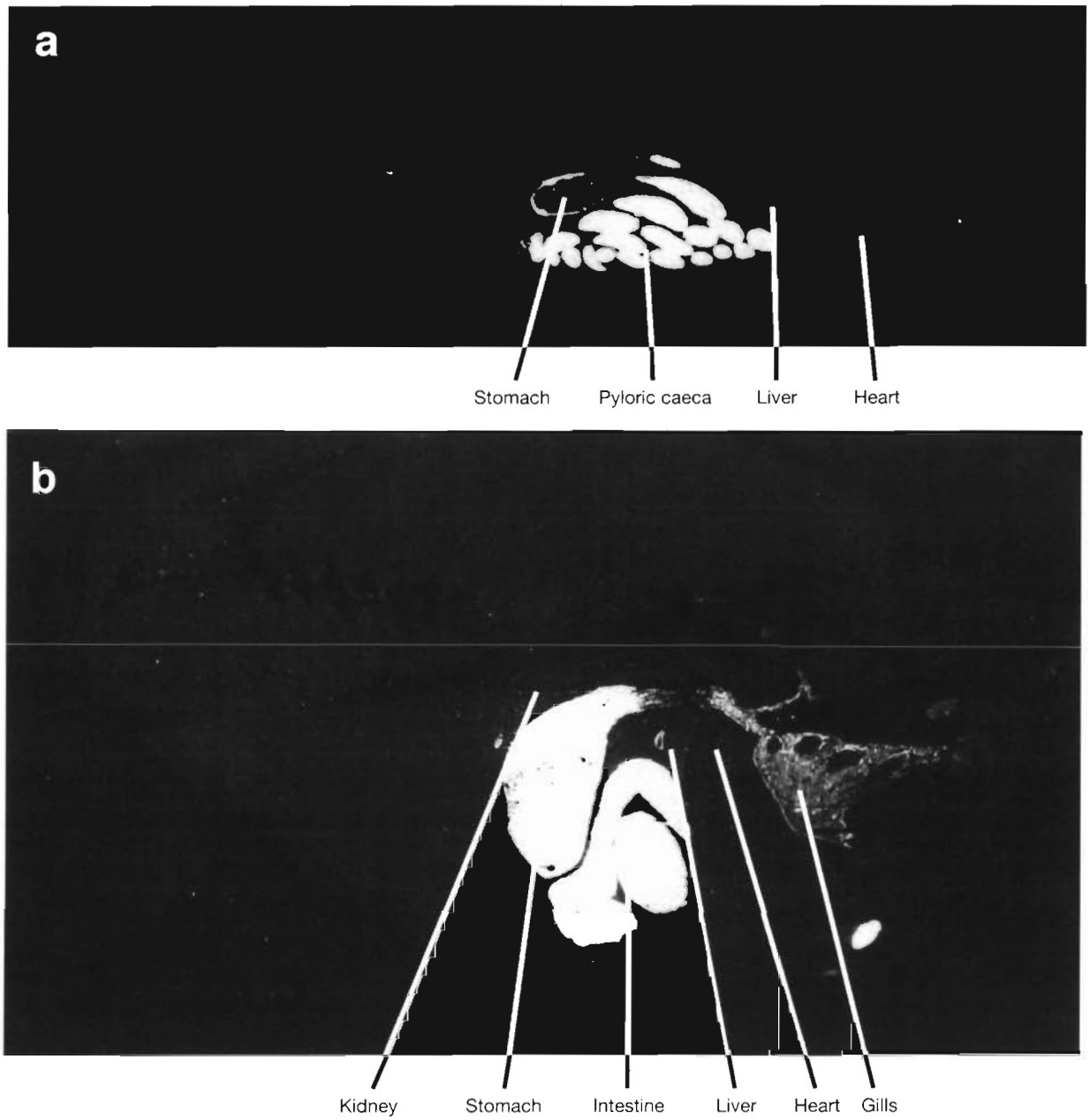

Fig. 5. *Oncorhynchus mykiss* and *Scophthalmus maximus*. Distribution pattern of sarafloxacin and its metabolites in (a) rainbow trout and (b) turbot, 12 h after oral administration. Note that radioactivity is mainly observed in the intestinal content

following oral administration are shown in Fig. 6. The average peak concentrations in blood, muscle, kidney, liver and bile after oral administration are presented in Table 3.

DISCUSSION

Due to the fact that only 1 or 2 fish were sampled at each time point, the results in the present study must be interpreted with caution. The study design does not allow pharmacokinetic parameters to be calculated, as the estimated concentrations at each time point are based on liquid scintillation counting of samples from only 1 or 2 individuals, and hence any calculated pharmacokinetic parameters would be insignificant and disputable. Despite this methodological limitation, some important information concerning the disposition of sarafloxacin in the different fish species can be obtained.

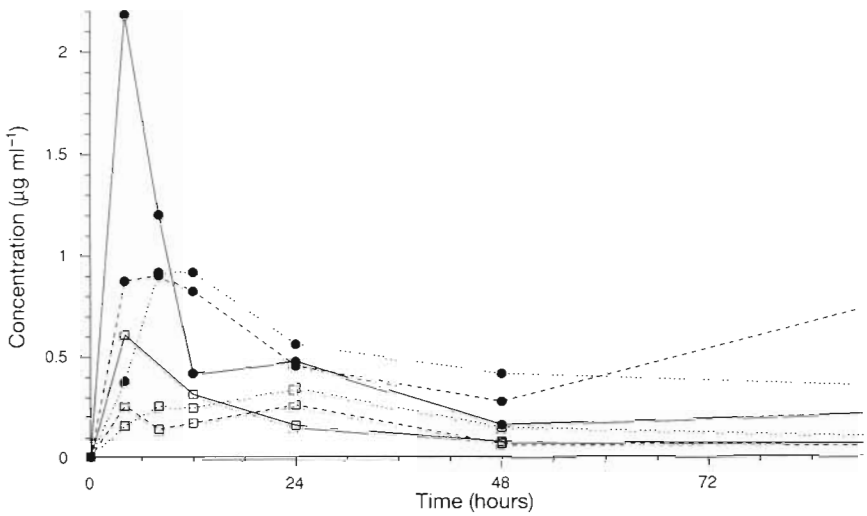

Fig. 6. *Salmo salar* and *Gadus morhua*. Concentration-time profile of sarafloxacin in blood (—), muscle (·····) and skin (- - -) in Atlantic salmon (□) and cod (●) following oral administration of 10 mg sarafloxacin kg^{-1}

The absorption of sarafloxacin was rapid, though incomplete, in all species tested. Irrespective of the time point chosen during the absorption phase, most of the radioactivity following oral administration to Atlantic salmon, rainbow trout and turbot was observed within the intestinal contents, thus showing that a major part of the administered drug was not absorbed. Incomplete absorption of sarafloxacin in Atlantic salmon has also been observed in a single-dose pharmacokinetic study (Martinsen et al. 1993), in which the highest mean plasma concentration ranged from 0.08 to 0.70 µg ml^{-1}, and the bioavailability from 3.6 to 23.9% depending on drug formulation. Furthermore, an apparent volume of distribution ($V_{d(area)}$) of 4.1 l kg^{-1} (Martinsen et al. 1993) indicated that tissue concentrations of sarafloxacin would be higher than those obtained in plasma. This suggestion is supported by the present study in which higher sarafloxacin concentrations were found in muscle, liver, bile and kidney than in blood.

A major species dissimilarity was recorded between cod and the other 3 species, cod showing much better absorption. The concentrations in blood and muscle tissue from fish sampled the first 2 d after oral administration were 2 to 4 times higher in cod than in Atlantic salmon. The reason for the better absorption of the drug in cod is at present unknown. However, species belonging to the codfish family (Gadidae) are reported to have a characteristic epithelial lining of the intestinal tract (Stoskopf 1992), and one might speculate whether this feature could influence the process of drug absorption. In addition, the pyloric caeca of cod, unlike those of salmonids, branch so that while there are many pyloric caeca, there are few openings into the intestine (Marrison & Zurbrigg 1992). This may increase the time for intimate contact between the absorbing mucosa of cod, and hence facilitate the absorption.

Interestingly, during treatment with the 4-quinolone oxolinic acid against furunculosis in a fish farm, Samuelsen et al. (1992) observed higher plasma and tissue concentrations of oxolinic acid in wild coalfish *Pollachius virens* outside the treated cages than in the treated Atlantic salmon. The authors explained this difference by a poorer appetite of the diseased Atlantic salmon. However, species differences in drug absorption of oxolinic acid might well also have contributed to their observation.

The distribution of sarafloxacin in cod was also different compared to the other species, with relatively low concentrations in the liver, and correspondingly high concentrations in muscle tissue (Fig. 2). An explanation for this species variation could be low accumulation of sarafloxacin in lipid-rich tissues. Cod is a lean fish species with the fat reserves in the liver, while salmonids have their main fat supplies in the intermuscular tissue and in the abdomen, to which insignificant quantities of sarafloxacin were distributed.

Table 3. Peak concentration (C_{max}) and observed time to reach C_{max} (T_{max}) of sarafloxacin and its metabolites in blood, muscle, kidney, liver and bile following oral administration of 10 mg kg^{-1}. Concentrations are means of 2 observations in units of µg ml^{-1} for blood and µg g^{-1} for tissues

Organ/ tissue	Atlantic salmon		Rainbow trout		Cod		Turbot	
	C_{max}	T_{max}	C_{max}	T_{max}	C_{max}	T_{max}	C_{max}	T_{max}
Blood	0.60	4 h	0.16	8 h	2.18	4 h	0.40	12 h
Muscle	0.34	24 h	0.09	8 h	1.33	4 d	0.29	12 h
Kidney	0.66	4 h	0.20	4 h	6.50	4 h	0.52	12 h
Liver	1.76	4 h	0.02	4 h	2.36	4 h	0.49	12 h
Bile	11.75	24 h	1.26	4 h	129.16	4 d	5.98	12 h

During the depletion phase, relatively high levels of radioactivity were recorded in the kidney, the urine and in the bile. This indicates that excretion of sarafloxacin and/or its metabolites takes place via both the urinary and the biliary pathways.

The rapid elimination of sarafloxacin from plasma ($t_{1/2}$ = 15.9 h) reported by Martinsen et al. (1993) was supported by this study, as no radioactivity was detected in blood or muscle in fish sampled later than 7 and 14 d after administration respectively. However, a significant amount of radioactivity in the skin and the kidney was still observed in all 4 species 28 d after administration. This is in accordance with Steffenak et al. (1991), who observed sarafloxacin residues in skin and bone in Atlantic salmon 80 d after treatment with a dose of 30 mg kg^{-1} body weight daily for 10 d.

The binding of antimicrobial compounds to melanin has previously been described in rainbow trout for sulphadiazine and trimethoprim (Bergsjø et al. 1979). The retention of sarafloxacin and/or its metabolites in the skin and the kidney, as observed in the present study, might also be explained by binding to melanin, as melanin is present in both types of tissue. However, further investigations are needed in order to determine whether sarafloxacin binds to melanin or to other tissue components in kidney and skin.

In view of the previously reported species differences in pharmacokinetic properties (Guarino et al. 1988, Nouws et al. 1988, Grondel et al. 1989), and the present dissimilarities observed between cod and the other 3 species, extrapolation of pharmacokinetic data from one fish species to another should be performed with great caution.

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LITERATURE CITED

Bergsjø, T., Nafstad, I., Ingebrigtsen, K. (1979). The distribution of ^{35}S-sulphadiazine and ^{14}C-trimethoprim in rainbow trout, *Salmo gairdneri*. Acta vet. Scand. 20: 25–37

Grondel, J. L., Nouws, J. F. M., Schutte, A. R., Driessens, F. (1989). Comparative pharmacokinetics of oxytetracycline in rainbow trout (*Salmo gairdneri*) and African catfish (*Clarias gariepinus*). J. vet. Pharmacol. Therap. 12: 157–162

Guarino, A. M., Plakas, S. M., Dickey, R. W., Zeeman, M. (1988). Principles of drug absorption and recent studies of bioavailability in aquatic species. Vet. hum. Toxicol. 30 (suppl. 1): 41–44

Johnson, M. R., Smith, K. L., Boyle, C. R. (1992). Field efficacy trials of the antibacterial sarafloxacin hydrochloride (A-56620) for treatment of *Edwardsiella ictaluri* infection in channel catfish. J. aquat. Anim. Health 4: 244–251

Markwardt, N. M., Klontz, G. W. (1989). A method to eliminate the asymptomatic carrier state of *Aeromonas salmonicida* in salmonids. J. Fish Dis. 12: 317–322

Marrison, C. M., Zurbrigg, R. E. (1992). Cold-water commercial marine fish, anatomy and physiology. In: Stoskopf, M. K. (ed.) Fish medicine. W. B. Saunders, Philadelphia, p. 672–685

Martinsen, B., Horsberg, T. E., Sohlberg, S., Burke, M. (1993). Single dose kinetic study of sarafloxacin after intravenous and oral administration of different formulations to Atlantic salmon (*Salmo salar*) held in sea water at 8.5 °C. Aquaculture 118: 37–47

Martinsen, B., Myhr, E., Reed, E., Håstein, T. (1991). In vitro antimicrobial activity of sarafloxacin against clinical isolates of bacteria pathogenic to fish. J. aquat. Anim. Health 3: 235–241

Nouws, J. F. M., Grondel, J. L., Schutte, A. R., Laurensen, J. (1988). Pharmacokinetics of ciprofloxacin in carp, African catfish and rainbow trout. Vet. Quarterly 10: 211–216

Plumb, J. A., Vinitnantharat, S. (1990). Dose titration of sarafloxacin (A-56620) against *Edwardsiella ictaluri* infection in channel catfish. J. aquat. Anim. Health 2: 194–197

Samuelsen, O. B., Lunestad, B. T., Husevåg, B., Hølleland, T., Ervik, A. (1992). Residues of oxolinic acid in wild fauna following medication in fish farms. Dis. aquat. Org. 12: 111–119

Stamm, J. M. (1989). In vitro resistance by fish pathogens to aquacultural antimicrobials, including the quinolones difloxacin (A-56619) and sarafloxacin (A-56620). J. aquat. Anim. Health 1: 135–141

Steffenak, I., Hormazabal, V., Yndestad, M. (1991). Reservoir of quinolone residues in fish. Food Add. Contam. 8(6): 777–780

Stoskopf, M. K. (1992). Fish histology. In: Stoskopf, M. K. (ed.) Fish medicine. W. B. Saunders, Philadelphia, p. 31–47

Thune, R. L., Johnson, M. C. (1992). Effect of oral sarafloxacin dose and dose duration on *Edwardsiella ictaluri*-infected channel catfish. J. aquat. Anim. Health 4: 252–256

Ullberg, S. (1954). Studies on the distribution and fate of S^{35}-labelled benzylpenicillin in the body. Acta radiol. Suppl. 118: 1–110

Ullberg, S. (1977). The technique of whole body autoradiography. Cryosectioning of large specimens. Science Tools (the LKB instrument journal): special issue on whole-body autoradiography. Stockholm, p. 1–29

Ullberg, S., Lindquist, N. G., Sjöstrand, S. E. (1970). Accumulation of chorioretiotoxic drugs in the foetal eye. Nature 227: 1257–1258

Wilson, J. C., MacMillan, J. R. (1989). Evaluation of two arylfluoroquinolones against bacterial pathogens of channel catfish. J. aquat. Anim. Health 1: 222–226

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