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Experimental validation of geosmin uptake in rainbow trout, *Oncorhynchus mykiss* (Waldbaum) suggests biotransformation

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Abstract

The bioconcentration of waterborne geosmin in rainbow trout, *Oncorhynchus mykiss* (Waldbaum) was assessed. Fifty rainbow trout with a mean (*SD*) weight of 226.6 (29.0) g and lipid content of 6.2 (0.6) % (w/w) were exposed to geosmin in static water for 0, 2, 4, 6, 8, 12, 24, 36, 48 and 120 hr, with one tank containing five fish for each exposure period. Geosmin concentrations were measured in fish tissue and water samples collected over time. With time the geosmin concentration in the fish increased and decreased in the water. However, the total absolute amount of geosmin in the system declined over time which could be explained by induction of biotransformation. This is in accordance with the decreasing lipid normalized geosmin levels in the liver compared with the liver-free carcass. Geosmin distribution within rainbow trout clearly is not exclusively governed by the lipid content of tissues. In vivo geosmin bioconcentration in rainbow trout is slower and the body burden reached is lower than the generally accepted theoretical model predicts.

KEYWORDS
bioconcentration, biotransformation, geosmin, off-flavour, rainbow trout, water-lipid partitioning

1 | INTRODUCTION

Off-flavour is the presence of undesired sensory properties in food items. Most common in aquaculture products are earthy-musty off-flavours caused by the presence of the lipophilic chemicals geosmin (4*S*,4a*S*,8a*R*)-4,8a-Dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-4a-ol) and 2-methylisoborneol (1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol, MIB) in fish tissues (Howgate, 2004). Geosmin and MIB are secondary metabolites produced by a wide range of microbiota common to land-based aquaculture systems. Actinomycetes and cyanobacteria are considered the most important geosmin and MIB producers in aquaculture systems (reviewed by Krishnani, Ravichandran & Ayyappa, 2008). Off-flavour in farmed fish is one of the most significant economic problems for land-based aquaculture (Robin, Cravedi, Hillenweck, Deshayes & Vallod, 2006; Vallod, Cravedi, Hillenweck & Robin, 2007).

Fish rapidly bioconcentrate waterborne lipophilic chemicals in their tissues (reviewed by Streit, 1998). Geosmin and MIB are assumed to be predominantly exchanged between water and fish by passive diffusion via the gills (Howgate, 2004) given their respective octanol/water partition coefficients of 3.57 and 3.31 (Clark, Gobas & Mackay, 1990). Diffusion of the chemicals is driven by the difference in chemical potential or fugacity between water and lipid fractions in the system. Equilibrium is reached when the fugacities in water and lipid fractions are equal (Howgate, 2004). Distribution of lipophilic chemicals over different tissues within an organism is influenced by their lipid content and perfusion (Nichols et al., 1990). Ultimately lipophilic chemicals reach equilibrium in the lipid fraction of different

tissues in an organism (Bertelsen et al., 1998; Tietge et al., 1998; Gobas et al., 1999; all in Arnot & Gobas, 2006) and the lipid normalized chemical concentrations are then equal among tissues.

Howgate (2004) presented a one-compartment model to describe the time-kinetics of geosmin and MIB bioconcentration in fish, including theoretical uptake and excretion rate constants for rainbow trout. In this model, uptake and depuration are the two dominant processes in geosmin and MIB bioconcentration. Biotransformation, growth dilution and faecal egestion are assumed to be insignificant and therefore not included in the model. In Howgate's (2004) model, the assumptions and theoretical rate constants have not been validated experimentally.

The first goal of the present study was to assess the accuracy of model-predicted geosmin bioconcentration in rainbow trout based on Howgate's (2004) theoretical rate constants. We hypothesized that experimental geosmin bioconcentration equals model-predicted bioconcentration. The second goal was to verify the assumption that uptake and depuration are the two dominant processes in geosmin bioconcentration. We predicted the uptake by the fish of geosmin from the water to follow first order kinetics. The geosmin removal from the water then equals the accumulation of geosmin in the fish. The third goal was to verify the assumption that within the fish geosmin is distributed according to the lipid content of tissues. We hypothesized that lipid-normalized geosmin concentrations in the whole fish and the liver are equal.

To test these hypotheses we exposed rainbow trout, *Oncorhynchus mykiss* (Waldbaum) to waterborne geosmin for 0 up to 120 hr in air-tight tanks with static water and monitored geosmin levels over time in water, whole fish, liver and head space air of the tanks.

2 | MATERIALS AND METHODS

2.1 | Bioconcentration experiment

The bioconcentration experiment was performed in 180 L polyester tanks with static water. The design of the geosmin exposure tanks aimed to minimize and quantify any geosmin losses via volatilization and adsorption to the tank and its auxiliary equipment (system losses). To this end each tank was covered by a 6 mm thick glass cover sheet and sealed air-tight to the tank by Duct tape. The glass covers were equipped with a circular hatch (diameter 150 mm) to allow for introduction of fish. The hatches were covered by glass sheets (200 × 200 mm) during the experiment.

Head space was minimized by maximum filling of the tanks with water. Teflon tubing was used where contact with tank water was inevitable (aeration, water sampling). Tubing (in and outflowing air, water sampling) entered the tank via air-tight transits. Each tank was equipped with an air-pump set at an air inflow of 300 ml/hr for oxygen supply. Air was extracted from each tank by a central vacuum pump at a flow rate slightly above that of the incoming air to create an under-pressured head space in the tank. For each tank extracted air was washed over a glass gas washing bottle filled with 1 L methanol to collect any volatilized geosmin.

One day before the start of the bioconcentration experiment, a single batch of 1,900 L local tap water was spiked with 8,143 μl of a 70 μg/ml geosmin (Sigma Aldrich) stock solution in acetone and mixed well, resulting in a nominal geosmin concentration of 308 ng/L. Four hours before the start of the experiment, nine exposure tanks were filled with 170 L of the geosmin solution. According to preliminary model predictions (see below) an initial geosmin level in the water above 300 ng/L should result in geosmin levels in water and fish above analytical detection limits at all sampling points. Fifty rainbow trout with a mean (*SD*) weight of 226.6 (29.0) g and a mean (*SD*) lipid content of 6.2 (0.6) % (w/w) were randomly split into ten groups of five fish. One group served as $t = 0$ fish sample. The other nine groups were randomly divided over nine exposure tanks at $t = 0$. Fish were exposed to geosmin for ca. 2, 4, 6, 8, 12, 24, 36, 48 and 120 hr with one tank for each exposure time prior to sampling.

A control treatment without fish was not included in the experiment as the stability of the geosmin concentration in the water of the exposure tanks had already been established in a preliminary stability study. The stability study was conducted in accordance with the OECD guideline for bioconcentration studies in fish (OECD, 2012), using two of the 9 identical exposure tanks filled with 170 L water spiked with geosmin. Tank water was aerated as described for the bioconcentration experiment. Water samples for geosmin analysis were collected from both tanks at $t = 0$ and $t = 120$ hr. Geosmin concentrations were 160 and 155 ng/L and $t = 0$ and 160 and 150 ng/L at $t = 120$ hr, which demonstrates that 97%–100% of the geosmin concentration measured at $t = 0$ remains in the exposure tanks during 120 hr.

Water samples for geosmin analysis (250 ml in glass containers) were collected from each geosmin exposure tank at $t = 0$ and just before fish sampling and stored at 4°C. Upon fish sampling approximately 99% of the water volume was quickly drained from the exposure tank. Fish were euthanized by adding 3 ml/L phenoxy ethanol to the remaining tank water and then removed from the tank. Livers were dissected, pooled per tank and stored in glass containers at −80°C. Liver-free carcasses (the entire fish except for the liver) were pooled per tank, homogenized using a refrigerated mincer (DRC C10, PSV Group, Genainville, France) and stored at −80°C in glass containers. Total biomass per tank was measured at $t = 0$; individual fish weight was measured upon fish sampling (Mettler PM40).

Dissolved oxygen concentration, and water temperature (Hach Lange Multimeter) were measured in each tank at $t = 0$ and upon fish sampling. Overall mean (*SD*) dissolved oxygen concentration was 5.5 (0.9) mg/L. Overall mean (*SD*) water temperature was 18.8 (0.3)°C.

Gas washing bottles were sampled upon fish sampling. Immediately after each fish sampling, the tank, air-stone, glass cover sheet and tubing were rinsed with 50 ml methanol to wash off any adsorbed geosmin. All methanol samples were stored in glass bottles at 4°C prior to geosmin concentration measurement.

The treatment of the fish was in accordance with Dutch law concerning animal welfare, as approved by the ethical committee for animal experimentation of Wageningen UR Livestock Research.

2.2 | Geosmin and lipid analysis

Liver-free carcass samples and liver samples were thawed overnight at 4°C. From each sample a subsample of approximately 1 g was taken. To each subsample 100 μl of internal standard solution (D5-geosmin in water, 1 μg/ml, Sigma Aldrich) was added. Samples were extracted by accelerated solvent extraction (ASE, Dionex, Amsterdam, The Netherlands) at 40°C using a 15:85 (v/v) pentane-dichloromethane mixture. After extraction, 1 ml of hexane was added to the extract. Extracts were concentrated to 1 ml by gently evaporating the pentane-dichloromethane mixture (Rotavap, Heidolph) and stored in 2 ml amber coloured glass vials at −20°C until geosmin concentration measurement.

To each water sample (250 ml) 100 μl of internal standard solution (D5-geosmin in water, 1 μg/ml) was added. Methanol samples originating from the gas washing bottles and the rinsing of equipment were diluted approximately 20 times in demineralized water and subsequently treated the same as the water samples. Water samples were extracted via an extraction cartridge (Sep-Pak® Vac 6 cc (1 g) Certified tC18) which were then eluted with 5 ml diethyl ether. Water was removed from the collected diethyl ether by addition of dried sodium sulphate. Diethyl ether samples were then separated from the sodium sulphate by manually transferring the liquid to another glass tube. The sodium sulphate was washed three times with 5 ml diethyl ether to ensure full transfer of geosmin. The extracts were concentrated to 1 ml under a gentle nitrogen gas flow and stored in an amber coloured glass vial at −20°C until geosmin measurement.

Geosmin concentrations were measured on a Shimadzu GCMS2010 (GC) coupled to a GCMS-QP2010 Ultra (MS) detector (Shimadzu, 's Hertogenbosch, The Netherlands) as described in detail in Schram, Schrama, Kusters, Kwadijk and Palstra (2016). The method for geosmin concentration measurement was validated in low fat (6% w/w) fish samples according to NEN 7,777 (Anonymous, 2011) and established a limit of detection of 6.1 ng/g, a recovery of 93.5%–99.2% and an extended uncertainty (U) of 27.8%. The 95% confidence interval of measured geosmin concentration equals the measured value ± U/2.

Lipid content of the liver-free carcass samples and liver samples was determined using the gravimetric method according to Bligh and Dyer (1959) modified by De Boer (1988).

2.3 | Calculations and statistics

The total amounts of geosmin in the system compartments (liver-free carcass, liver, water and gas washing bottles) were calculated for each sampling point by multiplying the measured geosmin concentrations with the respective masses or volumes.

Bioconcentration can be described mathematically by an organism-water two compartment model:

$$\frac{dC_B}{dt} = k_1 C_{WD} - (k_2 + k_E + k_M + k_G)\, C_B \quad (1)$$

where C_B is the chemical concentration in the fish (g/kg), C_{WD} the chemical concentration in the water (g/L), k_1, k_2, k_E, k_M and k_G the rate constants (days^{-1}) for the uptake from the water k_1, excretion to the water k_2, faecal egestion k_E, metabolic biotransformation k_M and growth dilution k_G. The sum of k_2, k_E, k_M and k_G represents the total elimination or depuration rate constant k_T (Arnot & Gobas, 2006). As the fish in our study were starved prior to the experiment and not fed during the experiment, we excluded the rate constants for faecal egestion and growth dilution from the model. In contrast to Howgate (2004) we do not rule out biotransformation of geosmin and thus maintain the metabolic biotransformation rate constant k_M in the model. Equation 1 then simplifies to:

$$\frac{dC_B}{dt} = k_1 C_{WD} - k_2 C_B - k_M C_B \quad (2)$$

In experimental settings the chemical concentration of lipophilic compounds in the water declines over time due to the uptake of chemicals by organisms (Arnot & Gobas, 2006). Since our experimental system had static water and a high biomass to water volume ratio, we predicted a declining geosmin concentration in the water over time due to uptake in the fish. To account for a decline of the geosmin concentration in the water in the current experiment, we extended the bioconcentration model by allowing C_{WD} to vary over time, which is described by:

$$\frac{dC_{WD}}{dt} = zk_2 C_B - zk_1 C_{WD} \quad (3)$$

where the first term describes the increase in concentration as a result of depuration from fish and the second term is the change rate of the concentration in the water as a result of uptake by fish. The parameter z is the ratio of fish biomass to water volume, which is used to account for the different masses. We assume that fish and water have an identical density of one. We solved the system formed by Equations (2) and (3) analytically using Mathematica 9.0 (Wolfram Research, Champaign, Illinois, USA) to yield equations for $C_{B(t)}$ and $C_{WD(t)}$:

$$\begin{aligned} C_{B(t)} = \frac{1}{2D} e^{-\frac{1}{2}t\,(k_2 + k_M + k_1 z + D)} \big(& k_2 C_{B0} + k_M C_{B0} - 2k_1 C_{WD0} \\ & - k_2 C_{B0} e^{tD} - k_M C_{B0} e^{tD} + 2k_1 C_{WD0} e^{tD} - k_1 C_{B0} z \\ & + k_1 C_{B0} e^{tD} z + C_{B0} D + C_{B0} e^{tD} D\big) \end{aligned} \quad (4)$$

$$\begin{aligned} C_{WD(t)} = \frac{1}{2D} e^{-\frac{1}{2}t\,(k_2 + k_M + k_1 z + D)} \big(& - k_2 C_{WD0} - k_M C_{WD0} \\ & + k_2 C_{WD0} e^{tD} + k_M C_{WD0} e^{tD} - 2k_2 C_{B0} z + k_1 C_{WD0} z \\ & + 2k_2 C_{B0} e^{tD} z - k_1 C_{WD0} e^{tD} z + C_{WD0} D + C_{WD0} e^{tD} D\big) \end{aligned} \quad (5)$$

where $D = \sqrt{-4\,k_1\,k_M z + (k_2 + k_M + k_1 z)^2}$, C_{B0} is the chemical concentration in the fish (g/kg) at $t = 0$, C_{WD0} the chemical concentration in the water (g/L) at $t = 0$ and t is time (days).

Experimental rate constants for uptake (k_1), depuration (k_2) and biotransformation (k_M) were estimated by fitting the equations for $C_{B(t)}$ and $C_{WD(t)}$, respectively, to the observed geosmin concentrations in the fish and the water by non-linear regression analysis. The liver-free carcass samples were used to represent the geosmin concentration in the intact fish C_B, which is appropriate given the minimal contribution of the liver (~1.5%) to the total mass and geosmin

content of the intact fish. Theoretical uptake (k_1) and depuration (k_2) rate constants representative for the experimental fish were calculated according to Howgate (2004), taking into account mean fish body weight, fish lipid content and water temperature in the experiment. As Howgate (2004) does not consider geosmin biotransformation, the theoretical metabolic biotransformation rate constant k_M was set at zero.

The equations for $C_{B(t)}$ and $C_{WD(t)}$ were used to predict the development over time of the geosmin concentrations in fish and water, using the experimentally determined (experiment based prediction) and theoretical (theory based prediction) rate constants. For hypothesis-testing the 95% confidence intervals of the experimental estimates for the rate constants were compared. Rate constants were considered significantly different at $p < .05$ when 95% confidence intervals showed no overlap.

Geosmin concentrations in liver-free carcass samples and liver samples were normalized for lipid content by dividing the measured geosmin concentration by its lipid content. The geosmin concentrations in the lipid fractions of the liver-free carcass and the liver were predicted to increase with exposure time (Howgate, 2004; OECD, 2012). The measured geosmin concentrations were related to natural logarithm transformed exposure times by linear regression analysis. We hypothesized that geosmin is distributed over the liver-free carcass and the liver according to their lipid contents and reaches equilibrium in the lipid fractions, in which case the ratio between the lipid normalized geosmin content of the liver and the liver-free carcass R equals 1. To assess the development of this ratio towards 1 with increasing exposure time, the lipid-normalized liver:liver-free carcass ratio was related to exposure time t using Equation (6).

$$R = R_0 \times e^{(-k\,t)} + A \tag{6}$$

where R_0 equals the ratio between the lipid normalized geosmin content of the liver at $t = 0$, k a term for the change in the ration over time and A the asymptote. The model parameters were estimated by non-linear regression analysis.

All statistical procedures were performed in SAS 9.1.

3 | RESULTS

3.1 | Geosmin in water and fish

Geosmin concentrations, volumes and masses of system components, and the total amounts of geosmin in the various system components are presented per exposure time in Table 1. The total amount of geosmin in the water declined over time while in the fish the total amount of geosmin increased (Table 1). The total amount of geosmin in the system declined over time and the loss of geosmin from the system exceeded geosmin accumulated in the fish (Table 1). During the first 12 hr of the experiment no geosmin was detected in the gas washing bottles, and this increased from <0.1 at $t = 24$ hr to 860 ng at $t = 120$ hr. In total less than 4% of the total amount of geosmin in water and fish together was found in the gas washing bottles. No geosmin was detected in the methanol used to

TABLE 1 Geosmin mass balance. Absolute amounts of geosmin (ng) in the different system components per exposure time (hr)

	Liver-free carcass			Liver			Water			Gas washing bottle filled with methanol				
Exposure time	Geosmin (ng/g)	Mass (g)	Total geosmin (ng)	Geosmin (ng/g)	Mass (g)	Total geosmin (ng)	Geosmin (ng/L)	Volume (L)	Total geosmin (ng)	Geosmin (ng/g methanol)	Mass (g)	Total geosmin (ng)	Total geosmin (ng)	Geosmin losses (ng)
0	0	1,023	0	1.9	10.2	19	308	170	52,360	–	–	–	52,379	
1.8	5.5	1,251	6,881	9.5	12.5	119	180	169.5	30,510	n.a.	725	–	37,509	14,870
3.8	8.7	1,201	10,449	8.2	12.0	98	160	169.2	27,072	n.a.	714	–	37,619	14,760
5.9	9.5	1,096	10,412	7.5	11.1	82	140	168.5	23,590	n.a.	751	–	34,084	18,295
7.8	11	941	10,351	5.5	9.4	52	140	168.1	23,534	n.a.	682	–	33,937	18,442
12.5	13	1,184	15,392	7.6	11.8	90	110	167.7	18,447	n.a.	646	–	33,929	18,450
23.9	15	1,100	16,500	7.9	11.0	87	85	166.9	14,187	0.1	638	64	30,773	21,606
36.5	18	939	16,902	6.6	9.4	62	82	166.5	13,653	0.6	513	308	30,617	21,762
47.9	21	1,033	21,693	7	10.3	72	67	166.3	11,142	0.8	488	390	32,907	19,472
120.9	14	1,101	15,414	5.3	11.0	58	45	165.7	7,457	2.3	377	867	22,929	29,450

rinse the exposure tanks and auxiliary equipment upon termination of the geosmin exposure. Geosmin losses due to evaporation and adsorption to rinse the exposure tanks and auxiliary equipment were therefore almost negligible.

3.2 | Theoretical versus experimental geosmin bioconcentration

Exposure of rainbow trout to waterborne geosmin resulted in an increase in the geosmin concentration in the fish (Figure 1a) and a steady decline of the geosmin concentration in the water (Figure 1b). The equations for $C_{B(t)}$ and $C_{WD(t)}$ provided significant fits to, respectively, the observed geosmin concentrations in the liver-free carcass and to the observed geosmin concentrations in the water. The estimates for the uptake, excretion and biotransformation rate constants all show large 95% confidence intervals (Table 2). The theoretical rate constant for geosmin excretion by rainbow trout does not differ from the two experiment-based estimates. The theoretical uptake rate constant for geosmin by rainbow trout is higher than the uptake rate constant estimated from the observed geosmin uptake by the liver-free carcass but lower than the uptake rate constant estimated from the observed geosmin decline in the water (Table 2). The theoretical predictions consequently show a faster geosmin uptake in the fish (Figure 1a) and a slower decline of the geosmin concentration in the water (Figure 1b). In the theoretical prediction the biotransformation rate constant (k_M) was set at zero and geosmin levels in fish and water reach steady-states. For both experiment based predictions k_M was estimated to be larger than zero. Consequently the model predicts constant geosmin removal from the system and the concentrations in fish and water do not reach a steady-state. Instead the geosmin concentration in the fish first peak and then start to decline (Figure 1a). The geosmin concentration in the water continues to decline at the point where the geosmin concentration in the theoretical prediction stabilizes (Figure 1b).

3.3 | Geosmin uptake: liver-free carcass versus liver

Geosmin exposure of rainbow trout resulted in a significant increase over time of the geosmin concentration in the lipid fraction of the liver-free carcass, while the highest geosmin concentration in the lipid fraction of the liver was reached already at the first sampling point (2 hr) and declined thereafter (Figure 2a). The ratio between the lipid-normalized geosmin concentration in the liver and the liver-free carcass initially declined and then stabilized after ca. 20 hr at approximately 0.5 (Figure 2b), showing that the geosmin concentration was consistently lower in the liver than in the liver-free carcass.

4 | DISCUSSION

Based on the generally accepted model for geosmin bioconcentration in fish (Howgate, 2004), the predicted uptake of geosmin by rainbow trout exposed to waterborne geosmin will result in a decline of the geosmin concentration in the water and an increase in the geosmin concentration in the fish. We indeed observed an increase in the geosmin concentration in the liver-free carcass of rainbow trout, which coincided with a decline of the geosmin concentration in the water. These findings are in accordance with the general consensus on the uptake by fish of waterborne lipophilic chemicals in general (Nichols et al., 2007) and geosmin in particular (Howgate, 2004) and were previously reported by Robertson, Hammond, Jauncey, Beveridge and Lawton (2006).

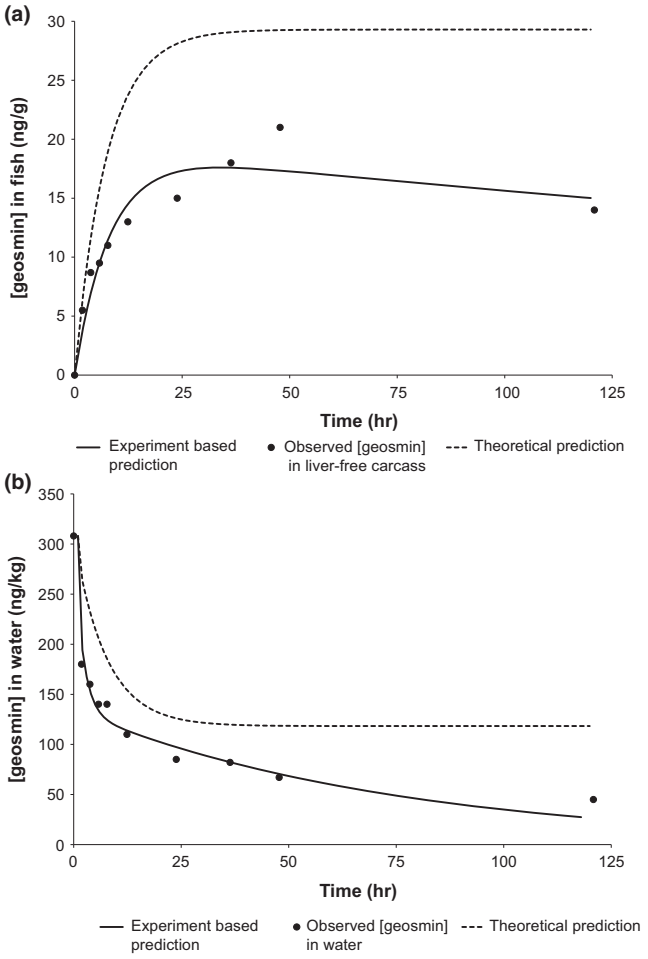

FIGURE 1 Observed and predicted geosmin bioconcentration in rainbow trout (a) and the concurrent decline of the geosmin concentration in the water (b). The predictions are based on the equations for $C_{B(t)}$ (a) and $C_{WD(t)}$ (b). The theoretical predictions are based on theoretical uptake (k_1) and excretion (k_2) rate constants according to Howgate (2004), adapted to the mean fish body weight, fish lipid content and water temperature in the experiment. The experiment-based predictions are based on the experimental uptake, excretion and biotransformation rate constants estimated by fitting the equation for $C_{B(t)}$ to the observed geosmin concentrations in the liver free carcass (a) and the equation for $C_{WD(t)}$ to the observed geosmin concentrations in the water (b). Both are significant fits, respectively to the observed geosmin concentrations in the liver-free carcass and water (non-linear regression analysis, $p < .0001$)

TABLE 2 Theoretical and experimentally determined geosmin uptake rate constants (k_1), excretion rate constants (k_2) and metabolic biotransformation rate constant (k_M) for rainbow trout

	k_1			k_2			k_M		
Background	**Estimate**	***SE***	**95% CI**	**Estimate**	***SE***	**95% CI**	**Estimate**	***SE***	**95% CI**
Theoretical (Howgate, 2004)	307[a]			1.24			0[a]		
Experimental ($C_{B(t)}$)	180[b]	35.2	97–263	1.69	0.64	0.17–3.23	0.12[ab]	0.10	−0.11–0.36
Experimental ($C_{WD(t)}$)	1,073[c]	236.1	515–1,631	4.87	1.98	0.19–9.55	0.56[b]	0.23	0.02–1.11
Experimental ($C_{B(t)}$) excluding t = 0*	100[b]	20.8	49–151	0.0017	0.25	−0.62 to 0.62	0.21[b]	0.07	0.046–0.38
Experimental ($C_{WD(t)}$) excluding t = 0*	162[b]	21.1	111–214	0.43	0.17	0.004–0.86	0.12[ab]	0.11	−0.15 to 0.38

Experimental uptake, excretion rate and metabolic biotranformation constants were estimated by fitting the observed geosmin uptake in the liver-free carcass C_B to the equation for $C_{B(t)}$ and by fitting the observed geosmin concentration in the water C_{WD} to the equation for $C_{WD(t)}$. Estimates without overlap in their 95% confidence intervals were considered significantly different from each other and these are indicated by different letters in superscript.
*See Discussion.

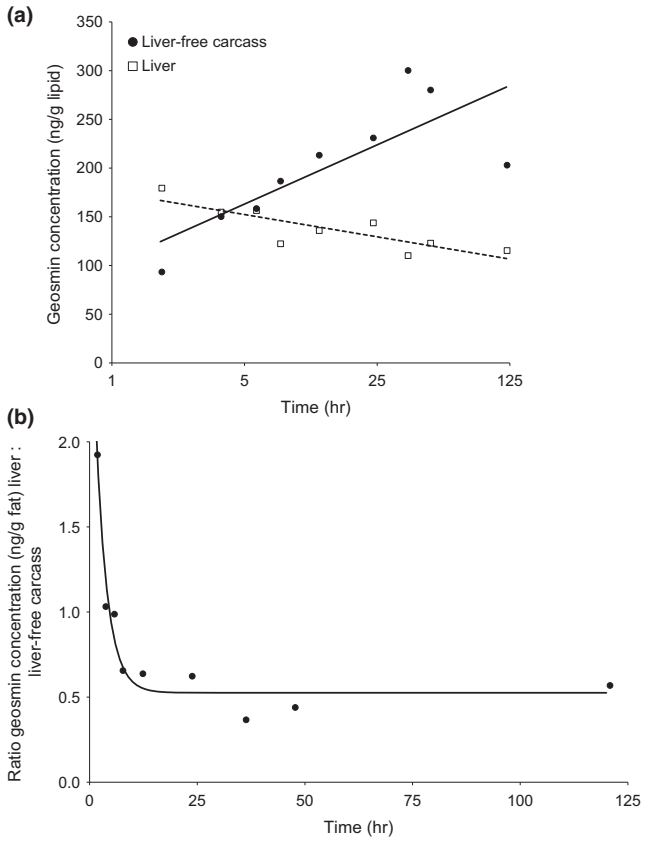

FIGURE 2 (a) Lipid-normalized geosmin concentration (ng/g lipid) over time in the liver-free carcass and the liver of rainbow trout during geosmin exposure. (b) The ratio between lipid-normalized geosmin concentrations (ng/g lipid) in liver and whole body over time during geosmin exposure. Ratio = $2.70 \times e^{(-0.37 \times \text{Time})} + 0.52$. Model p-value = .0002

We exposed rainbow trout to geosmin in closed systems with static water of limited volume (high biomass:water ratio). During exposure in static water, the uptake of the chemical by the fish is expected to cause a decline in the water exposure concentration, especially when both the biomass:water volume ratio and the affinity of the chemical for the fish are high. Advantage of exposure in static water is that it requires far less of the target chemical than exposure in flow through systems (e.g. according to the OECD 305 test guideline). The uptake and depuration rate constants, however, are intrinsic properties of the organism and therefore independent from the exposure system. A static system thus provides a cost-effective alternative for flow through exposure systems when the studied chemical is expensive, as is the case for geosmin. Monitoring the decline of the chemical concentration in the water over time ($C_{WD(t)}$) next to the increase in the concentration in the fish over time ($C_{B(t)}$), provides and additional data set that describes the time-kinetics of the bioconcentration of the chemical. In theory, both data sets yield the same estimates for the rate constants of the bioconcentration process, provided that the chemical partitions to no other compartments than the fish and water compartment and that biotransformation, growth dilution and faecal egestion are absent or taken into account.

In the current bioconcentration experiment, the observed increase in the geosmin concentration in the fish ($C_{B(t)}$) and the decline of the geosmin concentration in the water ($C_{WD(t)}$) lead to conflicting modelling results. The observed geosmin uptake in the fish was lower than the theoretical rate constants predict, which implies a lower concurrent decline of the geosmin concentration in the water than the theoretical rate constants predict. However, the opposite was observed: the geosmin concentration in the water declined faster than predicted by the theoretical rate constants. The observed decline of the geosmin concentration in the water implies a faster geosmin uptake in the fish than predicted by the theoretical rate constants and observed in the fish. These conflicting modelling results are reflected by the significant differences among the estimates and theoretical values for the uptake rate constant k_1. The observation that the decline of the geosmin concentration in the water is not reflected by a corresponding geosmin increase in this fish suggests that the rate constants estimated from the observed geosmin concentration in the water are overestimations or that some process is overlooked. The rate constants estimated from the observed geosmin concentrations in the fish seem a better representation of the actual values. Oxygen saturation in the experiment was rather low at 60%–65% saturation. Oxygen concentration in the water is an important determinant of the gill ventilation rate (Neely, 1979) and higher gill ventilation rates result in higher uptake (k_1) and depuration (k_2) rate constants (equations in Howgate, 2004). The experimental oxygen conditions thus probably led to relatively fast

geosmin uptake in the fish and high values for the uptake (k_1) and depuration (k_2) rate constants compared with uptake at 100% oxygen saturation. However, the final geosmin concentration reached in the fish was probably not affected by the low oxygen saturation as the bioconcentration factor (k_1/k_2) does not depend on the gill ventilation rate. Jointly taken, it appears that net geosmin uptake in vivo is less than theoretical rate constants predict for rainbow trout. It should be noted that although oxygen concentration and temperature do not appear directly in the model we used, these factors are accounted for since the estimated rate constants are specific for the experimental conditions.

Clearly the decline of the geosmin water concentration cannot be fully explained by the uptake of geosmin in the fish. Therefore, biotransformation in the fish and other geosmin sinks have to be considered. Given the stable concentration of waterborne geosmin in the preliminary stability study without fish, system losses of geosmin due to volatilization, adsorption to the tanks or geosmin removal by microbial degradation (Ho, Hoefel, Bock, Saint & Newcombe, 2007) seem unlikely. Growth dilution and faecal egestion may contribute to the loss of chemicals from fish (OECD, 2012), but not from the system. Growth dilution and faecal egestion are unlikely as the exposure period was short, the fish were not fed the day before and during geosmin exposure and no faeces were observed in the exposure tanks. Any faeces present inside the fish would be included in the liver free carcass sample.

Most geosmin was lost from the system between $t = 0$ and $t = 1.8$ hr. In this period, the geosmin concentration in the water showed a strong decline which did not result in a corresponding increase in the geosmin concentration in the fish. The strong initial decline of the geosmin concentration in the water therefore cannot be entirely attributed to uptake in the fish; other, unknown geosmin sinks also seem to play a role. We therefore excluded the observed geosmin concentrations in fish and water at $t = 0$ and used the observed levels at $t = 1.8$ hr (5.5 ng/g and 180 ng/L) as the initial geosmin concentrations in fish and water to estimate the rate constants by fitting the equations for $C_{B(t)}$ and $C_{WD(t)}$ to, respectively, the observed geosmin concentrations in the fish and the water. The results are included in Table 2. The so obtained estimate for the uptake rate constant based on the observed geosmin levels in the fish does not differ from the estimate including the data observed at $t = 0$. The uptake rate constant based on the geosmin decline in the water is strongly affected by excluding the $t = 0$ data: the estimated rate constant for uptake from the water no longer differs from the estimate based on the observed geosmin uptake in the fish. Excluding the observations at $t = 0$ does not change the notion that net geosmin uptake in vivo is less than theoretical rate constants predict for rainbow trout.

Although assumed to be absent in fish (Howgate, 2004) biotransformation of geosmin has in fact never been investigated. Biotransformation of lipophilic compounds such as geosmin is very likely as biotransformation pathways have very low substrate specificity and almost any non-polar (lipophilic) compound can be metabolized (Jakoby & Ziegler, 1990), and several biotransformation pathways have been established in fish (Kleinow, Melancon & Lech, 1987). We therefore did not rule out geosmin biotransformation and maintained the metabolic biotransformation rate constant in the bioconcentration model. The model-based equations we used to describe the kinetic profiles of geosmin in water and fish provided both significant as well as visually satisfactory fits to the observed data. Biotransformation will result in a lower bioconcentration of compounds than predicted based on their lipophilicity (Lech and Bend, 1980; Kleinow et al., 1987), which is exactly what we observed. Rather than reaching the equilibrium states as would be the case in absence of biotransformation, the measured levels in water and fish suggest removal of geosmin from the system, which is in accordance with the notion of induced biotransformation. Our kinetic profile for geosmin bioconcentration shows large resemblance with the kinetic profile for trifluralin bioconcentration in rainbow trout, for which biotransformation has been established (Schultz & Hayton, 1999).

Biotransformation affects the distribution and accumulation of chemicals in fish (Kleinow et al., 1987). Presence of significant geosmin biotransformation in fish would thus open new opportunities for off-flavour mitigation and management next to geosmin depuration. Large variations in biotransformation pathways have been demonstrated among fish species but also among individuals of the same species (Kleinow et al., 1987). Variation in biotransformation capacity can both be inherent, have a genetic basis, and be the result of induction or inhibition by environmental factors and ubiquitous chemicals (Kleinow et al., 1987). Variation with a genetic basis opens opportunities to increase biotransformation capacity by selective breeding. Induction of biotransformation during off-flavour depuration may enhance geosmin elimination and thereby reduce the required depuration time as well as improve depuration results. All this clearly requires further investigations, with establishing the actual presence of geosmin biotransformation and its relative contribution to geosmin elimination being first priorities.

The geosmin concentration in the lipid fraction of the liver at the end of the experiment was approximately twofold lower than the geosmin concentration in the lipid fraction of the rest of the body. Following absorption from the water by the gills, lipophilic compounds are distributed throughout the fish' body via the circulatory system and exchanged with all vascularized tissues and organs (Streit, 1998). Circulating lipophilic compounds accumulate rapidly in highly perfused organs such as the liver. Accumulation is slower in organs with lower blood flow, such as muscle and adipose tissue (Barron, 1990, 1995; Bickel, 1984; Gunkel and Streit, 1980; all in Streit, 1998). The relatively high levels of geosmin we observed in the rainbow trout liver shortly after the start of the exposure to geosmin may thus be explained by the high perfusion of this organ. The following decline of the geosmin content of the liver relative to the rest of the fish body can be explained by induced biotransformation in the liver, known for its high biotransformation capacity. This quite plausible mechanism could explain the development over time towards a decreasing geosmin concentration in the liver lipids compared with the liver-free carcass lipids (ratio from ~1.5 at $t = 2$ hr to ~0.5 at $t = 120$ hr). Clearly, geosmin distribution within the fish is not exclusively governed by the lipid content of tissues.

5 | CONCLUSION

The current study reveals that rainbow trout bioconcentrates waterborne geosmin, but in vivo bioconcentration is less than the generally accepted model predicts based on theoretical rate constants assuming passive distribution based on lipophilicity only. Clearly, geosmin distribution within rainbow trout is not exclusively governed by the lipid content of tissues, given the different lipid-normalized geosmin concentrations in liver compared with the liver-free carcass. Geosmin removal from the water exceeded the concurrent geosmin bioconcentration in the fish. The observed geosmin concentrations in the water and liver free carcass can be described by model-based equations that include biotransformation. The liver usually is the main site of biotransformation, which can explain the relatively low lipid-normalized geosmin levels in the liver, after initial fast uptake, compared with those in the rest of the fish. Because biotransformation affects the distribution and accumulation of chemicals in fish, we advise to perform dedicated biotransformation studies aimed at detecting geosmin and 2-methylisoborneol metabolites to confirm the suggested biotransformation of both off-flavour causing compounds.

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