Table 1. Uptake Rate, Depuration Rate Constants (k_d), Half-Life ($t_{1/2}$), Assimilation Efficiency (α), Steady-State Bioaccumulation Factor (BAF), and Predicted Time to Achieve 85% Steady State (t_{ss})[a]

compound	exp. conc. (ng g^{-1})	tissue	uptake rate (pg g^{-1} d^{-1})	$k_d \times 10^{-2}$ (d^{-1})	$t_{1/2}$ (d)	α (%)	BAF	t_{ss}
P-CTX-1	19.1 ± 1.91	muscle	33.7 ± 2.53 (0.89)	2.52 ± 0.215 (0.97)	27.5 ± 2.34			
		skin	66.3 ± 3.49 (0.94)	0.966 ± 0.475 (0.58)	71.7 ± 35.3			
		brain	48.5 ± 2.77 (0.93)	5.92 ± 1.40 (0.86)	11.7 ± 2.78			
		gill	45.5 ± 3.03 (0.91)	1.67 ± 0.501 (0.92)	41.5 ± 12.4			
		liver	95.0 ± 6.12 (0.92)	16.5 ± 1.27 (0.98)	4.19 ± 0.323			
		intestine	48.8 ± 2.14 (0.96)	2.74 ± 0.409 (0.96)	25.3 ± 3.78			
		carcass	25.7 ± 1.38 (0.94)	2.91 ± 0.287 (0.97)	23.8 ± 2.35	6.01	0.0619	65.2
		whole fish	25.2 ± 1.30 (0.95)	3.23 ± 0.32 (0.97)	21.4 ± 2.12	6.13	0.0569	58.7
P-CTX-2	6.75 ± 1.06	muscle	5.00 ± 0.26 (0.94)	2.70 ± 1.10 (0.67)	25.6 ± 10.4			
		skin	11.3 ± 0.898 (0.88)	1.57 ± 1.57 (0.33)	44.1 ± 44.1			
		brain	8.65 ± 0.587 (0.91)	8.29 ± 0.435 (0.99)	8.36 ± 0.439			
		gill	11.6 ± 3.55 (0.33)	1.51 ± 1.50 (0.25)	45.9 ± 45.6			
		liver	6.27 ± 1.79 (0.53)	16.1 ± 1.30 (0.98)	4.31 ± 0.349			
		intestine	10.8 ± 0.817 (0.89)	2.00 ± 0.840 (0.74)	34.6 ± 14.5			
		carcass	3.96 ± 0.249 (0.92)	3.18 ± 0.801 (0.84)	21.8 ± 5.48	2.88	0.0271	59.7
		whole fish	3.51 ± 0.210 (0.93)	3.23 ± 0.826 (0.84)	21.5 ± 5.50	2.61	0.0243	58.7
P-CTX-3	4.00 ± 0.28	muscle	1.37 ± 0.0866 (0.92)	2.11 ± 1.22 (0.60)	32.9 ± 19.1			
		skin	4.07 ± 0.295 (0.90)	0.557 ± 0.571 (0.24)	125 ± 128			
		brain	3.00 ± 0.382 (0.74)	2.07 ± 1.08 (0.55)	33.5 ± 17.5			
		gill	2.78 ± 0.272 (0.83)	1.33 ± 1.57 (0.42)	52.0 ± 61.2			
		liver	1.50 ± 0.284 (0.56)	10.6 ± 2.59 (0.94)	6.53 ± 1.59			
		intestine	2.54 ± 0.165 (0.91)	1.66 ± 1.07 (0.55)	41.9 ± 26.9			
		carcass	1.12 ± 0.0786 (0.90)	1.82 ± 1.57 (0.31)	38.1 ± 32.8	1.28	0.0210	104
		whole fish	0.956 ± 0.0714 (0.89)	1.83 ± 1.36 (0.37)	38.0 ± 28.3	1.15	0.0189	104

[a]Error represents ± standard error, and values in parentheses represent the coefficient of determination (r^2) for the corresponding regression analysis.

$$\frac{C_{fish}}{C_{feed}} = \frac{\alpha F}{k_d}(1 - e^{-k_d t}) = BAF(1 - e^{-k_d t}) \quad (3)$$

where F is the feeding rate (F = 0.03 g food g^{-1} of fish d^{-1}). After long exposure times (i.e., $t \rightarrow \infty$), the equilibrium bioaccumulation factor (BAF) can be estimated through $\alpha F/k_d$.[33] Regarding the biotransformation between P-CTX-1, -2, and -3, a calculation[34] was conducted and the results indicated that the biotransformation of P-CTX-2 and -3 to P-CTX-1 in our exposed fish during the course of the experiment was negligible as shown in the Supporting Information.

RESULTS AND DISCUSSION

No orange-spotted groupers died during the experiment in either the control or treatment (exposed) groups. No detectable P-CTX-1, -2, and -3 were found in orange-spotted groupers in the control group at any time point during the entire accumulation−elimination phase (60 d). Significant differences in whole fish growth were observed between the exposed and control groups ($p < 0.005$). In addition, in the exposed group, the fish growth rate calculated at day 60 (0.0144 g d^{-1}) was significantly lower than that at day 30 (i.e., the accumulation end day, 0.0211 g d^{-1}) ($p < 0.001$). To address the dilution effect of toxin concentrations due to fish growth during the entire experiment, tissue P-CTX concentrations were corrected by multiplying a factor of $(1 + g \times t)$.[35] Levels of P-CTX-1, -2, and -3 in various exposed grouper tissues (muscle, skin, gill, brain, liver, and intestine) were therefore growth-corrected using the two growth rates (i.e., day 30 and day 60), and the levels are shown in Figure 1. In the following discussion, the concentrations are all growth-corrected.

Uptake and Depuration Kinetics of P-CTXs. For P-CTX-1, during the uptake phase from 0 to 30 d, the detectable concentrations of P-CTX-1 in six tissues of exposed grouper followed a linear increase with linear coefficients (r^2) in the range of 0.89−0.96, as shown in Table 1. Except for liver (r^2 = 0.53) and gill (r^2 = 0.33), r^2 of the concentrations of P-CTX-2 for a linear increase in the other four tissues was in the range of 0.88−0.94. In regard to P-CTX-3, except for the liver (r^2 = 0.56), r^2 of the concentrations for a linear increase in the other five tissues was in the range of 0.74−0.92. Furthermore, the concentrations of the three P-CTXs in all of the tissues did not reach a steady state during the 30 d exposure. An estimation of the time required to reach steady state (t_{ss}) was mathematically calculated for the whole fish and carcass (weight of carcass = weight of whole fish − weight of liver − weight of intestine).[35] According to eq 3, at steady state, C_{fish}/C_{feed} approaches BAF asymptotically and, by the mathematical definition, never equals BAF (until $t \rightarrow \infty$).[33] To obtain t_{ss}, we assumed 0.85 BAF (i.e., 85% to steady state) to replace C_{fish}/C_{feed} and predict t_{ss} for P-CTXs during the uptake phase. As a result, the t_{ss} values of the three P-CTXs in the whole fish and carcass were in the range of 58.7−104 d, which exceeded 30 d (Table 1). Therefore, our experimental uptake data did not appear to level off during the 30 d exposure.[33,36] Similar results were reported in a previous study in which juvenile *N. brevirostris* dietarily exposed to ciguatoxic dinoflagellate daily achieved a steady state at approximately 8−16 weeks.[24]

During the elimination phase, P-CTX-1, -2, and -3 levels in muscle, brain, and liver of exposed orange-spotted groupers declined exponentially from day 32 to day 60. In the skin, intestine, and gill, however, the observed elimination curves indicated two stages of elimination kinetics. The concen-

https://dx.doi.org/10.1021/acs.est.9b07888
Environ. Sci. Technol. 2020, 54, 4475−4483