Bioconcentration and Elimination of Sulfamethazine and Its Main Metabolite in Sturgeon (*Acipenser schrenkii*)

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A steady-state bioconcentration and elimination of sulfamethazine (SM_2) in the sturgeon (*A. schrenkii*) was conducted in flow-through aqueous conditions. Two treated groups of fish were exposed to concentrations of 1.00 and 0.10 mg/L of SM_2, respectively. SM_2 and its main metabolite, N^4-acetyl-SM_2, were determined in both fish muscle and water during the 8-day uptake period and the subsequent 6-day elimination period. Rapid uptakes of the drug were observed in both treated groups. Muscle tissue residues plateaued after ∼3 days. The bioconcentration factor in muscle (BCF_m) in the low-concentration drug solution was 1.19 and that in the high-concentration-treated level was 0.61. The calculated biodegradation index was 3.72%. The elimination half-times ($t_{1/2}$) of the two treatment levels were 19.44 and 23.52 h, respectively. The result indicates that SM_2 will neither bioconcentrate in individual aquatic organisms nor biomagnify in the food chain, although the BCF_m was relatively higher under the low-concentration exposure.

KEYWORDS: Sulfamethazine; bioconcentration; elimination; metabolite; sturgeon; HPLC

INTRODUCTION

Sulfamethazine (SM_2) is widely used in food production animals for therapeutic, prophylactic, and/or growth promotion purposes (*1*−*3*). SM_2 produces thyroid follicular tumors in rodent bioassays. Whether it could lead to similar toxicity from residue levels has not been tested. Large amounts of SM_2 and its metabolites may also be excreted in feces. The possibility exists that plentiful application of the drug could result in the introduction of the drug into nearby bodies of water, leading to uptake by aquatic organisms. Occurrence and distribution of SM_2 from the pharmaceutical industry have been described in water down-gradient from a contaminated landfill (*4*). To assess the potential impact of SM_2 on the environment, the bioconcentration and elimination were examined in the current study. Through the evaluation of the natural distribution, bioconcentration, and elimination of SM_2 residues following fish exposure to the treated water, the safety of these residues with respect to environmental and human exposure can be better assessed.

It is assumed that hydrophobicity, as indicated by the 1-octanol/water partition coefficient (k_{ow}) of a compound, is often the primary screening value for initial prediction of the tendency of a chemical to bioconcentrate in aquatic species (*5, 6*). On the basis of laboratory experiments, mathematical relationships have estimated bioconcentration (BCF). Using the regression equation derived by Neely (*7*)

$$\log BCF = 0.542 \log K_{ow} + 0.124$$

we estimate the BCF of SM_2 to be ∼1.28.

Another physicochemical property that has been used to predict the bioconcentration is aqueous solubility. An observed correlation for the trout between BCF and the aqueous solubility of a variety of compounds, both chlorinated and nonchlorinated hydrocarbons, was described by the regression equation (*6*)

$$\log BCF = 3.41 - 0.508 \log S$$

S equals the aqueous solubility in μmol/L. Thus, S for SM_2 is 1366 μmol/L at neutral pH, which suggests that the potential bioconcentration is 1.96.

On the basis of the regression equation from the two models above, SM_2 has little potential to bioconcentrate, but the model based on the equations might not be predictive for compounds outside the original databases. Furthermore, the model is only an approximation and useful mainly for neutral organic compounds (*5, 7*−*9*). SM_2 is an ionizable compound and would not be expected to have a predictable BCF, because the ambient pH value would be a contributing factor. Thus, the superior approach is to actually determine the BCF value from a real biological system.

Sulfamethazine is known to undergo metabolism to mainly form N^4-acetyl-SM_2 in numerous species of domestic animals, including fish and humans (*10*−*14*). Metabolic alteration of the

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parent compound by fish to more polar species or to less polar species can have an impact upon bioconcentration (*5*), so N^4-acetyl-SM$_2$, which is less polar than SM$_2$, was used as a biodegradation marker of the drug in the test organism. The sturgeon is delicious, reared for food in China nowadays, and easily available in most areas in China. No literature about the biotransformation of SM$_2$ in sturgeon has been reported. Thus, we selected it as the test organism.

Under natural conditions, the drug concentration would go through a change from high to low, and nontarget organisms may be exposed to different residual levels. To fully evaluate the potential for bioconcentration, we designed the experiment using two drug levels.

MATERIALS AND METHODS

Chemicals. Sulfamethazine was from Sigma (St. Louis, MO). N^4-Acetyl-SM$_2$ was synthesized according to the method of Vree et al. (*12*). Sulfamethazine sodium was obtained from the second pharmaceutical factory in Beijing, China, and the purity was 99%. Unless otherwise indicated, chemicals used were all of analytical or HPLC grade.

Preparation of Standard Solutions. Each standard (10 mg) was accurately weighed into a 100-mL volumetric flask and diluted with acetonitrile. Subsequent dilutions (0.01, 0.02, 0.05, 0.10, 0.50, 1.00, and 2.50 μg/mL) were made with mobile phase.

Test Organism. Juvenile sturgeons of both sexes from the same year class, with the estimated age of 4−5 months, were obtained from Miyun County fishery in Beijing. The fish had an initial mean length of 22.4 ± 3.5 cm and an initial mean weight of 28.2 ± 5.1 g and were initially held to acclimate for 20 days at 20 ± 1 °C in flow-through tap water, which had been dechlorinated by exposure to air-bubbling in advance. Sturgeons were fed standard fish granule food (obtained from a fishery feed company of Qingdao Oceanic University in China) daily, and excess food was siphoned from test chambers after feeding.

Test System and Sampling Procedures. The bioaccumulation of sodium SM$_2$ in sturgeon was studied in glass aquaria containing 528 L of water. The aquaria (one controlled and two treated) were temperature-controlled, held at 19−21 °C, and the flow-through resulted in five volume changes per day in each aquarium.

The treated sturgeons were divided into two groups of high- and low-dose levels. A primary stock aquarium was prepared with a solution of SM$_2$ directly dissolved. A peristaltic pump controlled by a rotation-meter delivered SM$_2$ stock solution to mixing chambers assigned to the treatment groups. For the two treatment groups, the diluter nominal aqueous concentrations were 1.00 and 0.10 μg/mL, respectively, and the resulting water flowed into respective aquaria continuously.

Before study initiation, the test solution from the diluter system was passed through test aquaria for a 24-h equilibration period, and the concentrations were confirmed by HPLC analysis.

The uptake phase was initiated by introducing 45 fish into each of the two treated aquaria and the control aquarium. Immediately after the 8-day exposure, the flow of SM$_2$ solution to both treatment aquaria was terminated and the water was removed by siphoning until a depth of ∼10 cm of water remained in each aquarium. Aquaria were refilled and replaced with equal volumes of untreated water. Fishes in both treatment groups were then exposed to the flow-through clean water for 6 days. Randomly selected water and fish (three fish and duplicate 10-mL samples of water at each time point) were sampled during exposure (i.e., days 0.25, 0.5, 1, 2, 3, 4, 5, 6, and 8) and elimination (i.e., days 8.5, 9, 10, 11, 12, and 13) periods from each aquarium and then assayed by HPLC.

Extraction and Determination of Residues. *For Muscle Residue.* Extraction and cleanup of muscle tissue were performed according to modified processes reported previously (*15−18*). Each sample of fish muscle (without adhering skin) was minced and homogenized in a homogenizer at high speed for 2 min, and 2 g was accurately weighed into 50-mL polypropylene centrifuge tubes and mixed with anhydrous sodium sulfate (∼5 g). Twenty-five milliliters of acetonitrile was added to each sample, swirled for 2 min, and shaken for 20 min at room

Table 1. Recoveries and Precisions of the Two Drugs from Fish Muscles ($n = 5$)

drug	added (μg/kg)	av recovery (%)	CV (%)
SM$_2$	20	80.4	8.4
	100	85.6	5.3
	2000	89.1	2.6
N^4-acetyl-SM$_2$	20	106.2	7.9
	100	86.0	4.4
	2000	87.6	2.1

temperature, and then centrifuged for 10 min at 2500 rpm; the supernatant was decanted to a 100-mL separatory funnel containing 40 mL of hexane. The separatory funnel was gently shaken for at least 2 min and allowed to stand to separate the layers at room temperature. The lower aqueous layer was collected to a 50-mL pear-shape flask. Another 25 mL of acetonitrile was added to the muscle cake and the above procedure was repeated. The two aqueous phases were combined and evaporated to near dryness under rotary low-pressure evaporator at 40 °C, and the residue was dissolved with 5 mL of acetonitrile/ water (95:5, v/v) and applied to an alumina B cartridge (Waters Sep-Pak Vac 12 cm^3, 2 g) that had been pretreated with 12 mL of acetonitrile/water (95:5, v/v). The column was cleansed with 8 mL of acetonitrile/water (95:5, v/v) and then eluted with 5 mL of acetonitrile/ water (75:25, v/v). The elution was collected in a 5-mL tube and evaporated to dryness under a stream of dry nitrogen. The residue was dissolved in exactly 2 mL of mobile phase, the solution was filtered through a 0.45-μm membrane, and subsequently 50 μL was injected into the HPLC system for quantitation.

For Water Residue. Extraction of the chemical residues in water was achieved by introducing 10 mL of water sample (the pH was adjusted to 6.5 with diluted acetic acid) into a 100-mL separatory funnel, followed by a 20-mL aliquot of ethyl acetate (*19*). The mixture was shaken vigorously for 5 min and the vapor exhausted; the mixture and layers were allowed to stand to separate, and the upper, ethyl acetate layer was collected. Another 20 mL of ethyl acetate was added to the separatory funnel; it was shaken, allowed to separate, and collected as before. Then the collected organic portion was evaporated by rotary evaporation at 40 °C to dryness. Exactly 10 mL of mobile phase was added to the flask and swirled to mix well. The solution was filtered through a 0.45-μm membrane, and 50 μL was subsequently injected into an HPLC system for quantitation.
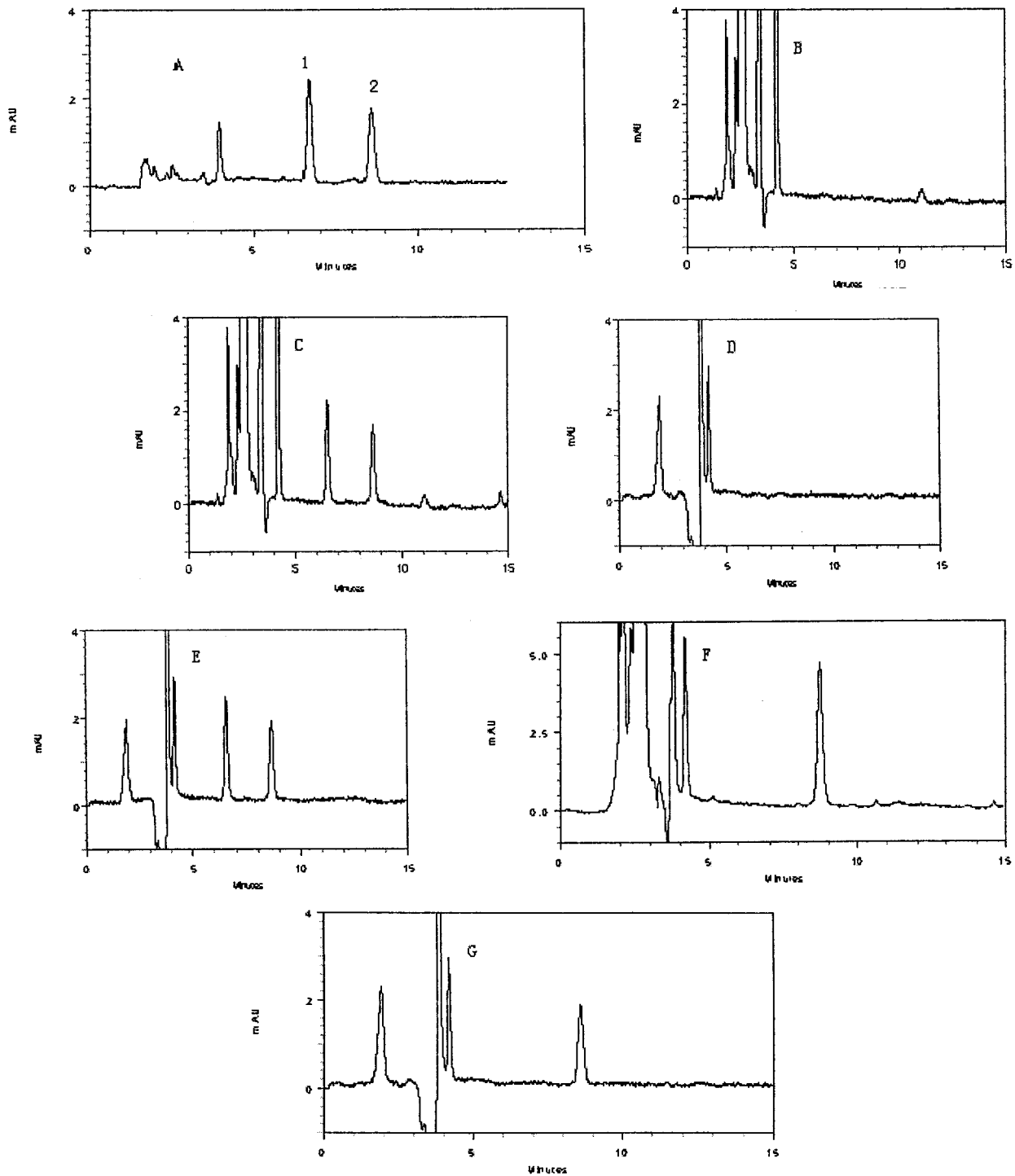
Liquid Chromatographic Conditions. Characterization of the isolated residue of the two agents was simultaneously conducted with a Shimadzu HPLC (consisting of an SCL-10 AVP system controller, a class VP 5.032 Chemstation, and an SPD-M10 AVP diode array detector). The separation was performed on Inertsil C$_{18}$ (5 μm, 250 × 4.6 mm i.d.) with acetonitrile/water/acetic acid (24:76:0.05, v/v) as mobile phase at a flow rate of 1.0 mL/min at room temperature. Typical chromatograms are presented as **Figure 1**.

RESULTS AND DISCUSSION

To test the applicability of the method for the simultaneous determination of two sulfonamides, the standard curves (concentration versus chromatographic peak area) were constructed with standard solutions over a concentration range from 0.01 to 2.50 μg/mL for two drugs. Fifty microliters of each standard solution was injected in an HPLC system. The linear correlation coefficients are 0.9995 for SM$_2$ and 0.9998 for N^4-acetyl-SM$_2$.

Fortified at 0.02, 0.10, and 2.00 μg/g in muscle, recoveries were 80.4−89.1% for SM$_2$ and 87.6−106.2% for N^4-acetyl-SM$_2$, with coefficients of variation (CVs) of 2.6−8.4% (SM$_2$) and 2.1−7.9% (N^4-acetyl-SM$_2$). The results are shown in **Table 1**. The detection limits were 0.01 μg/g for each drug. The detection limits of the assay were calculated to be 3 times the peak area of the baseline noise from the drug-free sample.

Fortified at 0.02, 0.10, and 2.00 μg/mL in water, recoveries were 93.7−96.4% for SM$_2$ and 91.1−102.6% for N^4-acetyl-

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Figure 1. Typical chromatograms of (**A**) 0.10 μg/mL mixed working solution, (**B**) blank sturgeon muscle, (**C**) 0.10 μg/g from fortified sturgeon muscle, (**D**) blank water, (**E**) 0.10 μg/mL from fortified water, (**F**) sample extracted from treated sturgeon muscle on day 1 of the 1.00 μg/mL exposure group, and (**G**) sample extracted from treated water on day 1 of the 0.10 μg/mL exposure group. Detection wavelength = 265 nm. Peaks: 1 = N^4-acetyl-SM$_2$; 2 = SM$_2$.

SM$_2$, with CVs of 2.6−6.5 and 2.4−5.9%, respectively. The results are shown in **Table 2**. The detection limits were 0.01 μg/mL for each drug.

In the whole experiment, the ranges of daily measured temperature, dissolved oxygen concentration, and pH were 19− 21 °C, 6.4−7.9 μg/mL, and 7.4−7.8, respectively, in all of the test aquaria, and no statistically significant variation was calculated between control and treated aquaria. During the whole test period, neither mortality nor adverse behavior was observed in treated groups, and no other difference between the controls and treated was observed either.

Table 2. Recoveries and Precisions of the Two Drugs from Water ($n = 5$)

drug	added (μg/L)	av recovery (%)	CV (%)
SM$_2$	20	93.7	6.5
	100	94.5	5.3
	2000	96.4	2.6
N^4-acetyl-SM$_2$	20	91.1	5.9
	100	97.2	4.0
	2000	102.6	2.4

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Table 3. Mean Values of Calculated Uptake Rate Constants (K_1) and Elimination Rate Constants (K_2), Muscle Bioconcentration Factors (BCF_m), Elimination $t_{1/2}$, and Biodegradation Index (BI) for SM_2 in Sturgeon Muscle

fish muscle	K_1 (h^{-1})	K_2 (h^{-1})	BCF_m	$t_{1/2}$ (h)	BI (%)
low-dose group (0.10 μg/mL)	20.16	16.80	1.19 (1.00–1.45)	23.52	not detected
high-dose group (1.00 μg/mL)	12.24	20.40	0.61 (0.44–0.70)	19.44	3.72
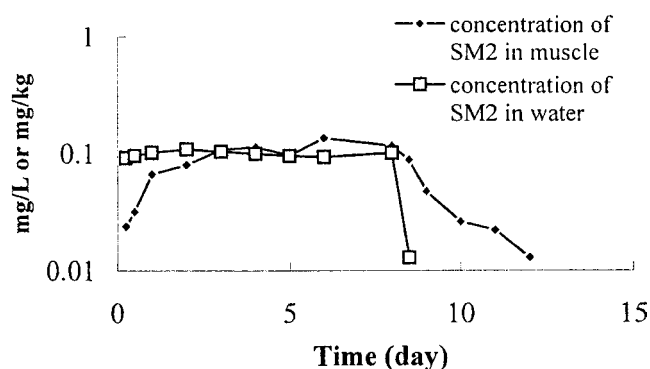
Figure 2. Measured SM_2 residue levels (mean ± SD) in muscle and water of 0.10 mg/L exposure level to sturgeons during 8-day exposure and 6-day elimination test.
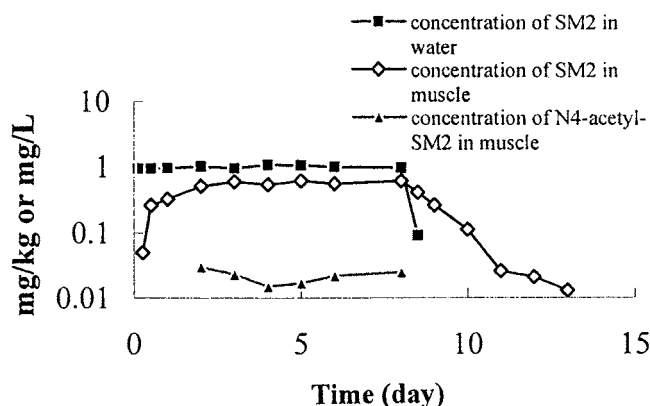
Figure 3. SM_2 and N^4-acetyl-SM_2 residue levels (mean ± SD) in water and muscle at 1.00 g/L exposure concentration during 8-day exposure and 6-day elimination in sturgeons.

The mean concentrations of SM_2 in water, as determined by HPLC, in the exposure solutions (uptake phase) were 0.101 ± 0.004 and 1.008 ± 0.036 μg/mL (mean ± SD), respectively, for the low and high concentrations, with no value deviating more than 7.6 and 5.2% from the means, respectively. Concentrations of SM_2 and its main metabolites, N^4-acetyl-SM_2, in water and in the muscle of sturgeon in the two treated groups over time during an 8-day bioconcentration and 6-day elimination study are presented in **Figures 2** and **3**. The acetyl metabolite was not detected in fish muscle from the low-level exposure group or in any treated water sample. Concentrations of SM_2 in fish muscle were below the detection limit from day 13. Thus, clearances for fish muscle until elimination day 5 were both >90%. The data reported in this study were not corrected for recovery.

The compound was found to fit first-order kinetics with high correlation coefficients ($r > 0.90$). The elimination data of the two groups from days 8–13 were fit to the "exponential curve" of $Y = C_m e^{-k2t}$ (*21*), where C_m is the mean concentration of SM_2 in muscle at day 8 and Y is the concentration of SM_2 in the muscle versus time. The uptake data, days 0–8, were iteratively fit to the "negative exponential growth curve" of $Y = (k_1/k_2)C_w(1 - e^{-k2t})$ (*22*), where C_w is the mean solution

concentration of SM_2. Fitting was performed using the "procnlin marquardt" program for SAS 8 statistical software system (*23*).

The bioconcentration factors in fish muscle (BCF_m) at the high and low drug levels, calculated from the relationship K_1/K_2, were 0.61 and 1.19, respectively. These values both agreed with the day 8 BCF_m values from the ratio between the steady-state residue concentrations in fish muscle and in water, which were 0.60 and 1.21, respectively. The time required to get 90% of the steady-state fish muscle concentration from the program above by regression equation was ~3 days. The mean biodegradation index (BI) was calculated from $C_{N4-axcetyl-SM2}/(C_{SM2} + C_{N4-acetyl-SM2})$ in fish muscle from steady-state concentration data; N^4-acetyl-SM_2 was counted as the biodegradation product; the BI was 3.72%. The K_1, K_2, the mean elimination half-time values calculated from the elimination curve, and the bioconcentration factors calculated from C_m/C_w (equivalent to K_1/K_2) are also listed in **Table 3**.

Rapid uptake by the fish was observed, and the residue plateaued by ~3 days. The steady-state bioconcentration factors for SM_2 in fish muscle (BCF_m) at the two concentrations were so low as to be considered of little environmental concern and are not expected to bioconcentrate into tissues consumed by humans nor biomagnify in fish consumed by fish predators.

Eliminations in the two exposure levels were also rapid. Elimination half-lives ($t_{1/2}$) for SM_2 were 19.44 and 23.52 h in the muscle, respectively, under the high- and low-drug levels. More than 90% of the drug was eliminated within 3–4 days after the fish in both exposure levels were placed in uncontaminated water.

It is important to note that there are significant differences between parameters of the two exposure groups, such as K_1, K_2, and BCF_m. These parameters were determined with two-sample *t* test ($a = 0.05$). As seen in **Table 3**, we could conclude that an ambient concentration exposure to the organism can have an impact upon pharmacokinetic processes. The comparatively higher BCF for sturgeon from the lower SM_2 concentration means that low BCFs may be obtained by testing with high concentrations and vice versa, which is in agreement with reuslts for other chemicals summarized by Franke (*24*). Relating the data to critical body burden concentrations for the respective ecotoxicological effects will be a better approach for risk assessment than considering the BCF alone.

In the in *vivo* study, the rapid uptake of sulfamethazine and its low persistence in sturgeon demonstrate that it would neither bioconcentrate in individual fish nor biomagnify in the food chain, even if it was introduced into a body of water. Such a result is similar to that reported with mosquito fish by Coats et al. (*20*).

The sturgeon is a good test model organism for it is easy to rear and easily available. It can be bred and cultivated in fish farms and in laboratories. With the further development of aquaculture, more will be available.

ABBREVIATIONS USED

SM_2, sulfamethazine; N^4-acetyl-SM_2, N^4-acetylsulfamethazine; BCF_m, bioconcentration factor in muscle; CV, coefficient of variation.

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