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Measuring the Elimination of Arsenic by the Gills of Rainbow Trout (*Salmo gairdneri*) by Using a Two Compartment Respirometer

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Arsenic occurs naturally in minerals and ores from which it may be released during dissolution, weathering and erosion. Other than the natural sources, large quantities of arsenic are released into the environment as a result of industrial and agricultural activities. From these sources arsenic finds its way into the aquatic environment where it may be taken up by the components of the aquatic ecosystem, including fish.

In mammals, loss of arsenic occurs mainly via the urine, with the fecal route accounting for only a relatively small portion of the total (Charbonneau et al. 1978). This may not be the case for fish since an earlier study by Oladimeji et al. (1979) indicated that urinary and biliary excretion together could account for only a minor portion of arsenic ingested by rainbow trout. On this basis it was suggested that arsenic could have been lost by the gills. The present study is designed to examine the role of gills in arsenic excretion and also to obtain a quantitative estimate of this route for arsenic elimination. The proportion of the ingested dose eliminated via the urine was also estimated.

MATERIALS AND METHODS

Rainbow trout (57-82g) were obtained from Goosen's Fish Hatchery, RR2, Otterville, Ontario. On arrival at the laboratory, fish were held at 10 ± 1°C in refrigerated fibreglass tanks (Frigid Unit, Toledo, Ohio) with approximately 1g of fish per 150 ml of water. These tanks were aerated and supplied with a continuous flow of 24l/h dechlorinated filtered water having a pH of 7.2-7.4, hardness of 45gm l^{-1} and dissolved oxygen of 10-11 mg l^{-1}0. During experimentation fish were fed once daily with Purina Trout Chow at a daily rate of 2% of their body weight.

The radioisotope arsenic (^{74}As) used for this study was in the form of carrier-free arsenic acid in 0.04N HCl and was purchased from Amersham Searle, Arlington Height, Ill. The specific activity of the arsenic was 0.5-1.0 ug/mCi.

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One week prior to dosing, fish were tagged (fingerling fish tags, 5mm x 3mm) through the musculature anterior to the dorsal fin for subsequent identification.

Measurement of arsenic excreted via the gill was achieved by the use of the two compartment respirometer of Boddington et al. (1979). Modifications in design and operation, as indicated by Fig. 1, were incorporated to achieve this function.

The respirometer with diaphragm installed as described by Boddington et al. (1979) was placed in a bath of running water 12 ± 1°C. The fish was placed in the restrainer and located horizontally with its head projecting through the hole in the diaphragm and into the head chamber until the opercula just cleared the diaphragm. Note that in this application, the fish was located horizontally, not at an angle of 30° to the horizontal as described by Boddington et al. In this position relative to the diaphragm exhalant water was returned to the head chamber, movement of jaw muscles was not restricted by the diaphragm and there was no leakage across the diaphragm with the fish in situ tested with methylene blue.

Oxygen in slight excess to maintain saturation was continuously bubbled through the water in the head region through A_1 (Fig. 1.1) vented at A_2 through a bulb containing activated charcoal to absorb any volatile arsenic in the effluent gas. The bulb was counted for radioactivity associated with adsorbed arsenic at the end of each run. The opening, C_1, in the head region was fitted with a probe for monitoring oxygen concentration which was maintained near saturation at all times during measurements. A vertical tygon tubing attached to A_2 served as a manometer for the tail end to monitor and ensure maintenance of pressure differences between the two compartments.

Duplicate 5 ml water samples were removed from the head and tail compartments through B_1 and B_2 respectively at 30 minute intervals. The radioactivity associated with the arsenic in the water was measured in the gamma counter and the samples returned into the respective chambers.

For this part of the study fish were not anaesthetized. Instead eight starved (24h) fish were fed arsenic-containing diet to satiation individually in separate aquaria. The amount of arsenic (^{74}As) ingested by each fish ranged from 1.5 ng-3.5 ng (3-7 uCi). Fish were placed in the respirometer 20 h postdose. Since preliminary runs made with fish in the respirometer prior to 20 h postdose resulted in regurgitation of arsenic contaminated food in the head chamber of the respirometer. No regurgitation was observed during the runs. This is not expected since according to Windell et al. (1969) gastric evacuation would have been completed by 20 h postdose under the conditions of this experiment.

Fish were kept in the respirometer for period of times ranging from 1.5-4.0 h and a number of runs were carried out on each fish

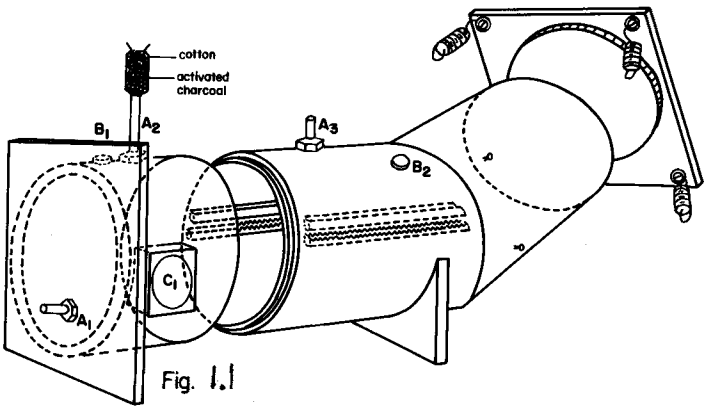

Fig. 1.1

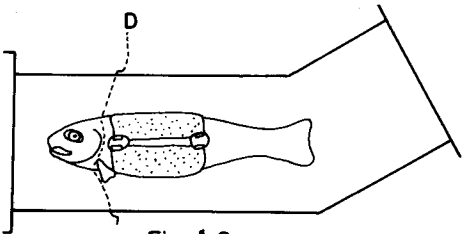

Fig. 1.2

Fig. 1.1 Apparatus used for measuring the excretion of dietary arsenic via the gill of rainbow trout.

Fig. 1.2 Orientation of the restrainer and fish. (See text for description and operation of the apparatus.)

at various times between 20-288 h postdose. Measurements of arsenic in the head compartment was made every 30 minutes of each run. No anaesthetic was used since the most common anaesthetic, MS-222, has been shown by various studies including Houston et al. (1979) to reduce blood flow and movement of water across the gills.

Fish used for kidney excretion of arsenic had an average weight of 57.4 ± 0.5 g. Each fish was anesthetized lightly (50 ppm MS-222) and force-fed one # 4 gelatin capsule containing 500 mg of pulverized trout chow to which 25 ug of arsenic was added. ^{74}As (10 uCi) and 24.99 ug stable arsenic acid, used as carrier. After ingestion of the capsule, each fish was placed in a 120 litre aquarium and watched for 2 h in case any regurgitation took place. Fish that regurgitated were discarded. The body content of arsenic 2 h postdose was regarded as the dose.

Urine collection was carried out by insertion of a polyethylene tube (PE90) through the urinary papilla of lightly anaesthetized fish as described by Fenwick (1974). The tube was fixed in place by suturing it with a thread to the skin and musculature surrounding the vent. Urine collection began 24 h after catheterization. At the beginning of each period of urine collection, each fish was anaesthetized and the finger-cot which has been previously washed with water was deflated and tied over the end of the catheter. At the end of each period, the urine volume was measured.

The amount of arsenic excreted in the urine was determined by radioassaying 0.5 ml portions of urine and the amount of residual arsenic (^{74}As) in the emptied cot was also estimated.

Whole body retention of the input dose of arsenic was determined for each fish by measuring its content of arsenic (^{74}As) at various times (postdose) when the urine was collected or elimination via the gill measured using an Ortec gamma counter which had a counting efficiency of approximately 40%. The arrangement of the apparatus for whole body counting of arsenic is shown in Fig. 2.

RESULTS AND DISCUSSION

Data for the excretion of arsenic via the gills are presented in Table 1. The results show that the rate of excretion into the head compartment of the respirometer via the gills was very high during the first half-four of each run probably due to excitement of the fish since the more rapid opercular movements during this period suggest an increased metabolic rate. During the second and third half-hour periods, opercular movements slowed down to a more normal rate. Excretion rates from the gill reported in Table 1 were therefore based on the 2nd and 3rd half-hour periods in the respirometer.

The mean rate of elimination via the gills based on the measurements made during the first two days postdose ranged from 0.77 -

Table 1. Excretion of an ingested dose of arsenic[a] via Gill tissue by rainbow trout, Salmo gairdneri[b], held at 12 ± 1°C.

Time post-dose (h)	Mean dose ngAs/fish	Gill excretion of arsenic[b] Fractional clearance % dose/h	% of remaining body burden/h	Arsenic loss from whole[c] fish, fractional clearance % dose/h
20	2.6 0.5	0.77±0.13	1.72±0.29	1.3±0.16
44		0.46±0.03	1.52±0.12	0.7±0.12
90		0.23±0.02	1.42±0.18	0.3±0.03
164		0.07±0.01	1.40±0.10	0.2±0.03

[a]Specific activity = 3.52 x 10^6 cpm/ng As.
[b]Mean wet weight of fish, 82±6g.
[c]Based on whole body content of arsenic at end of each indicated time period.

Table 2. Urinary excretion of arsenic[a] by rainbow trout[b], Salmo gairdneri, following an oral dose of 10 uCi^{74} (25 ug arsenic, as arsenic acid) in 500 mg trout chow. Fish were held at 10 ± 1°C.

Collection period h, postdose	Urine output (ml per 100 g fish/h) Control n = 2	Treated n = 4	Urinary excretion[c] of arsenic % of dose	% of remaining body burden	Remaining body[d] burden of arsenic % of dose
0-24	0.63	0.37±0.10	1.4±0.4	2.2±0.4	75.3±6.2
24-48	0.55	0.47±0.06	2.0±0.3	2.6±0.3	54.1±8.2
48-72	0.50	0.53±0.09	5.2±2.5	10.9±4.9	38.2±6.4
72-96	0.43	0.43±0.07	3.2±0.1	11.0±5.5	29.9±4.7
96-120	0.35	0.25±0.06	1.6±0.7	5.2±2.2	21.1±3.0
120-144	0.40	0.27±0.05	1.7±0.5	7.2±1.9	16.3±2.7
144-168	0.30	0.24±0.07	1.3±0.6	6.7±2.7	13.3±2.2

[a]The arsenic dose consisted of 0.10 ug^{74}As as arsenic acid plus 24.9 ug arsenic as non-radio active arsenic acid.
[b]Mean wet weight of fish, 57.0±0.59.
[c]Values represent percent per collection period.
[d]Values represent percent remaining body burden at the end of each collection period.

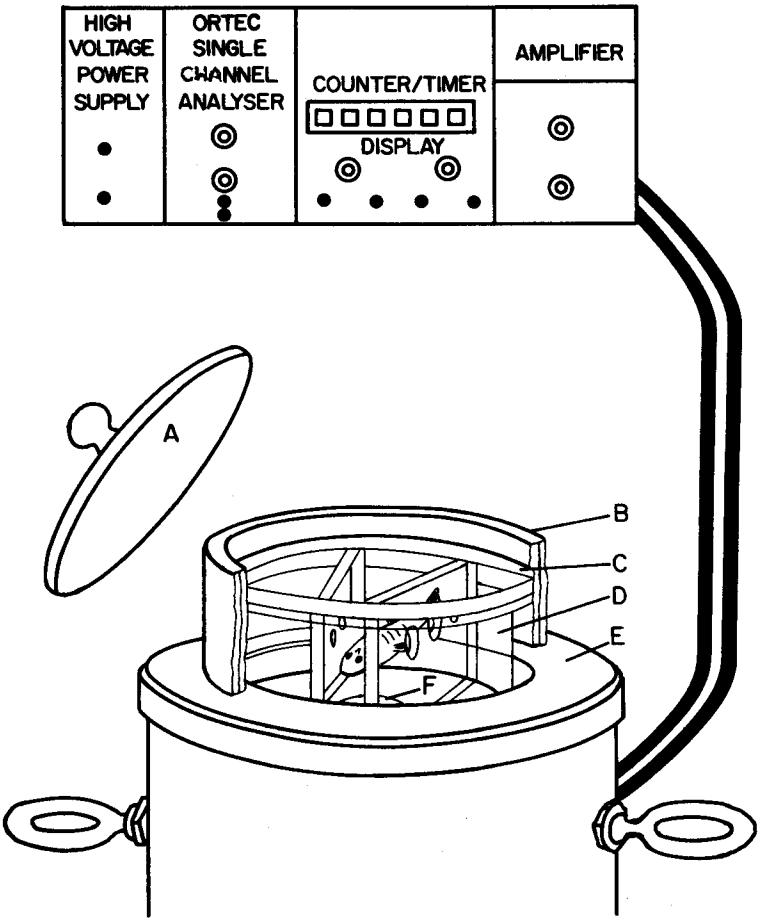

Fig. 2 Whole body counter for live fish.

A. Additional lead shield cover.
B. Additional lead shield on top of detector - cut away to reveal dish containing the fish.
C. Plastic lid for pyrex dish (partitioned to keep fish directly above F).
D. Pyrex glass dish containing 100 ml of water.
E. Original lead shield for detector.
F. Doughnut hole in NAI detector of counting vial when system is used as a manual deep well counter.

0.46% of dose h^{-1} with a mean of 0.62% h^{-1}. The values decreased to 0.23% h^{-1} and 0.07 at 90 h and 164 h postdose respectively. These values for clearance from the gill could account for approximately 40% of the ingested dose over a 7-day period. It is important to note that by 20 h postdose about 26% of the ingested dose had been cleared from these test fish as shown by the data in Table 1 and that the excretion of arsenic via the gills accounts for most of the initial fast-clearing phase.

Results in Table 2 demonstrate that the urine output of the control and the treated fish were similar. Using the urine output (ml per 100g fish h^{-1}) as a basis for evaluating renal function, the dose of 25 ug As/fish did not seem to affect renal function. Urinary excretion of arsenic during 7 days postdose (Table 2) accounted for about 15% of the ingested dose of arsenic. Extrapolation of the clearance rate of arsenic in urine to post exposure times longer than 7 days suggests that during the two week period (8-21 days), arsenic loss via urine would have accounted for an additional excretion of no more than 5% of the ingested dose.

The excretion of orally administered inorganic arsenic in man (Coulson et al. 1935; Hunter et al. 1942; Mealey et al. 1959; Ray Bettely and O'Shea 1975) and monkeys (Charbonneau et al. 1979) has been shown to be primarily via the kidneys. In the present study an average of 16% of the ingested dose was excreted in the urine by each fish within a 7 day period postdose. However, there are marked individual differences between the fish. For instance, fish No. 3 (Table 2) excreted 25% of the administered dose in 7 days whereas the excretion rates in others were much lower. Similar differences were reported in monkeys by Charbonneau et al. (1978). The authors attributed the variation to differences in individual's metabolism.

The excretion of arsenic from the gills was rapid, accounting for approximately 40% of the administered dose over a period of 7 days after feeding. Calculations based on the measurements taken during the 1st and 2nd half hour of each run appear to be an overestimate of the "normal" excretion rate. Measurements made during the third half-hour during which the fish appeared to have settled down were therefore used in the calculations. Measurements of oxygen consumption were not made during this study and consequently, there is no quantitative measure of excitation. However, measurements made by Boddington et al. (1979) indicated that the metabolic rate of rainbow trout made in this respirometer were 9.6 ug O_2 min^{-1} $g^{-0.8}$ for the first and 8.0 ug O_2 min^{-1} $g^{-0.8}$ for the second set of fish. These values were considered high by Boddington et al. (1979) but within the 'scope' when compared to the data of Kutty (1969), Evans (1970), Griffiths (1978) of 2,5 and 13 ug O_2 min^{-1} $g^{-0.8}$ for standard, routine and sustained active metabolism. Values presented here may therefore be considered a reasonable approximation of the actual rate of excretion of arsenic from the gills of free-swimming rainbow trout.

The results of this study show that the respirometer could be used effectively to study the elimination of pollutants via the gills of fish. The apparatus is simple and easy to operate. The only major problem is the initial high metabolic rate of the fish, which is minimised if they are allowed to calm down before the measurements are made.

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