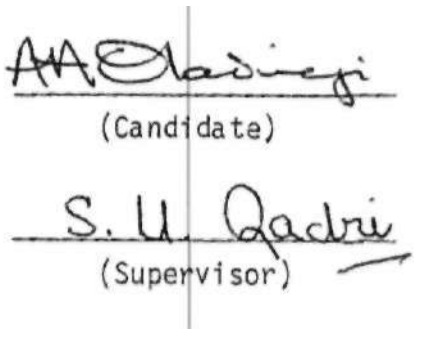
BIOACCUMULATION AND METABOLISM OF ARSENIC BY RAINBOW TROUT, *SALMO GAIRDNERI*

BY

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ABSTRACT

Uptake of inorganic arsenic from water and food and its retention by rainbow trout, Salmo gairdneri, were determined using radioactive tracer methods. Arsenic taken up from both vectors was accumulated into two compartments which ware distinguishable by differences in their fractional clearance rates. The uptake rates of arsenic from water, though low were directly related to the concentrations of arsenic in water. However, arsenic accumulation from food was more efficient, with a minimum assimilation of 50% of ingested dose.

Clearance of arsenic was in two phases following uptake from both water and food. The fast compartment, which comprised from 19-30% of the dose had a half-life of less than 1 day. The slow-clearing compartment had a half-life of 4.3-5.0 arjd 6.4 days following uptake from water and food respectively. There was no effect of route of exposure on the relative size of the fast and slow-cllearing compartments.

The results on the routes of arsenic elimination by rainbow trout demonstrate that fecal and gill routes can be of equal magnitude and could account for 80% of total arsejnic loss, urinary excretion accounting for 20% or less. Urine, unlike in mammals, seems to play a minor role in the elimination of arsenic by rainbow trout.

Chemical speciation of arsenic was examined by radiotracer and chromatographic techniques applied to muscle, liver, gill, kidney, blood, bile and urine of trout following an oral dose of

^{74}AS as arsenic acid. The ratio of total organic to inorganic arsenic increased rapidly with time in all tissues, with the organic fraction accounting for about 50% at 6 h postdose and over 80% at 24 h. An organoarsenic metabolite was shown by thin layer chromatography (TLC) to have the same Rf value as ethylarsenobetaine and, this compound accounted for tie major portion of the total arsenic in all tissues as well *as* urine and bile sampled 24-96 h postdose.

The effect of acclimation to sublethal dose of arsenic on whole body clearance rates, tissue distribution and binding of a subsequent dose of ^{74}As was examined. Whole body clearance of ^{74}As was twice as fast in preexposed fish. The liver and bile of preexposed fish contained higher concentrations of arsenic than those for the control. Gel chromatography of liver cytosol proteins revealed no differences in arsenic-protein binding pattern in control and preexposed fish. Arsenic elution was in two peaks, the first peak bound to proteins of molecular size 19,000 daltons and the other corresponding to moleculer size 3,000 daltons.

Trout exposed to arsenic levels of 20 mg As kg^{-1} diet for a duration of 2 to 8 weeks showed reduced growth Compared to the control. The liver contained the highest level of arsenic among the tissues analysed.

RESUME

L'absorption d'arsenic inorganique à partir de l'eau et de la nourriture et sa rétention par la truite arc-en-ciel, Salmo gairdneri, ont été estimées par dépistage radioactif. L'arsenic absorbé à partir des deux vecteurs fut accumulé dans deux compartiments que l'on peut distinguer par les différences dans leurs taux d'élimination fractionnelle. Les taux d'absorptions d'arsenic à partir de l'eau, quoique faibles, furent directement proportionelles aux concentrations d'arsenic dans l'eau. Néanmoins, l'accumulation d'arsenic à partir de la nourriture fut très efficace avec un minimum d'assimilation de 50% de la dose ingérée.

L'élimination de l'arsenic s'éffectue en deux phases suivant son absorption à partir de l'eau et de la nourriture. Le compartiment rapide, qui comprend de 19-30% de la dose, avait une demie-vie de moins d'un jour. Le compartiment à élimination lente avait une demie-vie de 4.3-5.0 et 6.4 jours suivant son absorption à partir de l'eau et de la nourriture respectivement. Il n'y eut aucun effet de route d'exposition sur la grandeur relative des compartiments d'élimination rapide et lent.

Les résultats sur les routes d'élimination d'arsenic par la truite arc-en-ciel démontrent que les routes fécale et branchiale peuvent être de grandeur égale et responsable de 80% de l'arsenic éliminé, l'excrétion urinaire n'étant responsable que de 20% ou moins. L'urine, à l'opposé de chez les mammifères, semble jouer un role de second importance dans l'élimination d'arsenic par la truite arc-en-ciel.

La spéciation chimique de l'arsenic fut examinée par des techniques de dépistage radioactif et chromagraphique auxquelles furent soumis les muscles, le foie, les reins, le sang, la bile et l'urine de truite suivant une dose orale d'^{74}As sous forme d'acide arsénieux. Le rapport d'arsenic organique total à arsenic inorganique total augmente rapidement avec le temps dans tous les tissus, la fraction organique étant responsable pour approximativement 50% 6 h suivant la dose et au-dessus de 80% à 24 h. Un métabolite organo-arsénique fut démontré par chromatographie sur couches minces (CCM) comme ayant un Rf semblable à l'ethylarsenobetaine et, ce composé fut responsable pour la plus grande fraction de l'arsenic total dans tous les tissus de même que dans l'urine et la bile échantillonnées 24-96 h après la dose.

L'effet d'acclimation à des doses sous-léthales d'arsenic sur les taux d'élimination à la grandeur du corps, sur la distribution d'arsenic dans les tissus et sur l'attachement d'une dose subséquente d'^{74}As fut examiné. L'élimination d'^{74}As à la grandeur du corps fut deux fois plus rapide dans les poissons pré-exposés contenaient de plus hautes concentrations d'arsenic que ceux du témoin. La chromatographie sur gel des protéines du cytosol du foie n'a révélée aucune différences dans le type d'attachement arsenic-protéine dans les témoins et les poissons pré-exposés. L'éluat d'arsenic donna deux sommets, le premier sommet attaché à des protéines de 19,000 daltons de grosseur moléculaire et l'autre correspondant à 3,000 daltons de grosseur moléculaire.

Les truites exposées à des niveaux d'arsenic de 20 et 30 mg

As Kg^{-1} dans leur diète pour une période de 2 à 8 semaines démontrèrent une croissance réduite comparé au témoin. Le foie contenant le plus haut niveau d'arsenic parmi les tissus analysés.

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GENERAL INTRODUCTION

Arsenic occurs naturally in minerals and ores from which it may be released during dissolution, weathering and erosion. As much as 10-15 percent arsenic may be associated with certain gold ore and coal, while up to 1 percent may be present in galena (PbS) ores used principally in lead smelting. Ferguson and Gavis (1972) estimated that 41 x 10^9 g arsenic are released annually from rocks through geochemical processes.

Other than the natural sources, large quantities of arsenicals are released into the environment as a result of industrial and especially agricultural activities. The mining, refining and smelting of ores of gold, lead and copper, with which arsenic is usually associated, result in release of arsenic, chiefly as arsenic trioxide. The use of pesticides containing arsenic also contributes to the environmental load of arsenic. Paris green (copper acetoarsenite) was the first pesticide widely used in modern agriculture (Carriker et al. 1976) and several arsenic compounds continue to be used today. All these sources of arsenic may result in high concentrations of arsenic in the atmosphere, soil and the aquatic environment.

Arsenic levels in natural waters vary widely, ranging from 0 to 400 ppb (NRCC No. 15391, 1978). Waldbott (1973) suggested that the arsenic content of uncontaminated surface waters averages less than 10 ppm in general. However, groundwaters are higher in arsenic than surface fresh and marine waters. Hot and cold spring waters containmuch higher levels of arsenic when they percolate

through volcanic ash and rocks, which may contain up to 7.7 percent arsenic (NRCC No 15391, 1978).

Arsenic occurs in the aquatic environment in several forms. Inorganic forms of arsenic prevail in most waters. Arsenate has been reported to be the most stable form of inorganic arsenic in freshwater and is most common under equilibrium conditions. The chemical forms of arsenic present in the aquatic media depend on the physicochemical characteristics of the water such as pH, Eh and dissolved oxygen content. At low pH, the lower oxidation states of arsenic (3,0,-3) are prevalent. More basic conditions result in an increase in the percentage of arsenic present in the pentavalent state (Gulledge and O'Connor, 1973). Arsenic in the 0 form is quite rare and the -3 forms exist only at extremely low Eh values. The two most common forms are the +3 and +5 forms of which the +3 is the more toxic (Ferguson and Gavis, 1972).

The accumulation of arsenic by aquatic organisms is well documented. In seawater containing arsenic levels ranging between 0.05 and 5 µg l^{-1}, marine plants are reported to have arsenic concentrations ranging between 1 and 12 mg kg^{-1}, while marine animals have concentrations usually between 0.1 and 50 mg kg^{-1} (Cox, 1925; Wedepohl, 1969) Chapman (1926) found arsenic levels as high as 200 mg kg^{-1} in shrimps and lobsters. Concentrations in freshwater organisms are generally much lower than in marine species. Woolson (1975), in a recent review, reported a bioaccumulation ratio in the order of 3-30 for freshwater fish and 500-5400 for marine species. The difference between freshwater and marine systems may be a function

of the arsenic concentration to which the organisms are continuously exposed as well as its chemical form. Differences in osmoregulatory systems may also be involved.

The chemical nature of arsenicals in the medium, and in the organism, affects the retention, and extent of arsenic toxicity. Arsenate (As + 5) usually elicits fewer signs of toxicity than arsenite (As + 3) (Ferguson and Gavis, 1972). Naturally occuring organic forms are known to be less toxic than the inorganic, and as such, biotransformation into the organic forms is considered a detoxification mechanism.

Since fish may take up arsenic from the environment directly from the water via the gills, or by ingesting sediment and other food items contaminated with arsenic (Penrose, 1975), it may be assumed that the exposure vector will influence accumulation and subsequent kinetics of arsenic in both freshwater and marine organisms. Direct uptake of arsenic from freshwater by fish has been shown to be relatively inefficient compared to methyl mercuric chloride (CH_3HgCl), for example (deFreitas and Hart, 1975), whereas uptake from food is very efficient with assimilation efficiency of more than 50% (Lunde 1972; Penrose 1975). In the decapod crustacean, Lysmata seticaudata, an assimilation efficiency of ingested inorganic arsenic of 88% was observed and elimination kinetics differed from those following uptake directly from water (Fowler and Unlu, 1978).

One important factor, besides physicochemical paramaters such as temperature, pH and alkalinity, that may influence the

uptake and subsequent handling of a toxicant by an organism is the recent environmental history of the organism in terms of exposure to arsenic. Previous contact with toxic agents must be taken into account before tolerance experiments are performed since previous encounters in some cases may render an animal more sensitive (Warren, 1971). However, the tolerance of an animal to a toxicant is usually increased by a recent exposure to sublethal levels of the toxicant. The stress resulting from sublethal levels elicits some adaptive responses within the organism which renders it more tolerant to low stress condition and even to subsequent, more critical ones. The organism is said to have adapted and become acclimated to the conditions. Fish have been shown to acclimate to a variety of environmental pollutants, including zinc (Lloyd, 1960; Edwards and Brown, 1967; Sinley et al. 1974) and cadmium (Spehar, 1976; Pascoe and Beattie, 1978). It was suggested by Pascoe and Beattie (1978) that synthesis of metallothionein, a metal protein complex produced in response to the ions of cadmium, zinc and mercury, could have been responsible for the increased resistance to cadmium observed in rainbow trout. Acclimation to arsenic has also been reported for mammals (Schwartze, 1922; Kuhs et al. 1939) and fish (Dixon, 1979). The extent of acclimation of rainbow trout fingerlings to arsenic was shown to be a function of level as well as the duration of exposure (Dixon, 1979). There is no evidence yet that arsenic induces the production of metallothionein, but it is possible that one of the processes involved in the acclimation of rainbow trout to arsenic could be generally similar to that for cadmium and related metals. Increased

tolerance could be due to an induction of the mechanism involved in the clearance of arsenic or the removal of arsenic from circulation by a metallothionein-type arsenic binding protein. If the former is the case, the clearance of a subsequent dose of arsenic after the fish have been conditioned to sublethal doses should be faster. In the latter case however, specific tissues involved in the binding and immobilisation of arsenic would be expected to contain relatively higher levels of arsenic. The mechanisms of acclimation is not yet understood. Acclimation has an ecological significance not only to the organisms, but to the entire community. A lack of information on acclimation of fish to chemicals could be a major obstacle preventing accurate application of laboratory toxicology results to field situations.

The common practices whereby mining effluents are accumulated during winter months and released in early May (Falk et al. 1973) and spraying of pesticides at regular time intervals raises the question of acclimation. If previous exposure to sublethal levels of arsenic released from the sediments over a long period increased the tolerance of the fish to arsenic, accumulation and hence the toxicity of subsequent discharges containing relatively high levels of arsenic could be affected.

Although considerable information has been published on the toxicity of arsenic most of the data has been related to lethality, not sublethal physiological stress. Therefore, the long term effects of arsenic on growth and other physiological functions were also examined in this study as the stress from

sublethal concentrations of arsenic have been reported to have serious biochemical, behavioural and physiological effects (Gilderhus, 1966; Weir and Hine, 1970; Speyer, 1975; Sorensen, et al., 1979). The mechanism whereby physiological stresses are brought about have been shown to vary with the form of arsenic. Trivalent arsenic forms covalent bonds with carbon and sulphur. The covalent bonding of arsenic +3 to sulfhydryl (SH) groups of cysteine in enzymes, causing its inactivation is the primary mode of toxicity (Ferguson and Gavis, 1972). Arsenate (As + 5) does not bind to sulfhydryl groups, but it can be reduced to trivalent arsenic in organisms (Schroeder and Balassa 1966). Arsenate uncouples oxidative phosphorylation in the triose oxidation step of glycolysis (Lehninger, 1972), during which the enzyme can utilize arsenate instead of phosphate. However, no high energy phosphate group is generated although the overall oxidoreduction takes place. The presence of arsenate therefore creates a shortage of energy required for physiological processes. Finally, both arsenite and arsenate ions coagulate proteins by causing the shape or configuration of proteins to change.

In spite of the extensive literature on levels of arsenic in aquatic organisms, one major problem remains yet unsolved; the understanding of the kinetics of arsenic accumulation. Most reported values for levels of arsenic in aquatic organisms in the literature were obtained from organisms killed after laboratory exposure or caught in arsenic-contaminated environments. To date there is insufficient information on which to estimate the bioaccumulation potential of arsenic in terms of the uptake

efficiency from food and water, metabolic fate, action and elimination of arsenic in aquatic organisms, the understanding of which is essential to reach an evaluation of the associated risks to aquatic organisms and other parts of the ecosystem. To this end, this study was undertaken to examine the bioaccumulation potential of arsenic within the following framework: 1) efficiency of uptake from water and food; 2) retention index or fractional clearance from whole body with special emphasis on routes of clearance; 3) metabolic products of arsenic and their influence on the clearance pattern; 4) adaptation of rainbow trout to arsenic as reflected by the elimination of arsenic from whole body, tissue distribution and binding of arsenic to liver proteins; 5) the accumulation of arsenic under long-term equilibrium conditions and 6) physiological effects.

Chapter 1

Bioaccumulation kinetics of arsenic in rainbow trout, Salmo *gairdneri*.

INTRODUCTION

The presence of arsenic in both marine and freshwater organisms is well documented. Chapman (1926) reported 8.0, 7.0 and 3.5 ppm arsenic in plaice, sole and dab (wet weight of fish) respectively caught from salt water containing 0.28 ppm of arsenic. Ellis et al. (1941) listed arsenic content in whole fish of 0.28 to 1.30 ppm for 15 freshwater fish species in Southern United States. The authors reported tissue distribution pattern for only large mouth bass which contained 0.66 ppm in whole body (wet weight), 0.50 ppm in eviscerated fish and 6.06 ppm in liver. More recently, Falk et al. (1973) and Wallace and Hardin (1975) reported arsenic levels in fish muscles from Grace, Likely and Keg Lakes (North West Territories, Canada) as 0.28 ppm in lake whitefish and 3.22 ppm in northern pike. A recent laboratory study by Sorensen et al. (1979) indicated a time-dependent increase in arsenic concentrations in the liver, spleen and kidney of green sunfish following a 6-day exposure to water containing 60 ppm arsenic at 20°C. The reported arsenic levels of 42.3 ppm for liver and 23.4 ppm for spleen of fish sampled after 4 days of exposure were significantly higher than those sampled after 2 days. The arsenic concentrations in these tissues after 6 days did not differ from those reported after 4 days.

The amount of a chemical toxicant an organism accumulates from the environment is controlled by a number of factors, which may be classified as "biological" or "physical" (deFreitas, 1977). The biological factors are those intrinsic to the fish itself such

as metabolic rate and the inherent biochemical and physiological systems involved in the uptake, detoxification and clearance of the toxicant. The dose of arsenic and the resulting tissue levels should be directly related to arsenic concentration in food, food consumption, assimilation efficiency of ingested arsenic, assimilation efficiency for uptake from water and clearance rates. The "physical" factors such as the concentration of the pollutant in water, the water temperature and also the chemical nature of the toxicant are external to the organism.

Arsenic found in tissues of fish and other organisms must have entered the body by direct uptake from water and assimilation of arsenic from arsenic-contaminated food. Whether or not biomagnification of arsenic from prey to predator occurs depends on the efficiency of uptake through the intestinal epithelium and clearance of tissue arsenic. Bioaccumulation from water likewise depends on the assimilation efficiency for uptake via gill tissue and in some cases, the general body surfaces. Arsenic has been reported in aquatic organisms that make up fish food such as algae, coelenterates, molluscs and crustaceans (Bowen, 1966 and Isensee et al. 1973). Another potential source of arsenic found in benthophagous fish is sediment which may be ingested with food, particularly since sediments in several lakes have been shown to contain high levels to arsenic (Seydel, 1972; Foley et al. 1978; Wagemann et al. 1978). The arsenic levels reported by these authors suggest that biomagnification of arsenic between trophic levels does not appear to take place in freshwater organisms though certain studies (LeBlanc and Jackson, 1973; Kennedy, 1976) that examined total

arsenic in marine crustaceans and their food suggest biomagnification in the food chain from zooplankton to their predator, the shrimp. However, there was a decrease in concentration up the food chain from shrimp to their predators, fish. In order to understand the reason for this apparent lack of food chain biomagnification, it is necessary to have information on the pharmacokinetics of arsenic in these organisms.

There is not much literature on the effect of the route of exposure on the kinetics of arsenic uptake in aquatic organisms, particularly fish. However, it has been demonstrated (Fowler and Unlu, 1978) that the marine shrimp, Lysmata seticaudata acquired most of its body burden of arsenic from the food.

Also, the route of uptake has been shown to modify the subsequent time course of tissue distribution and metabolism of arsenic. Four hours after an intramuscular injection of 20 µCi. ^{74}As-arsenic acid, elevated arsenic residues (up to 7.5% of total arsenic in muscle tissue present in organic form) appeared in blood, muscle and liver of rainbow trout (Penrose, 1975). Oral ingestion of a similar dose produced a slower increase in tissue levels and an increase in the organic form of the arsenic accumulated in liver tissue (up to 93%).

Upon entering the circulation of an organism and being bound to the tissues, the clearance of a toxicant from the whole organism should be directly related to the turnover of chemical components of the tissue, which in turn depends on the metabolic rate. For example, sensitivity to a toxicant may vary due to

individual differences in whole body retention and elimination rates, which probably reflects the capacity of specific tissues to detoxify or transform the toxic substance. For instance, it has been established that fish contain an adaptive protein which can bind large amounts of mercury, cadmium and zinc (Spehar, 1976; Brown and Parson, 1978; Pruell, 1977). The fish is therefore more tolerant of the metal as the binding removes it from the pool of metabolic constituents. Brown et al. (1977) and Brown and Parsons (1978) agree with Leber's (1974) hypothesis that tolerance to metals may result from the synthesis in the liver or kidney and possibly gut wall of metallothionein (MT) which binds the metals. These authors believed that detoxification by metallothionein is brought about, in part, by the displacement of the less toxic metal component (zinc) of MT by the more toxic metals. A "spillover" of toxic metals from metallothionein occurs when there is insufficient zinc-containing binding sites on metallothionein to immobilize the metals as may occur when rate of toxicant influx is greater than the rate of metallothionein synthesis (Brown and Parsons, 1978). There is no evidence that arsenic induces synthesis of metallothionein. However, if arsenic is immobilized by an arsenic-induced system similar to metallothionein one would expect the induction to produce a decreased whole body clearance and an increase in concentration of arsenic at the specific tissues involved in the immobilization.

In spite of the vast literature on arsenic in biological systems, there are no conclusive reports on the pharmacokinetics arsenic in fish on which to base uptake efficiency from water or food and rates of total elimination from whole fish, as well as by

specific excretory pathways. Excretion of drugs via the gills has been discussed with interest by pharmacologists and comparative zoologists over the years (Brodie and Maickel, 1962) but there are few quantitative reports on the subject. The routes of excretion of arsenic from fish have not been quantitatively defined in the literature. In a recent publication from our laboratory however, (Oladimeji et al. 1979), it was reported that urinary and biliary excretion could account for only a minor portion of the ingested arsenic and the suggestion was made that the gill may play an important role in the excretion of this element. A demonstration that the gill tissue functions as an excretory route for arsenic could have important implications to the toxicity of other metalloids such as selenium and the interactions between, for example copper and arsenic could be important, since copper and other heavy metals exert their toxic action on gill tissue (Baker, 1969 and Skidmore, 1970) by causing histological damage which may disturb its respiratory gas exchange function. In this case, synergism between arsenic and copper might be caused by copper preventing the excretion of arsenic by the gills. The present study was undertaken to define the bioaccumulation kinetics of arsenic by the rainbow trout. The following aspects of bioaccumulation kinetics were examined.

(a) The uptake of arsenic from food and water and its subsequent elimination

(i) the magnitude of the food and water vectors for arsenic uptake

(ii) whether the food vector can result in biomagnification of arsenic from prey to predator

(iii) the role of gill as an excretory route for arsenic and the proportion of total excretion which takes place via the gills and other routes.

(b) Induction of the synthesis of metallothionein-type proteins as detected by protein isolation chromatography and changes in arsenic retention and tissue distribution.

MATERIALS AND METHODS

General Procedures

Maintenance of the fish

Rainbow trout, Salmo gairdneri, (30-100 g wet weight), were obtained from Goosen's Fish Hatchery, RR2, Otterville, Ontario and Thistle Springs Trout Farm, Ashton, Ontario. On arrival at the laboratory, fish were held at 10 ± 1°C in refrigerated fibreglass tanks (Frigid Unit, Toledo, Ohio), at a density of approximately 1 g of fish tissue per 150 ml of water. These tanks were aerated, and supplied with a continous flow of 24 litres/h dechlorinated filtered water. Water quality parameter values are presented in Table 1. The values for those parameters which were not determined weekly are quite close to those carried out be the City of Ottawa Water Works. During experimentation fish were fed Purina Trout Chow once daily at the rate of 2% of their body weight (dry weight of food and wet weight of fish) or as specified for each experiment.

Measurement of radiactivity

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

The radioactivity present in whole fish, fish tissue and water was measured with gamma-counting spectrophotometers. Two counters were used in this study. A Nuclear Chicago instrument was used as well as an Ortec manual deep well counter, consisting of 3 X 3- in NaI detector crystal in a lead-shielded well. The

Table 1. Characteristics[a] of the water used for the study

Constituent	Concentration (mg/litre)
orthophosphate	0.52
metaphosphate	0.04
total phosphate	0.56
silica	6.50
Fe	0.10
As[b]	0.002
$N\text{-}NO_3$	8.00
NO_2	36.20
O_2 (temp. 10°C)[c]	10-11.00
hardness (total)[c]	41-48.00
pH[c]	7.2-7.4
Alkalinity[c]	18-20.00

[a]Determinations made according to standard methods as described by Lind (1974)

[b]Obtained from the results of the analysis of water samples taken during 1978 and 1979 at the City of Ottawa purification plants

[c]Range of values obtained from determinations carried out once every week during the tests are presented for these parameters, while those for others are means of measurements made twice during the experiments.

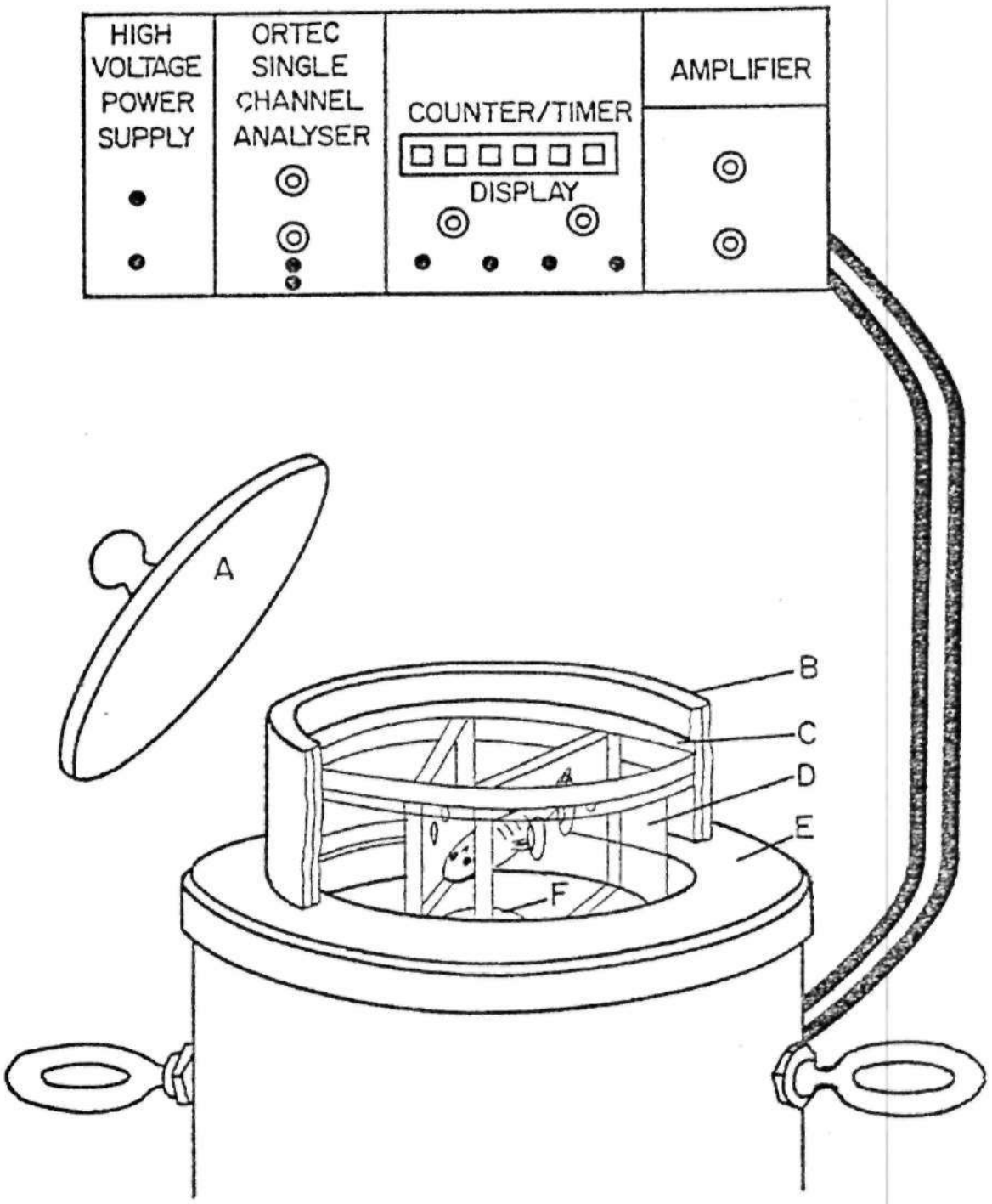
Ortec counter was used mainly for establishing the amount of radioactive ^{74}As in live fish. The crystal was connected to a single channel energy spectrum analyser.

Handling and measurement of whole body content of ^{74}As in live fish

One to 2 weeks prior to dosing fish were tagged[1] through the musculature anterior to the dorsal fin for subsequent identification. Whole body retention of the input dose of arsenic was determined for each fish by measuring its content of ^{74}As at various times postdose. For whole body retention, 5-8 fish dosed with arsenic in diet or water were separated into 120 litre glass aquaria supplied with flow through dechlorinated water (24 litres/h) maintained at 10 ± 1°C. Prior to being separated into the 120L glass aquaria for clearance studies, each fish was rinsed for 10 minutes in uncontaminated water and counted live. Two different methods were used for measuring radioactivity in the different sizes of fish. Following the rinsing, larger fish (31-100g) were placed in polyethylene bags containing 100 ml of dechlorinated water for whole body counting. Each of the smaller fish (20-30 g wet weight) was placed in a crystallization dish (Pyrex 100 x 50) containing 100 ml of water. The crystallization dish was partitioned to keep the fish directly above the well (Fig. 1). The crystallisation dish or polyethylene bag containing the fish was placed on top of the 3-in sodium iodide detector crystal. When the polyethylene bag was used, it was placed in a circular cardboard box partitioned to constrain the fish directly above the detector crystal. To facilitate the conversion of the measurements of radioactivity in

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[1] Fingerling fish tags, 5 mm X 3 mm. Floy Tag Manufacturing, Ltd., 4616 Union Bay Place N.E.' Seattle, Washington 98105


HIGH VOLTAGE POWER SUPPLY
ORTEC SINGLE CHANNEL ANALYSER
COUNTER/TIMER
DISPLAY
AMPLIFIER
A
B
C
D
E
F

whole fish made on top of the well to deep well counting efficiency, some fish were exposed to arsenic, counted live in polyethylene bags or crystallisation dishes and then sacrificed. The fish were then homogenized to liquid form with their weight of water using a Polytron homogenizer and one-gram subsamples of the homogenates counted.

Standardization of radioactivity measurements

The counting efficiencies of the gamma counters were determined by the use of standards consisting of accurately measured quantities of ^{74}As-labelled arsenic acid in standard counting vials (Ortec) or gamma tubes (Nuclear Chicago). The standard was used to determine the day to day variations in the counting efficiencies observed during the periods of measurements. For any given sets of measurements, observed counts were corrected for physical decay and variation in counting efficiency at the time of measurement as determined from the value of the standard.

Data analysis

The corrected whole body counts were averaged for each group of fish, at each time point during clearance experiments. Assuming first order kinetics, the averaged data and those for individual fish were fitted to the exponential equation

$$Yt = ae^{-bt}$$ where

Yt = body burden of arsenic resulting from exposure

a = a constant representing compartment size (or Y intercept of the fast or slow clearing compartment)

t = time in days

b = fractional clearance rate d^{-1}

The fractional clearance rate was obtained by regression analysis and the biological half-life ($t_{\frac{1}{2}}$) was derived from the relationship

$$t_{\frac{1}{2}} = \frac{\ln 2}{b} = \frac{0.693}{b}$$

where b = slope of the regression line

Values for biological half-lives or fractional clearance for fish exposed to the different concentrations of arsenic in water and diet were compared by means of Student's t test. The effect of preexposure and route of exposure on tissue distribution in the different groups was determined by an analysis of variance (ANOVA) between exposure groups.

Specific Procedures

Uptake of arsenic from water and its subsequent clearance

Studies on direct uptake of arsenic from water by fish were carried out in 15 litres of dechlorinated water contained in glass jars with a capacity of 25 litres. Fifteen tagged fish (ranging in body weight from 40 to 55 g) were divided into three groups of 5 fish each. Each group of 5 fish was exposed to one of three different initial concentrations of arsenic, 6, 12, and 25 pg ^{74}As ml^{-1} for 12 h during which the temperature was maintained at 10 ± 1°C. Measurement of ^{74}As in the exposure water was made at 6 and 12 h.

In order to determine the effect of duration of exposure on uptake and subsequent clearance of arsenic, three groups of 5

fish each were similarly exposed to a solution containing an initial concentration of 12 pg $^{74}As\ ml^{-1}$ for 6, 12 and 24 h as described above.

After the exposure period, fish were removed from the jars and rinsed for 10 minutes in uncontaminated water. Immediately following rinsing, the amount of arsenic taken up be each fish was estimated by measuring the radioactivity associated with the ^{74}As tracer in each whole live fish using the Ortec gamma counter. Subsequent clearance of arsenic from each fish was estimated by a series of postdose measurements.

Whole body retention of arsenic following uptake from food

Fish used for uptake and clearance of arsenic following uptake from food were starved for 24 h and then fed a single dose of arsenic-contaminated diet of 10 µCi ^{74}As (~10ng As)/100g of fish wet weight. For preparation of the contaminated diet, a measured amount of ^{74}As-labelled arsenic acid was added dropwise to the daily ration of Purina Trout Chow. After the addition of the arsenic, the food was allowed to stand for 10 minutes before it was fed to the fish. The amount of arsenic ingested by each fish, its assimilation efficiency from the gastrointestinal tract and its subsequent clearance were estimated by a series of postdose measurements of the radioactivity retained by each fish using the Ortec gamma counter. Measurements were made at 2 h postdose and subsequently at 1, 2, 4, 7, 14 and 21 days postdose. Fish were fed daily at 2% of their body weight (dry weight of food/wet weight of fish) during clearance.

Routes of arsenic excretion

Urinary excretion

Fish used for this study had an average weight of 57.5 ± 0.5 Each fish was anaesthetized lightly (50 ppm MS-222) and force-fed one #4 gelatin capsule containing 500 mg of pulverized trout chow to which 25 µg of arsenic was added. The 25 µg of arsenic consisted of a mixture of 0.01 µg ^{74}As (~10 µCi) and 24.99 µg stable arsenic, as arsenic acid, used as carrier.

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.

Excretion of arsenic via the gills

The 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 in Fig. 2 were incorporated to achieve this function.

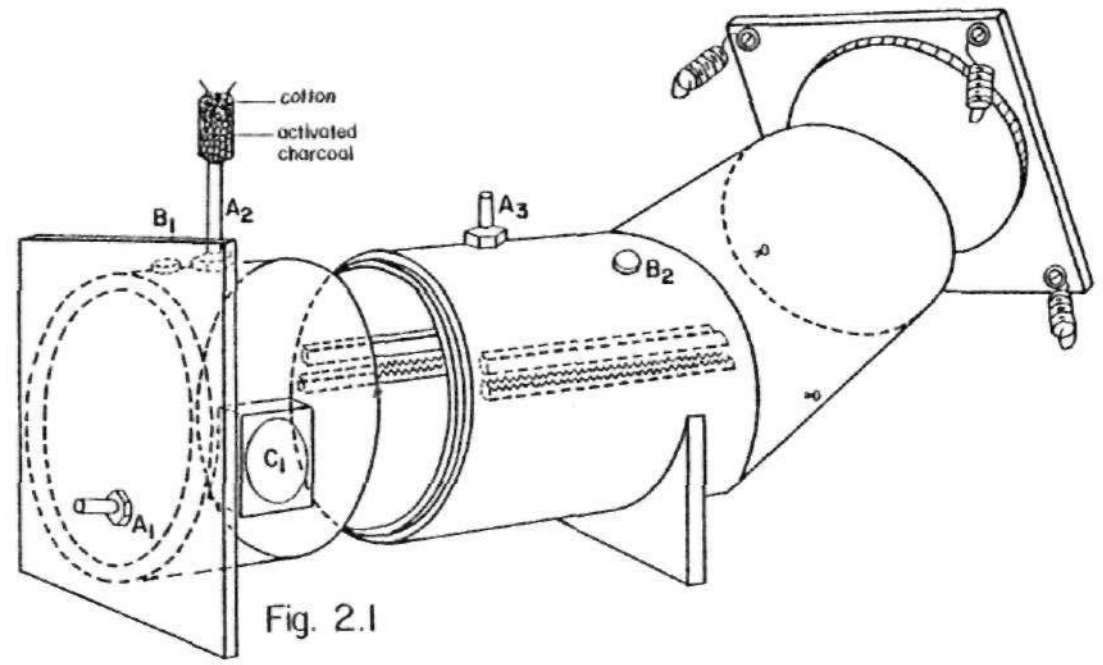


Fig. 2.1

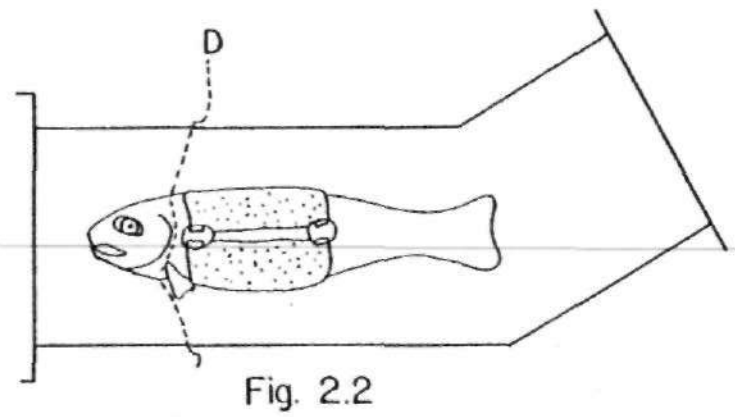


Fig. 2.2

Eight fish were fed arsenic-contaminated diet in group of 2 or 3 as previously described for whole body clearance. The amount of arsenic (^{74}As) ingested by each fish ranged from 1.5 ng - 3.5 ng (3-7 μCi). Since preliminary runs made with fish in the respirometer 8 h postdose resulted in regurgitation of food in the head chamber of the respirometer the runs were therefor commenced at 20 h postdose. No regurgitation was observed during the runs. This is not unexpected since according to Windell et al. (1969) gastric evacuation would have been or almost completed by 20 h postdose under the conditions of this experiment.

Duration of experiment varied from 1.5-4 h and a number of repetitions were carried out on each fish at various times between 20-288 h postdose. .Measurements of arsenic in the head compartment was made every 20 minutes of each experiment. No anaesthetics was used since the most common anaesthetic, MS-222, has been shown by various studies including that of Houston et al. (1971) to reduce blood flow and movement of water across the gills.

Operation of the modified respirometer (Fig. 2).

The respirometer with the diaphragm instralled as described by Boddington et al. (1970) was placed in a bath of running water at 12 ± 1°C. The fish was placed in the restrainer and located horizontally with its head through the hole in the diaghragm and projecting into the head chamber until the opercula just cleared the diaphragm. Note that in this application, the fish was not at an angle of 30° to the horizontal as described by Boddington et al. (1979), in which the differential concentration of O_2 and mercury in the inhalant

and exhalant water and the ventilation volume were being measured and in which the opercula did not have to clear the diaphragm into the front compartment. Movement of jaw muscles did not seem to be restricted in our application of the apparatus. According to Boddington's application, only a separation of the inhalant and exhalant water was necessary. There was no leakage across the diaphragm with the fish in situ when tested with methylene blue as described by Boddington et al. 1979.

Oxygen in slight excess to maintain saturation was continuously bubbled through the water in the head region through A_1 (Fig. 2.1) 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.

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.

Whole body retention and tissue distribution of ^{74}As in rainbow trout preexposed to ingested arsenic

Determination of the effect of preexposure on retention of arsenic in whole fish was carried out using three groups of fish.

These consisted of control (maintained under identical conditions in the absence of the toxicant) and two groups preexposed to 5.70 and 22.80 mg As/kg (as sodium arsenite) of diet respectively for 14 days during which they were fed at a daily rate of 2% body weight. Following the preexposure, the fish were starved for one day and fed a pulse dose of 10 µCi ^{74}As (~10ng)/100 g fish wet weight. To obtain this dose a measured amount of the ^{74}As-labelled arsenic acid was added dropwise to the regular daily ration (containing 0,5.70 and 22.80 mg As/kg diet respectively). The amount of arsenic ingested, assimilation efficiency and subsequent clearance were determined as previously described.

In order to follow the condition of the fish without enhancing clearance of arsenic as a result of handling, duplicates of the above exposure groups were run concurrently. These duplicates consisted of 20 fish each and the treatment groups were exposed to 5.70 and 22.80 mg As/kg diet for 4 weeks. Fish in each group were weighed at the beginning, at two weeks and at the end of the experiment. In all cases fish were not fed for 24 hours prior to weighing. A pooled sample of 4 fish from each of the treatment groups (including those used for hemoglobin determination) were killed at weekly intervals over a 4-week period. The arsenic content was determined[2] on freeze-dried tissue homogenates.

Blood hemoglobin content was measured at the beginning of the experiment on 5 fish before they were allocated to their treatment groups and on 4 fish from each group after two weeks of exposure and at the end of the experiment. The method used was that of Wintrobe

[2] Bondar-Clegg & Co., Ltd., 764 Belfast Road, Ottawa

Table 2. Tissue and organ weights expressed as percent[a] of total wet weight of rainbow trout.

Body Component	Percent of total wet weight
Blood[b]	3.12
Brain	0.72
Anterior gut	2.81
Posterior gut & pyloric caeca	4.70
Gill	3.60
Liver	1.20
Kidney[c]	0.82
Bile & Gall bladder	0.26
Skeletal muscle	45.51
Skin	8.02
Spleen	0.15
Heart	0.15
Remaining carcass	27.94

[a]Computed by weighing the organs of 30 fish (10 fish for muscle and skin) having an average weight of 26.5 g.

[b]Blood value from Holmes and Donaldson (1969)

[c]Kidney value from Giblin and Masaro (1973)

(1961), using a cyanomethemoglobin standard. Blood samples were obtained by severing the caudal peduncle of anaesthetized fish and collecting blood in heparinized capillary tubes.

Tissue distribution of arsenic following uptake of arsenic from food

Thirty-five fish were separated into each of three aquaria. One group served as the control, while the other two were preexposed to 5.70 and 22.8 mg As/kg diet for 14 days as described for whole body clearance. Following the preexposure, the fish were starved for 24 h and given a pulse dose of ^{74}As-labelled arsenic acid to give the same dose of 10 µCi/100 g fish as used for the whole body clearance. Groups of 4-5 fish each were killed with MS-222 at 2, 24, 48, 96, 168, 336 and 504 h postdose and tissue distribution of arsenic was determined on each fish. The caudal peduncle was severed and blood collected in heparinized capillary tubes which were in turn placed in counting vials for subsequent assay. Fifteen other tissues or organs were isolated, weighed in counting vials and counted. The amount of radioactivity in weighed portions of the homogenized muscle, blood, skin, gills and remaining carcass (homogenized) was determined in the manual deep well-type gamma detector. The brain, heart, anterior gut (stomach, portion of midgut to which pyloric gut (remaining midgut and hindgut), posterior gut, posterior gut content, spleen and gall bladder (ligatured) were excised in toto. Radioactivity in whole organ or tissue was expressed as the relative concentration distribution in tissues, which is defined as cpm per g tissue/cpm per g fish. This expression normalizes the variation due to differences in the amount of arsenic ingested initially by each

fish since they were fed in groups which resulted in each fish ingesting different amounts of arsenic. The distribution of radioactivity in the tissues was also expressed as a percentage of the amount of arsenic (^{74}As) remaining in the whole fish at the time of sacrifice. Counts were corrected for differences in counting efficiency resulting from differences in tissue size by using a set of standard weights of homogenized fish tissue containing a known amount of arsenic. Where direct weighing of the entire organ or tissue was not possible, table of weights expressed as percent of total fish wet weight was used (Table 2).

Effect of preexposure to dietary arsenic on binding of arsenic to liver proteins

Four control fish and 4 fish which have been preexposed to 22.80 mg As/kg diet for 14 days were each fed a single pulse dose of 5 µCi ^{74}As-labelled arsenic acid/fish in No. 4 gelatin capsules. Two fish from each group were sacrificed at 24 h and 48 h postdose respectively. The livers were excised, weighed and homogenized with chilled isotonic saline. The homogenate was centrifuged at 35,000 g for 30 minutes in a sorval refrigerated centrifuge to separate the residue. The supernatant was withdrawn, recentrifuged at 35,000 g for an additional 15 minutes with fresh saline and the final supernatant retained for chromatography. The final supernatant was applied directly to a 2.6 x 100 cm column of Sephacryl S-200 superfine (Pharmacia Fine Chemicals, Dorval, Quebec) and eluted at 10 ml h^{-1} with 0.1 M Tris-HCl buffer, pH 8 containing NaCl (0.5M). One hundred 2 ml fractions of the eluate were collected. Optical

absorbance of the eluate fractions was monitored with a spectrophotometer (Pye Unicam PS68-100) at 280 nm. Quantitative protein determination was not carried out, but the presence or absence of protein was determined by precipitation using 5% trichloroacetic acid (TCA). Precipitation on the addition of drops of TCA indicated presence of protein. Eluate fractions were subsequently assayed for arsenic (^{74}As) using the gamma counter.

RESULTS

Uptake of arsenic from water and its subsequent clearance

The concentration of arsenic remained relatively constant during the exposure period, dropping gradually to about 90% of the initial value over the 12 h exposure period. Fish appeared 'normal' during the 12 h exposure to concentrations of 6.2, 12.1 and 25.3 pg As ml^{-1}.

The body burden of arsenic in fish exposed to 6.2, 12.1 and 25.3 pg As ml^{-1} for 12 h increased significantly with a rise in the concentration of arsenic in water (Table 3). The observed net uptake rates of 2.2, 6.4 and 15.7 pg As per fish h^{-1} were directly related to concentration of arsenic in water. As a result, uptake rates expressed as transfer coefficients were relatively constant with values of 7.8, 11.1 and $12.0 \times 10^{-3} g h^{-1}$ for fish exposed to 6, 12 and 25 pg As ml^{-1} respectively, were independent of concentrations. The transfer coefficient is the amount of water, in ml or g, that is completely cleared of its arsenic by 1 g of fish, in one hour. Its units are g h^{-1}. A transfer coefficient value of 5.0 h^{-1}, for example, means that 5.0 g of water was cleared of its arsenic content by 1 g fish in 1 h. Use of the transfer coefficient facilitates comparisons of experimental results between experiments, since it normalizes the differences in fish biomass and concentrations of arsenic in water. The transfer coefficient, Tc, is defined by the relationship:

$$Tc = \frac{Y(t)}{W.t.C.},$$

where Yt is the arsenic content of the whole body, or fast or slow-

Table 3. The uptake of arsenic from water by rainbow trout at various concentrations of arsenic in water. The fish were exposed to the levels at 10 ± 1°C. Means ± S.E. are presented and means scored by the same line are not significantly different at $P < 0.05$.

As conc^{n} in medium	Wet weight of fish	As content of fish[b]	Net uptake rate	Total transfer coefficient[c]	Fast-clearing compartment		Slow-clearing compartment	
					As uptake rate	As uptake, transfer coefficient	As uptake rate	As uptake, transfer coefficient
pg As g^{-1}	g	pg As $fish^{-1}$	pg As/fish h^{-1}	$x10^{-3}$	pg As/fish h^{-1}	$x10^{-3}$	pg As/fish h^{-1}	$x10^{-3}$
6.2(5.9)[a]	50.4 ± 1.7	26.5± 2.9	2.2 ± 0.3	7.8 ± 0.7	1.9 ± 0.4	5.5 ± 0.7	0.7 ± 0.1	2.2 ± 0.2
12.1(10.1)	50.9 ± 3.6	76.4± 5.4	6.4 ± 0.4	11.1 ± 0.8	5.3 ± 1.0	8.5 ± 0.9	1.5 ± 0.3	2.6 ± 0.6
25.3(22.3)	50.9 ± 4.4	188.1± 52.2	15.7 ± 4.4	12.0 ± 2.1	13.4 ± 1.9	10.1 ± 2.3	2.6 ± 0.4	2.0 ± 0.3

[a] Figures in parenthesis represent final arsenic conc^{n} in water at the end of exposure.

[b] Measured after rinsing in uncontaminated dechlorinated water for 10 minutes postexposure.

[c] Transfer coefficient values represent the weight of water in ml or g completely cleared of its arsenic content by 1 g of fish in 1 h. Unit is ml g $fish^{-1}$ h^{-1}

clearing compartment of rainbow trout of wet weight W grams after t hours of exposure to water containing arsenic at concentration(C.) The transfer coefficient as used in Tables 3 and 4 represents only a net uptake rate rather than a true uptake rate since the expression contains no term for release of arsenic from fish.

The results in Table 4 show that as duration of exposure increased from 6-12h, the arsenic content of fish increased. The body burden remained the same following an additional 12h exposure. The proportion of the body burden that was contained in the slow compartment, on the other hand, was similar in fish exposed for 24h (Table 6). The transfer coefficient decreased from a value of 19.3×10^{-3} h^{-1} in fish exposed to arsenic for 6h to 6.3 h^{-1} for those exposed for 24h, reflecting the effect of rapid arsenic loss from the fast compartment. The explanation for this involves the biphasic clearance of arsenic as will be shown more clearly by the results on clearance (Fig. 3).

When trout that had been exposed to ^{74}As in water were placed in uncontaminated water, the arsenic loss was always in two phases (Fig. 3 and 4). The rate of arsenic loss was initially very fast but after about 2 days the loss rate declined markedly. This is an indication that some of the arsenic that had been accumulated must have been only loosely associated with the organism and was quickly lost while the remaining arsenic was more firmly bound. The fractional clearance rates for the slow and fast-clearing compartments in the three groups of fish exposed to the different concentrations of arsenic are presented in Table 5.

Table 4. Effect of duration of exposure on uptake[a] of arsenic from dechlorinated water containing 12 pg As ml^{-1} by rainbow trout, Salmo gairdneri held at 10 ± 1°C. Values represent means ± S.E. of 5 fish and means scored by the same line are not significantly different at $P < 0.05$.

Duration of Exposure	Arsenic conc[n] in water	Initial wet weight of fish	Concentration of arsenic[c]	Net uptake rate	Total transfer coefficient[d]	Slow-clearing compartment		Fast-clearing compartment	
						As uptake rate	As uptake, transfer coefficient	As uptake rate	As uptake, transfer coefficient
h	pg As ml^{-1}	g	pg As $fish^{-1}$	pg As fish h^{-1}	$x10^{-3}$ h^{-1}	pg As/fish h^{-1}	$x10^{-3}$ h^{-1}	pg As/fish h^{-1}	$x10^{-3}$ h^{-1}
6	12 (11.4)[b]	44.8 ± 2.4	58.8 ± 4.4	10.3 ± 0.6	19.3 ± 1.2	2.2 ± 0.22	4.1 ± 0.6	9.6 ± 0.80	15.0 ± 0.8
12	12 (11.4)	45.6 ± 2.8	82.2 ± 7.9	6.8 ± 0.7	12.5 ± 1.0	1.6 ± 0.11	2.5 ± 0.3	7.3 ± 0.72	10.0 ± 0.8
24	12 (11.5)	45.3 ± 2.7	81.0 ± 4.6	3.5 ± 0.2	6.3 ± 0.5	1.0 ± 0.12	1.8 ± 0.3	4.1 ± 0.45	4.5 ± 0.3

[a] 5 fish each were exposed to 12 pg As ml^{-1} in water.

[b] Figures in parenthesis represent final concentration of arsenic in water at the end of exposure.

[c] Measured after rinsing in uncontaminated dechlorinated water for 10 minutes postexposure.

[d] Transfer coefficient values represent the weight of water in grams completely cleared of its arsenic content by 1g wet weight of fish in 1 day.

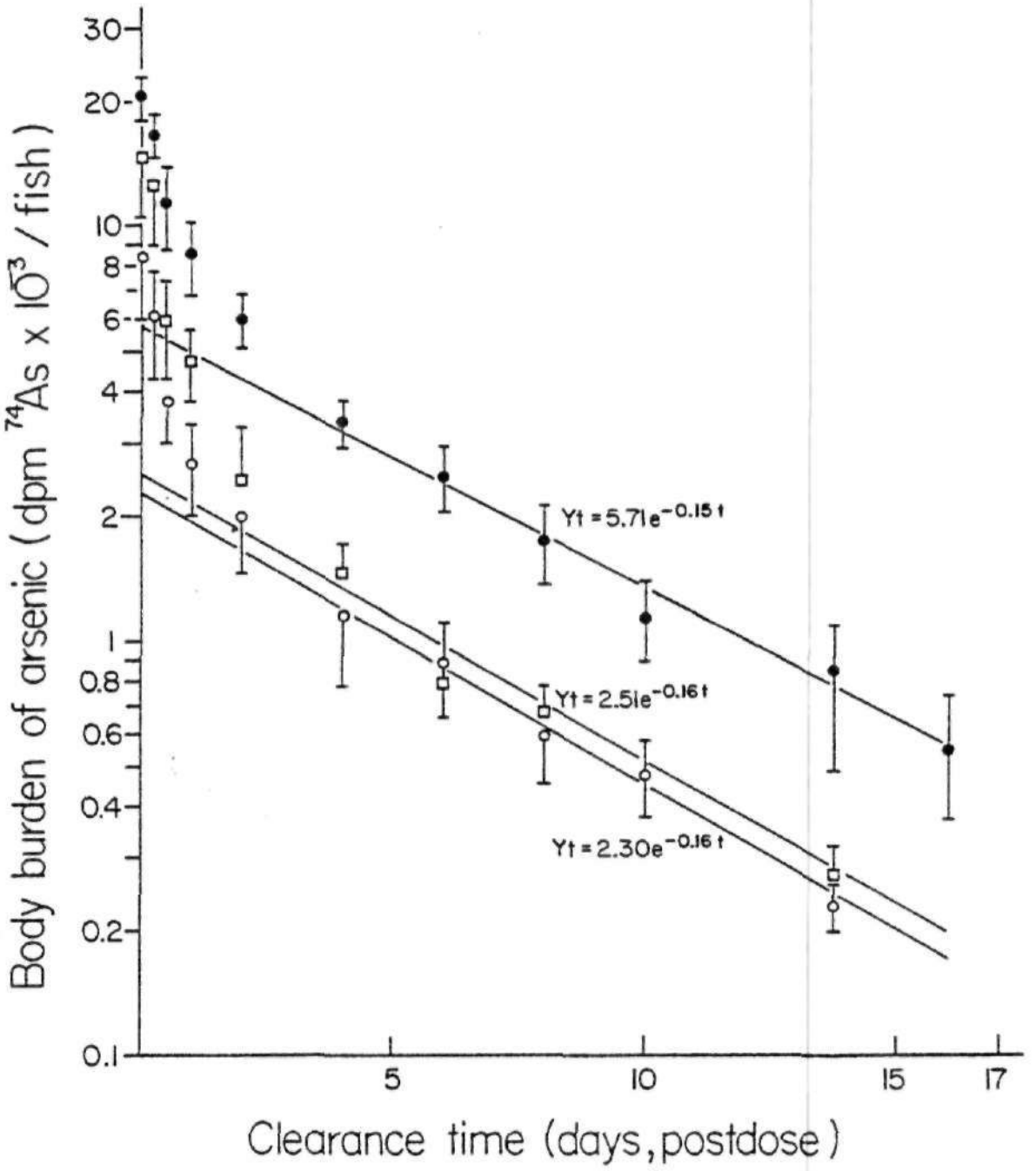

Body burden of arsenic (dpm ^{74}As x 10^{3} / fish)
30
20
10
8
6
4
2
1
0.8
0.6
0.4
0.2
0.1
Yt = 5.71e$^{-0.15t}$
Yt = 2.51e$^{-0.16t}$
Yt = 2.30e$^{-0.16t}$
5
10
15
17
Clearance time (days, postdose)

Table 5. Whole body clearance of ^{74}As-labelled arsenic acid following uptake from food and water by the rainbow trout, Salmo gairdneri held at 10 ± 1°C. Each value represent means ± S.E. Means scored by the same line are not significantly different at $P < 0.05$.

Route of exposure[a]	As conc[n]	Wet weight of fish[b]	Arsenic content	Fast-clearing compartment		Slow-clearing compartment		
				As loss, fractional clearance rate	Biological half-life	Compartment size	As loss, fractional clearance rate[c]	Biological half-life
	pg As g^{-1}	g	pg As $fish^{-1}$	d^{-1}	d	% of initial body burden	d^{-1}	d
Water	6.2	50.4 ± 1.7	26.5 ± 2.9	1.06 ± 0.23	0.60 ± 0.06	30.1 ± 0.7	0.15 ± 0.01	4.3 ± 0.33
Water	12.1	50.9 ± 3.6	76.4 ± 5.4	1.27 ± 0.16	0.57 ± 0.10	24.4 ± 3.9	0.16 ± 0.02	5.4 ± 0.61
Water	25.3	50.9 ± 4.4	188.1 ± 52.2	1.58 ± 0.40	0.47 ± 0.11	18.7 ± 4.5	0.16 ± 0.00	4.9 ± 0.18
Food	5000.0	26.8 ± 3.2	2556.0[d] ± 321.3	0.81 ± 0.00	0.89 ± 0.07	25.4* ± 2.8	0.11 ± 0.03	6.4 ± 0.80

[a] Duration of exposure to arsenic in water was 12h while arsenic in diet was given as a single dose of 10 ng ^{74}As 100 g^{-1} fish.

[b] 5 fish were used for uptake and retention of each level of arsenic in water and 8 for food.

[c] Value shown represent mean ingested dose.

[d] Value shown represent mean ingested dose.

* Compartment size not different from those for water.

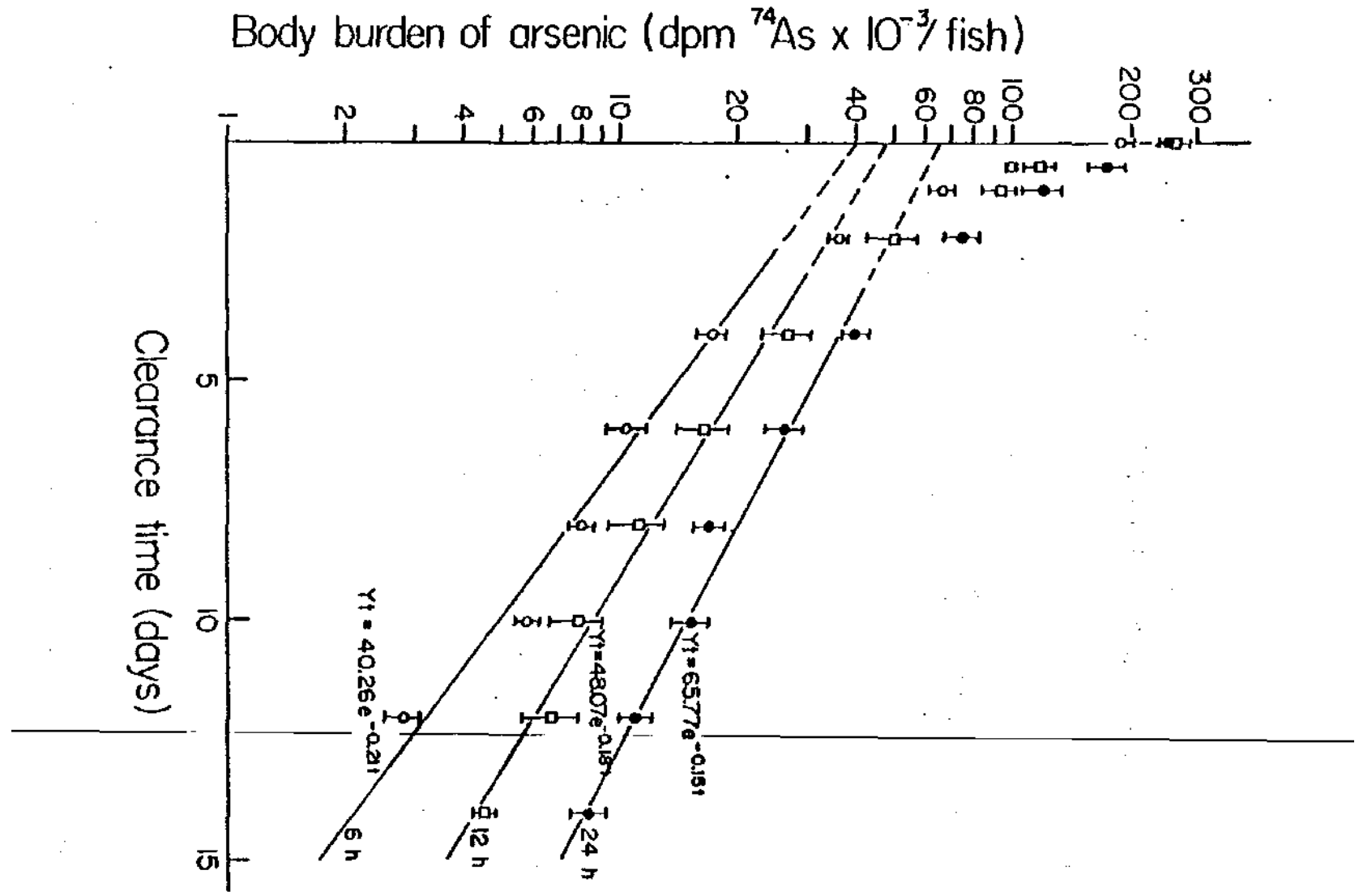

Body burden of arsenic (dpm $^{74}As \times 10^{-3}$/ fish)
1
2
4
6
8
10
20
40
60
80
100
200
300
Clearance time (days)
5
10
15
Y1 = 40.26e$^{-0.21}$
Y1=48.07e$^{-0.18}$
Y1=65.77e$^{-0.151}$
6 h
12 h
24 h

Table 6. Effect of duration of exposure on whole body clearance of arsenic following uptake[a] from water by rainbow trout, Salmo gairdneri held at 10 ± 1°C. Each value represents means ± S.E. for 5 fish. Values scored by the same line are not significantly different at $P < 0.05$.

Duration of	Mean wet weight of fish[b]	Retained dose	Fast Compartment		Slow Compartment		
			As loss, fractional clearance	Biological half-life	Compartment size	As loss, fractional clearance	Biological half-life
h	g	pg As/fish	d^{-1}	d	% of retained dose	d^{-1}	d
6	44.9 ± 2.4	58.8 ± 2.4	1.4 ± 0.12	0.50 ± 0.03	21.3 ± 2.2	0.21 ± 0.01	3.3 ± 0.15
12	45.7 ± 2.4	82.2 ± 7.9	1.1 ± 0.06	0.66 ± 0.04	19.7 ± 1.5	0.18 ± 0.01	3.9 ± 0.16
24	45.3 ± 2.6	81.0 ± 4.6	1.0 ± 0.08	0.70 ± 0.07	30.7 ± 3.2	0.15 ± 0.00	4.6 ± 0.19

[a] Fish were exposed to 12 pg As ml^{-1} in water

[b] Means of weights of fish at the beginning and end of clearance

There was no significant difference ($P<0.05$) between the fractional clearance rate of the slow-clearing compartment of trout that were exposed to arsenic in water for 6 and 12 hours and between those exposed to arsenic for 12 and 24 hours. The fractional clearance of the slow-clearing compartment for fish exposed to arsenic for 24 hours, however, was significantly lower than in fish exposed for 6 hours. (Fig. 6) The rate of excretion from the slow-clearing compartment was similar for arsenic taken up from water and from food, with biological half-lives of the slow-clearing compartment of 4.3-5.4. days and 6.4 days respectively as shown in Table 5.

Due to the biphasic nature of the clearance curve shown in Fig. 3 and 4, total uptake rate of arsenic from water can be usefully divided into two compartments, the slow and the fast, for purposes of calculating body burdens that approach a steady state with respect to uptake and elimination under long term exposure conditions. Transfer coefficient for uptake into slow and fast-clearing compartment and the corresponding values for clearance coefficients from these two compartments are shown in Tables 5 and 6.Using these values, equilibrium (steady state) concentration factors are reported (Table 7). The equilibrium concentration factors reported in Table 7 show that shortly after 2 days of continuous exposure, fast compartment equilibrium has been essentially achieved. Slow compartment equilibrium is achieved by 18-24 days of exposure and at this time, uptake into the fast compartment represents approximately 20% of the body burden.

The equilibrium bioconcentration ratio of 0.3-0.7 between water (Table 7) and fish for arsenic are low. This means that fish in water containing an arsenic concentration of 10 $\mu g\ ml^{-1}$, for instance, will not be

Table 7. Uptake of arsenic (^{74}As) by rainbow trout, Salmo gairdneri exposed to various concentrations of arsenic for periods ranging from 6-24 h at 10 ± 1°C.

Duration of exposure	Concentration of arsenic in water	Steady state body burden	Bioconcentration[a] ratio
h	pg ml^{-1}	pg As g^{-1} fish	
12	6.2	1.8	0.3
12	12.1	3.8	0.3
12	25.3	10.6	0.4
6	12.0	8.1	0.7
12	12.0	5.4	0.5
24	12.0	4.8	0.4

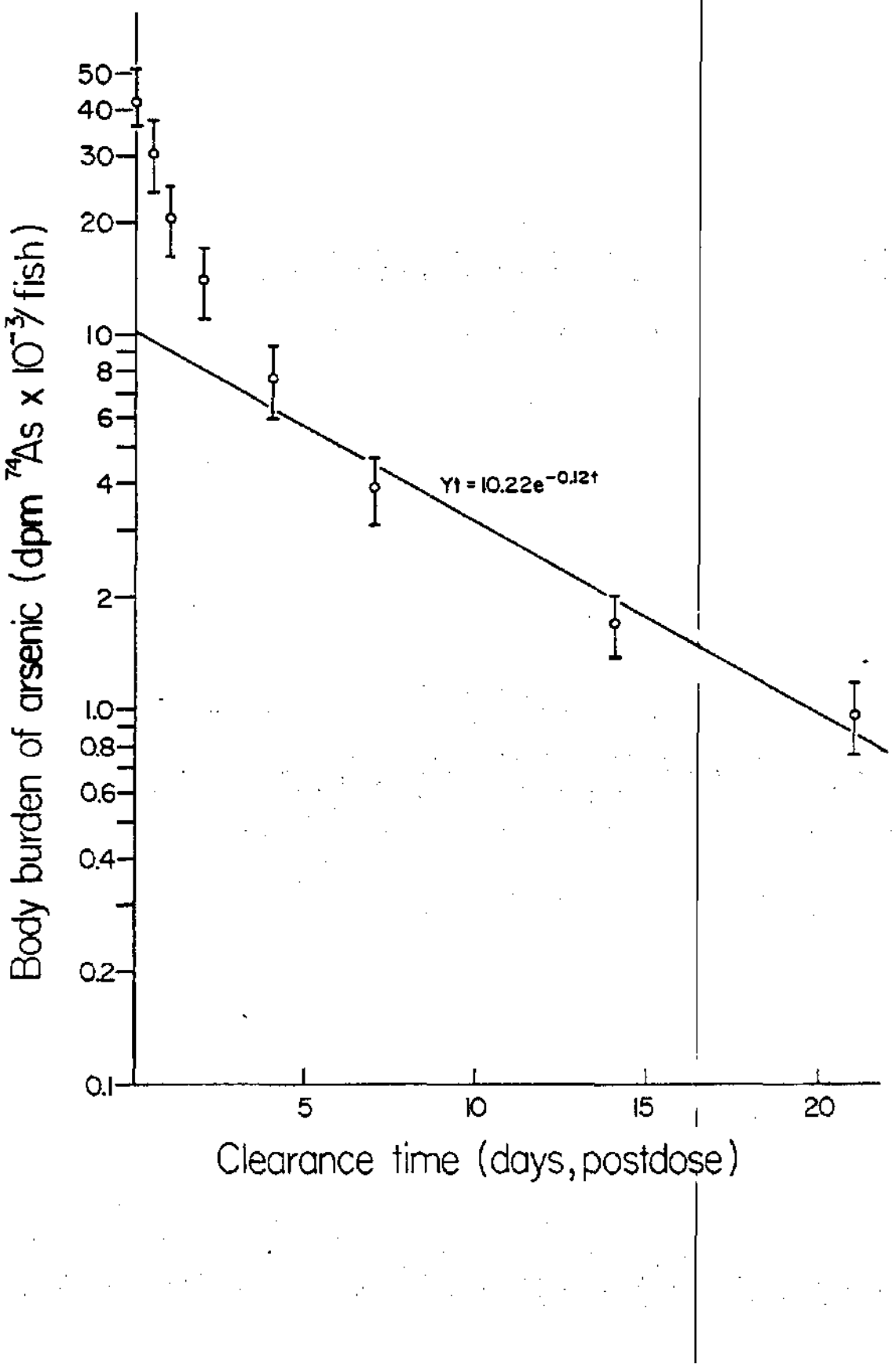
[a] Bioconcentration ratio = pg As per g fish/pg per g of water.

expected to contain more than 7 ppm arsenic at steady state.

Uptake of arsenic from food and its subsequent clearance

Uptake of arsenic from food was rapid and efficient. Tissue distribution data (presented later in this result section) indicated the presence of arsenic in most tissues as early as 2 hours after ingestion. An estimate of the assimilation efficiency from the gastrointestinal (GI) tract can be obtained by calculating the ratio, total arsenic in tissues/total ingested arsenic. The maximum value of this ratio should occur prior to the voidance time of unassimilated food. According to the observation made by Windell et al. (1969), gastric evacuation in rainbow trout maintained at 15°C was 86% at 20h after ingestion. The voidance time is therefore not expected to exceed one day. A second method of estimating the assimilation efficiency is to use whole body clearance curves by extrapolating the observed clearance occurring after voidance time back to zero time. The extrapolated value at 0-time is used as an estimate of the fraction of the ingested dose that was not lost during voidance of the meal, and therefore must have been absorbed by the tissues of the organism. However, for arsenic this results in an underestimate since (1) arsenic is cleared very fast and (2) the fast compartment corresponding to the voidance of unassimilated arsenic is not well-defined.

The data for the retention of arsenic following uptake from food demonstrate that the bulk of arsenic was rapidly eliminated (Fig. 5). Clearance data from Fig. 5 indicated that 2% of the ingested dose was retained 21 days postdose. Whole body clearance resulted in a curve that has at least two slopes.


Body burden of arsenic (dpm ^{74}As x 10^{-3}/fish)
50
40
30
20
10
8
6
4
2
1.0
0.8
0.6
0.4
0.2
0.1
$Y1 = 10.22e^{-0.12t}$
5
10
15
20
Clearance time (days, postdose)

The first set of postdose measurements represents the fast compartment and lasts for 2-3 days. For the reason given above, this compartment does not coincide with the voidance time of about 24h for passage of unabsorbed arsenic from the gastrointestinal tract. This fast compartment represented 75 ± 3% of the ingested dose and had a half-life of elimination of approximately 20 hours. The second, slow-clearing compartment represents the portion of the assimilated arsenic that remains firmly associated with the body tissues. As already explained above, the size of this latter compartment relative to the ingested dose is a useful measure of the net assimilation efficiency, in terms of calculating body burden during long term exposure. This second component accounted for 25% of the ingested dose and was eliminated at a much slower rate. Arsenic was eliminated from this slow pool with a biological half-life of 6.4 ± 0.01 d . The fast and slow compartments may represent two different chemical forms of arsenic in the tissues, each pool acting independently. This question will be examined in the discussion using information on chemical speciation and routes of excretion.

For reasons already explained, the value for assimilation efficiency of arsenic based on the extrapolation of the slow-clearing compartment curve is underestimated. Therefore, for an estimate of the actual assimilation efficiency, the maximum value of the ratio, total arsenic in tissues/total arsenic ingested was determined from the tissue distribution data. To obtain this value, 12 fish were dosed with arsenic (^{74}As) as previously explained for fish used in whole body clearance of arsenic. Three

fish each were sacrificed at 6, 12, 18 and 24h postdose and tissue distribution examined. A maximum value of 47 ± 6% of ingested dose was obtained at 12h postdose for the ratio. However, the data on tissue distribution and excretion of arsenic suggest that elimination of arsenic via the various routes must have begun much earlier than 12h. Therefore, for the purpose of estimating input of arsenic from the food vector under long term equilibrium conditions, a minimum of 50% assimilation efficiency is assumed.

Routes of Arsenic Excretion

Whole body elimination data have shown that arsenic was rapidly eliminated from whole body, elimination being a biphasic process with the fast-clearing phase accounting for the majority of the assimilated dose. Excretion through the gill, kidney, bile and/or intestinal epithelium represent the most probable routes.

Fecal excretion

By 24h postdose 80% or more of the arsenic in the gastrointestinal (GI) tract was in the posterior gut content. This proportion in the GI tract remains about the same over the following 19 days (Table 8). Approximately 10.8 percent of the body burden was contained in the gut content 24h postdose. It is possible that the lining of the GI tissue which contained a high proportion of arsenic may be shed thus enhancing fecal elimination. Taking into consideration the high arsenic content of the bile, the liver and the posterior gut content one can conclude that fecal elimination of arsenic represents an important clearance route and may account for 4.5% d^{-1} of the remaining body burden of arsenic during the first 5 days

Table 8. Arsenic (^{74}As) contained in the posterior gut content of rainbow trout, Salmo gairdneri following an oral dose (10ng As 100g fish^{-1}). Mean±S.E. for 4 fish are presented.

Time postdose hours(h) or days (d)	% dose	% of remaining body burden	% of total G.I. arsenic in posterior gut content
2h	4.9	5.1±0.6	6.8± 2.2
1d	6.4	10.8±1.5	80.0± 7.0
2d	1.6	5.4±1.0	78.0± 3.0
4d	0.7	4.5±0.8	90.0± 3.5
7d	0.4	4.5±0.4	93.0± 2.0
14d	0.1	2.3±0.5	77.0±15.0
21d	0.1	2.2±0.7	100.0± 0.0

of fast clearance, followed by reduced clearance rate of only about 2% of body burden d^{-1} during the subsequent two-week period (Table 8). This could result in as much as 35% of remaining body burden being lost by the fecal route.

Urinary excretion of arsenic

Using urine output (ml per 100 g fish h^{-1}) as a basis for evaluating renal function, the dose of 25 µg As/fish did not seem to affect renal function. The urine output for the control and the treated fish (Table 9) was similar. The data for the urinary excretion of arsenic by four rainbow trout is presented in Table 10. Urinary excretion during 7 days postdose accounted for 16 ± 3% of the ingested dose of arsenic. Extrapolation of the clearance rate to post exposure times longer than 7 days suggests that during the two week period 8-21 days, arsenic loss via urine accounted for an additional excretion of no more than 5% of the ingested dose.

Excretion of arsenic via the gills

The data for the excretion of arsenic via the gills are presented in Tables 11 and 12. The results show that the rate of excretion into the head compartment of the respirometer via the gills was high during the first half-hour of each run probably due to excitement of the fish. During this period, opercular movement was more rapid, suggesting an increased metabolic rate. During the second and third half-hours it slowed down to a more steady state. The excretion rates reported in Table 12 were therefore based on the 2nd and 3rd periods as detailed in Table 11. Whole body

Table 9. Urine output (ml per 100 g fish h^{-1}) by rainbow trout, Salmo gairdneri[a] held at 10±1°C.

Time Postdose (h)	Control n = 2	Treated[b] n = 4
0- 24	0.63	0.37±0.10
24- 48	0.55	0.47±0.06
48- 72	0.50	0.53±0.09
72- 96	0.43	0.43±0.07
96-120	0.35	0.25±0.06
120-144	0.40	0.27±0.05
144-168	0.30	0.24±0.07

[a] Mean wet weight of fish = 57.5±0.5 g

[b] Each fish was fed 0.01 µg ^{74}As-labelled arsenic acid plus 24.99 µg non-radioactive acid as carrier.

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

Fish No.	1		2		3		4		
Collection period postdose(h)	Remainaing body burden of arsenic[c]	Urinary excretion % of dose[d]	Remaining body burden of arsenic	Urinary excretion % of dose	Remaining body burden % of dose	Urinary excretion % of dose	Remaining body burden % of dose	Urinary excretion % of dose	Mean % urinary ±S.E.
0-24	80.6	2.0	81.2	0.9	82.6	0.4	56.7	2.2	1.4±0.4
24-48	65.0	1.9	62.6	1.6	58.9	3.0	29.7	1.5	2.0±0.3
48-72	47.9	2.4	45.2	0.8	40.2	12.2	19.6	5.3	5.2±2.5
72-96	33.5	2.7	37.9	1.2	31.9	3.5	16.4	5.3	3.2±0.1
96-120	20.1	3.1	27.1	0.7	23.7	2.8	13.3	0.1	1.6±0.7
120-144	16.1	1.6	21.2	2.5	19.1	2.4	8.8	0.2	1.7±0.5
144-168	12.7	1.3	17.1	2.9	15.9	0.8	7.3	0.1	1.3±0.6

[a] The arsenic dose consisted of 0.01 µg ^{74}As as arsenic acid plus 24.9 µg arsenic as non-radioactive arsenic acid.

[b] Mean wet weight of fish - 57.5 ± 0.5 g.

[c] Values represent percent remaining body burden at end of each collection period.

[d] Values represent % of dose per collection period.

Table 11. Excretion of arsenic through the gills of rainbow trout, Salmo gairdneri, following an oral dose of ^{74}As[a].

	Fish # 1 ($8.0x10^6$)[b]	Fish # 2 ($10.7x10^6$)	Fish # 3 ($6.5x10^6$)	Fish # 4 ($16.3x10^6$)	Fish # 5 ($16.3x10^6$)
Time postdose h	Dpm $^{74}As \times 10^3$				
20.0- 20.5	24.9	85.3	29.6	72.2	58.0
20.0- 21.0	21.3	78.2	27.2	58.0	58.0
21.0 21.5	20.1	56.8	24.9	41.5	52.1
44.0- 44.5	22.5	45.0	34.3	53.3	34.3
44.5- 45.0	20.T	28.4	21.3	49.7	19.0
45.0- 45.5	10.7	17.8	15.4	35.5	54.3
90 - 90.5	14.2	16.6	13.0	36.7	10.7
90.5- 91.0	9.3	14.2	9.5	28.4	14.2
91.0- 91.5	7.1	13.0	7.1	19.0	11.8
164.0-164.5	3.6	2.4	2.4	32.0	5.9
164.5-165.0	3.6	2.4	2.4	6.0	2.4
165.0-165.5	3.6	3.6	2.4	4.7	4.7

[a] specific activity of ^{74}As - 0.5 µg As/mCi

[b] values in parenthesis represent the dose of arsenic (dpm ^{74}As) ingested by each fish

Table 12. Excretion of an ingested dose of arsenic (^{74}As) via gill tissue by rainbow trout, Salmo gairdneri[a] maintained at 12°C.

Time postdose (h)	Fractional clearance through gill, % dose[c] h^{-1}						As loss from whole fish, fractional clearance rate[b]
	Fish # 1	Fish # 2	Fish # 3	Fish # 4	Fish # 5	Mean±S.E.	% dose h^{-1}
20	0.52 (1.15)[d]	1.26 (2.77)	0.80 (1.85)	0.61 (1.34)	0.68 (1.48)	0.77±0.13 (1.72±0.29)	2.3±0.16
44	0.37 (1.47)	0.43 (1.55)	0.56 (1.89)	0.52 (1.54)	0.44 (1.16)	0.46±0.03 (1.52±0.12)	0.7±0.12
90	0.21 (1.56)	0.25 (1.88)	0.25 (1.62)	0.29 (1.22)	0.16 (0.82)	0.23±0.02 (1.42±0.18)	0.3±0.03
164	0.09 (1.70)	0.06 (1.57)	0.07 (1.22)	0.07 (1.26)	0.04 (1.25)	0.07±0.01 (1.40±0.10)	0.2±0.03

[a] Mean wet weight of fish - 82±6g

[b] based on whole body measurements made at end of each of the indicated time period

[c] doses are the same as shown in Table 11.

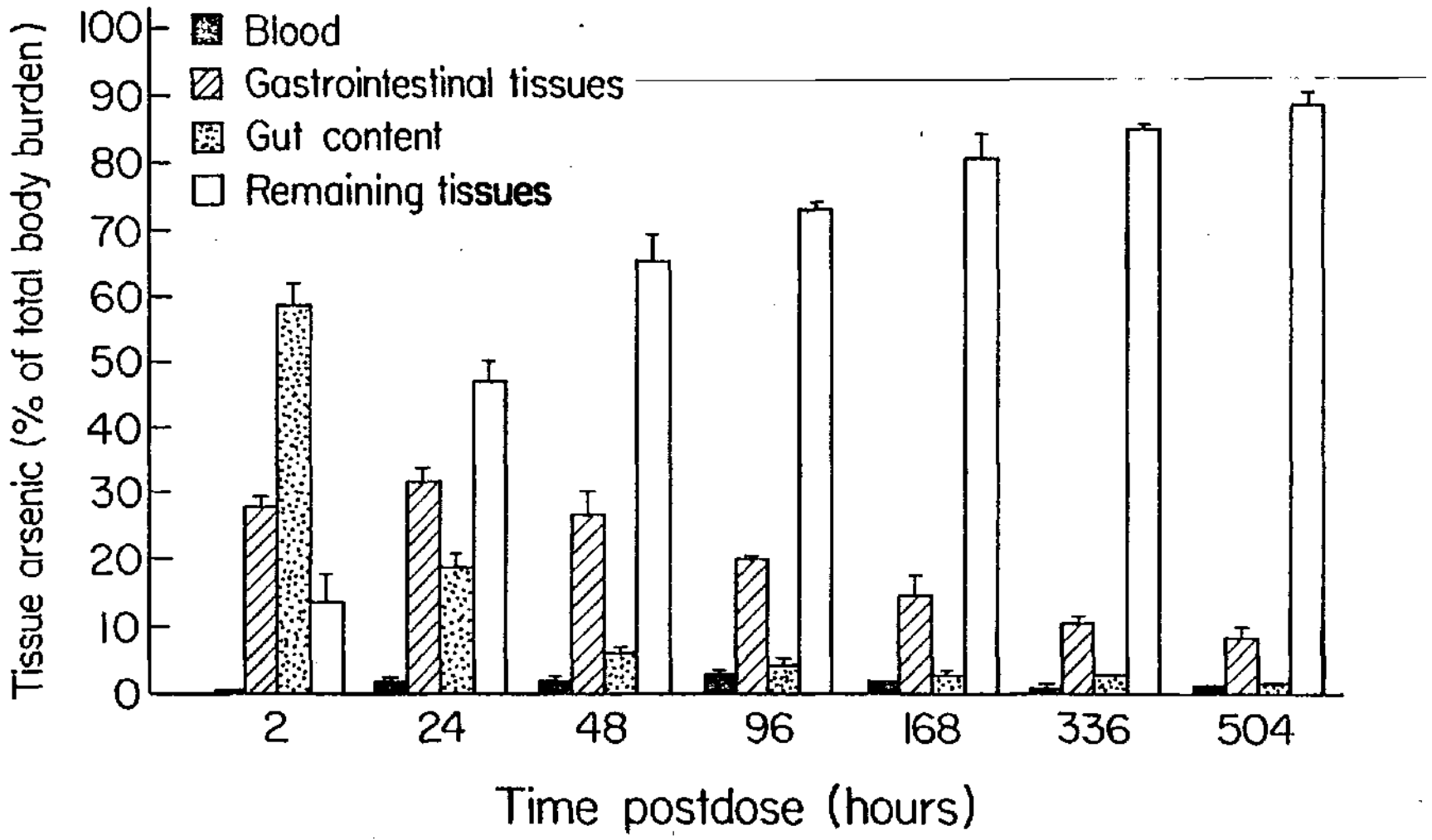
[d] Values in parentheses represent fractional clearance as a percent of remaining body burden of arsenic at time of measurement.

clearance of arsenic from the 4 test fish are reported in Table 12 and are in general agreement with values reported previously in uptake and clearance experiments (Fig. 5). Twenty hours postdose, 45% (at the rate of 2.28% h^{-1}-Table 12) of the ingested dose had been cleared from the whole fish. The mean rate of elimination via the gills based on the measurements made during the first two days postdose ranged from 0.77-0.46% of dose h^{-1} with a mean of 0.62% h^{-1}. The values decreased to 0.23% and 0.07 h^{-1} at 90h and 164h postdose respectively. These values for clearance from the gill represent approximately 40-50% of the ingested dose over a 7-day period, assuming that the clearance rates during measurements were representative of excretion via the gills if the fish were free-swimming.

The above results on routes of excretion of arsenic demonstrate that fecal and gill excretion might be of equal magnitude and could account for as much as 80% of total arsenic loss, urinary excretion accounting for between 15-20%. This present demonstration of gill excretion has important implications on the interaction effects of multiple toxicants such as copper which is known to affect gill function and this aspect of the problem will be treated more fully in the discussion.

Tissue Distribution of Arsenic Following Uptake from Food

The 'mass balance' of arsenic in the blood, gastrointestinal tissue, gut content and the remaining tissue is presented in Fig. 6. The highest proportion of arsenic in blood was reached at 4 days postdose when it contained 3% of total body burden of arsenic. The


Blood
Gastrointestinal tissues
Gut content
Remaining tissues
Tissue arsenic (% of total body burden)
100
90
80
70
60
50
40
30
20
10
0
2
24
48
96
168
336
504
Time postdose (hours)

proportion of arsenic in the gut wall tissue 24h postdose was very high, accounting for as much as 30% of the administered dose, but after 14 days the proportion was reduced to 10%, which is high considering the mass of tissues involved and the rapid turnover of intestinal epithelium. Arsenic in gut content was, as expected, highest at 2 hours and declined rapidly to a low value of less than 2% at 21 days postdose. In contrast, arsenic content in the remaining tissues increased gradually, reaching approximately 90% of the total body burden measured at 21 days postdose. The time course change in relative concentration (cpm ^{74}As per gram tissue/cpm ^{74}As per gram fish) distribution of arsenic is presented in Table 13. The nature of this unit (relative concentration) normalizes in a sense, the concentration values at various times postdose and the values are relative i.e. a higher value for arsenic at day 14 compared to day 2 does not mean that there is actually more arsenic in the tissue at day 14 than at day 2, but relative to the average concentration in the tissues at day 14, the concentration at day 2 is higher. Uptake of arsenic by trout was rapid. Two hours after dosing, significant levels of arsenic were measured in the blood, liver gill and kidney, with gill and blood reaching their peak values of 2.8 and 1.2 at 2 and 24h respectively. The liver reached maximum concentration of 1.8 at 4 days postdose. At one day after pulse dose, the bile, liver and kidney (decreasing order) contained the highest concentrations of arsenic. Levels of arsenic in the bile were much higher than in other tissues examined. The concentration in most tissues except the kidney in which it continued to increase, had decreased to less than half the

Table 13. Time course change in relative tissue concentration (cpm per g tissue/cpm per g fish) of arsenic[a] following a pulse dose (10ng ^{74}As $100g^{-1}$ fish) in rainbow trout held at 10 ± 1°C. Means ± S.E. of 4 fish are presented.

Tissue/organ	Time postdose, hours (h) or days (d)						
	2h	1d	2d	4d	7d	14d	21d
Blood	1.0±0.2	1.2±0.1	1.2±0.3	1.0±0.1	0.6±0.1	0.4±0.2	0.4±0.1
Brain	0.3±0.2	0.9±0.0	1.0±0.2	1.4±0.3	1.3±0.6	0.3±0.1	0.0±0.0
Gill	2.8±1.1	1.1±0.0	1.0±0.1	1.3±0.2	1.1±0.2	2.7±0.3	2.7±1.0
Liver	1.7±0.1	1.6±0.1	1.5±0.1	1.8±0.1	1.6±0.3	1.6±0.1	0.9±0.2
Bile & gall bladder	0.0±0.0	2.5±0.5	2.5±0.5	1.1±0.5	1.0±0.3	0.7±0.2	2.2±0.4
Kidney	1.1±0.3	1.4±0.3	1.6±0.2	2.3±0.9	1.9±0.3	3.2±1.0	2.0±0.6
Muscle & skin	0.2±0.0	0.8±0.0	0.9±0.0	0.9±0.0	0.7±0.1	0.6±0.0	0.4±0.2
Spleen	0.8±0.3	1.5±0.4	1.2±0.2	0.9±0.1	1.3±0.3	0.4±0.2	0.0±0.0

[a] Specific activity of arsenic - 0.5 µg mCi^{-1}

peak values by 21 days (Table 13). At all times postdose, the concentration of arsenic in the combined muscle and skin was low in comparison to the liver, gallbladder & bile and kidney.

The total amount of arsenic in each tissue is shown by the data in Table 14. It is important to note that the data in this table represent the amount of arsenic in each tissue as a porportion of total arsenic in whole body at time of sacrifice and not a proportion of ingested dose. Muscle and skin comprising 53% of the total weight of fish (Table 2) contained 38% of the total arsenic as a maximum at 4 days postdose. The gallbladder and bile which represents a relatively small percentage of the fish weight (0.26) contained only 0.56% as a maximum of the body burden of arsenic at 21 days postdose, although its relative tissue concentration (Table 13) is three times as high as that of the combined muscle and skin. In this particular experiment, the content of the gastrointestinal tract (GI) was not separated into anterior and posterior portions. However, the results in Table 8 obtained from a separate experiment, illustrate the role of feces as one of the excretion routes for arsenic. In quantitative terms, as reported in Table 8, the amount of arsenic in the posterior gut content represents a loss of arsenic that could account for as much as 35% of the initial ingested dose over the total period of 21 days required for essentially complete elimination.

Acclimation of Rainbow Trout to Arsenic

Whole body clearance of an ingested dose of ^{74}As in rainbow trout that were acclimated to sublethal doses of sodium arsenite for two weeks.

For all preexposure experiments, the trout were acclimated

Table 14. Time course change in tissue distribution[a] of an ingested dose (10ng As $100g^{-1}$ fish) of ^{74}As-labelled arsenic[b] acid in rainbow trout at 10 ± 1°C. Means ± S.E. of 4 fish are presented.

Tissue/organ	Time postdose, hours (h) or days (d)						
	2h	1d	2d	4d	7d	14d	21d
Blood	0.4 ±0.1	2.1±0.3	2.0±0.2	2.6±0.2	2.0±0.0	1.2±0.6	1.3±0.2
Brain	0.0 ±0.0	0.4±0.0	0.5±0.1	0.9±0.1	0.8±0.3	0.2±0.1	0.0±0.0
G.I. contents	58.7 ±3.0	18.9±1.1	6.3±0.8	4.4±0.1	2.8±0.5	3.1±0.2	1.7±0.3
G.I. tissue	28.3 ±1.8	31.8±1.9	26.9±3.3	20.3±0.4	14.8±2.8	10.8±0.6	8.6±1.5
Gill	4.4 ±2.6	2.2±0.2	2.8±0.2	3.8±0.6	3.4±0.2	8.5±1.1	9.1±3.0
Liver	0.3 ±0.1	1.1±0.1	1.3±0.1	1.7±0.2	1.2±0.2	1.6±0.2	0.8±0.1
Kidney	0.1 ±0.0	0.7±0.1	1.0±0.1	1.2±0.6	1.2±0.1	1.8±0.2	1.5±0.6
Muscle & skin	3.1 ±1.2	24.2±0.6	35.3±3.4	37.5±2.1	34.7±4.4	28.7±2.2	37.1±2.1
Gall bladder & bile	0.0 ±0.0	0.5±0.1	0.5±0.1	0.3±0.2	0.3±0.0	0.1±0.1	0.6±0.2
Spleen	0.0 ±0.0	0.1±0.0	0.2±0.0	0.1±0.0	0.3±0.2	0.0±0.0	0.0±0.0
Remaining carcass	5.38±1.8	18.2±2.0	23.4±1.9	27.1±2.3	38.7±1.3	42.9±2.1	37.2±4.9

[a] Expressed as a % of remaining body burden of arsenic at time of sacrifice

[b] Specific acitivty - 0.5 µg As mCi^{-1}

Table 15. Time course change in tissue distribution[a] of an ingested dose (10ng As $100g^{-1}$ fish) of ^{74}As-labelled arsenic[b] acid in rainbow trout held at 10±1°C.

Tissue/organ	Time postdose, hours (h) or days (d)						
	2h	1d	2d	4d	7d	14d	21d
Blood	0.4±0.1	1.0±0.2	0.7±0.1	0.5±0.0	0.2±0.1	0.1±0.0	0.1±0.0
Brain	0.0±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.0±0.0	0.0±0.0
G.I. content	56.3±2.9	9.1±0.6	2.1±0.3	0.8±0.0	0.3±0.0	0.1±0.1	0.1±0.0
G.I. tissue	22.8±4.2	15.4±0.9	8.9±1.0	3.7±0.1	4.0±2.5	0.4±0.0	0.3±0.1
Gill	4.2±2.5	1.1±0.1	0.9±0.1	0.7±0.1	0.3±0.0	0.4±0.1	0.2±0.1
Liver	0.3±0.1	0.5±0.1	0.4±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.0±0.0
Kidney	0.1±0.0	0.3±0.0	0.3±0.0	0.2±0.1	0.1±0.0	0.1±0.0	0.0±0.0
Muscle & skin	3.2±1.2	11.7±0.3	11.7±1.1	6.7±0.4	3.2±0.4	1.2±0.1	0.2±0.0
Gall bladder & bile	0.0±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Spleen	0.0±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Remaining carcass	5.5±1.4	8.9±1.0	7.7±0.6	4.2±0.9	3.5±0.1	1.8±0.1	1.4±0.4

[a] Mean values ± S.E. for 4 or 5 fish in each case are expressed as percentage of the single ingested dose.

[b] Specific activity - 0.5 µgAs mCi^{-1}

to sodium arsenite (oxidation state +3) for two weeks. The retention data is presented in Fig. 7. The fractional clearance rate and half-lives for the slow-clearing compartment for fish preexposed to 5.7mg As kg^{-1} diet for 14 days were not significantly different from those in the control fish. However, fish preexposed to 22.80 mgAskg^{-1} diet for the same period demonstrated a significantly higher ($P<0.05$) fractional clearance rate of 0.22 d^{-1} compared to the control rate of 0.12 d^{-1} (Table 16).

In order to avoid undue stress and possible acceleration of clearance as a result of handling (see previous result on gill excretion - Table 12) a replicate group of fish were used to monitor growth, blood hemoglobin levels and arsenic accumulation in tissues. These groups were maintained under identical conditions to those used for whole body clearance. Only the results for the control and 22.8 mgAskg^{-1} group are presented (Table 12) as some of the fish exposed to the lower arsenic concentration developed a fungal disease. There was no significant effect of this level of arsenic on growth and hemoglobin of fish measured at either 2 or 4 weeks of exposure (Table 17). A maximum arsenic concentration of 1.0 µg g^{-1} fish was observed after 14 days of exposure to 22.80 mgAskg^{-1} diet (Table 18). However, after 28 days of exposure to arsenic, concentration in the fish was lower than that for 14 days by almost 50%.

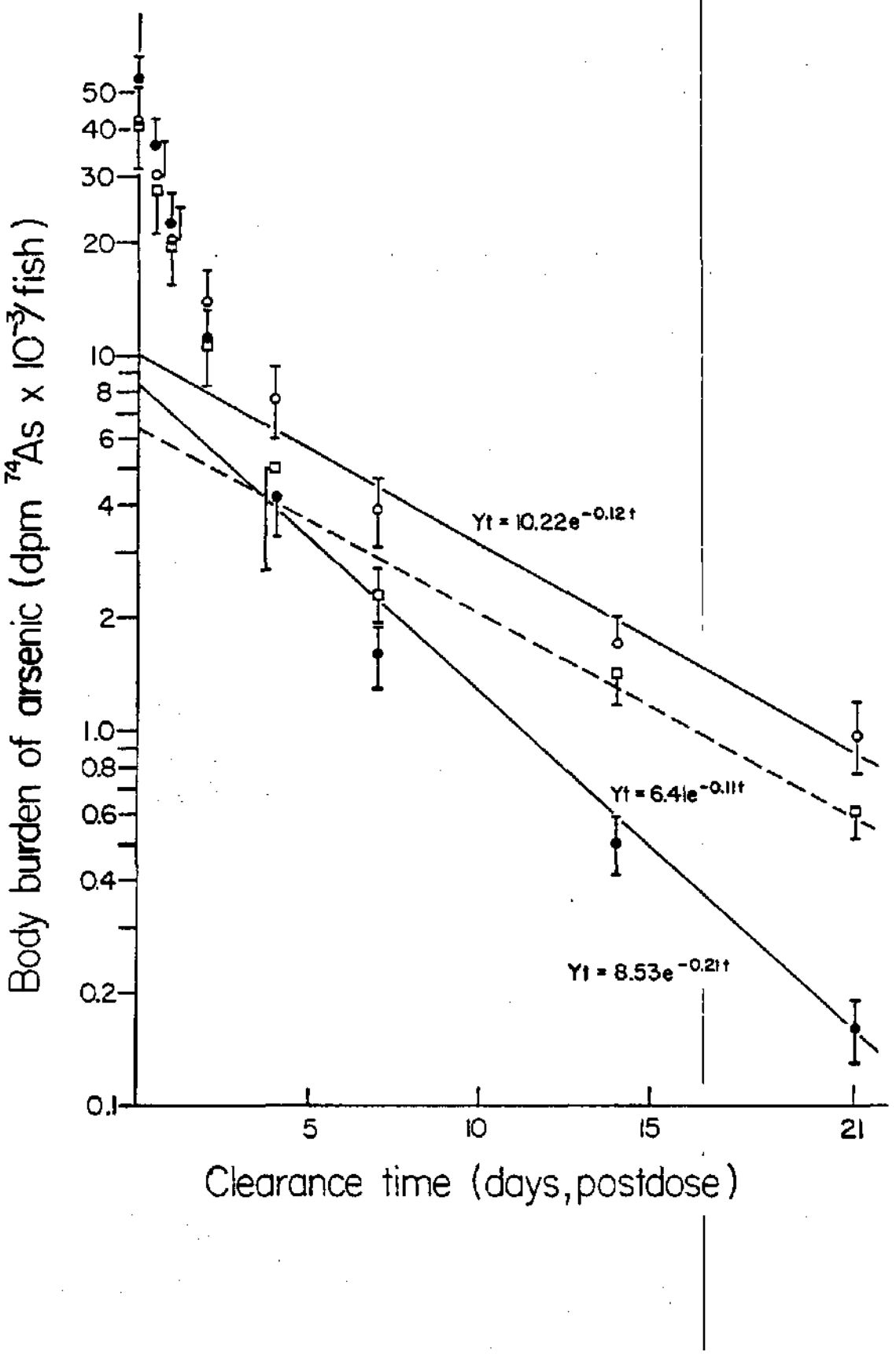

Body burden of arsenic (dpm ^{74}As x 10^{-3}/ fish)
50
40
30
20
10
8
6
4
2
1.0
0.8
0.6
0.4
0.2
0.1
Yt = 10.22e$^{-0.12t}$
Yt = 6.41e$^{-0.11t}$
Yt = 8.53e$^{-0.21t}$
5
10
15
21
Clearance time (days, postdose)

Table 16. Effect of preexposure to arsenic[a] on whole body clearance[b] of a subsequent ingested dose of 10 ng ^{74}As/100g fish (as arsenic acid[d]) by rainbow trout, Salmo gairdneri, held at 10 ± 1°C.

Preexposure dose	Mean wet of fish	No of fish	Retained dose	Fast compartment		Slow compartment		
				As loss, fractional clearance	Biological half-life	Compartment size	As loss, fractional clearance	Biological half-life
mgAs kg^{-1} diet[c]	g		Pg ^{74}As g^{-1} fish	d^{-1}	d	% of retained dose	d^{-1}	d
0 (control)	32.9±1.6	8	21.5	0.8 ± 0.05	0.90 ± 0.07	25.4 ± 2.8	0.11 ± 0.01	6.4 ± 0.4
5.70	29.1±1.3	6	29.7	0.9 ± 0.03	0.80 ± 0.03	16.6 ± 1.3	0.11 ± 0.00	6.2 ± 0.2
22.80	32.6±1.0	8	28.3	1.3 ± 0.14	0.60 ± 0.06	17.3 ± 2.3	0.22 ± 0.01	3.2 ± 0.1

[a]Fish were preexposed to arsenic as sodium arsenite for 2 weeks.

[b]Each value represents mean ± S.E. Means scored by the same line are not significantly different at $P < 0.05$ - student's t test.

[c]Fish were fed at the rate of 2% body weight/day during preexposure and 21-day clearance.

[d]Specific activity = 0.5 µg mCi^{-1}.

Table 17. Wet weight changes (g) and blood hemoglobin concentration in control and fish that were exposed to 22.8 mg As/kg diet for 4 weeks.

Duration of exposure (wks)	Mean wet weight of fish[1](g)		Mean hemoglobin[2] concn in blood (g/100 ml)	
	Control	Preexposed	Control	Preexposed
0(initial)	23.30±0.62	23.12 ±0.50	9.63±0.22[3]	----
2	28.00±0.66	27.12*±0.59	9.74±0.22	9.22*±0.32
4	29.04±0.85	29.40*±0.85	9.87±0.45	10.13*±0.58

[1] Mean ± S.E. of 9 fish measured throughout the duration of the experiment

[2] Mean ± S.E. of values obtained from 5 fish in each case

[3] Determined on blood of 5 fish sampled at the beginning of the experiment

* Not significantly different from controls at P < 0.05 (Students t-test)

Table 18. Arsenic retention by rainbow trout, Salmo gairdneri exposed to 22.8 mg As kg^{-1} diet for 4 weeks at 10 ± 1°C. Fish were fed at a daily rate of 2% body weight.

Duration of exposure (days)	Total arsenic ingested as $NaAsO_2$ µgAs g^{-1} wet wt.	Total arsenic retained[a] µg As g^{-1} wet wt.	Percent retention
0	0.00	Not detectable[b]	-
7	2.74	0.40	14.6
14	5.48	1.00	18.3
21	8.22	0.50	6.1
28	10.96	0.63	5.7

[a] Each value represents determination carried out on pooled samples of 5 fish at each time point.

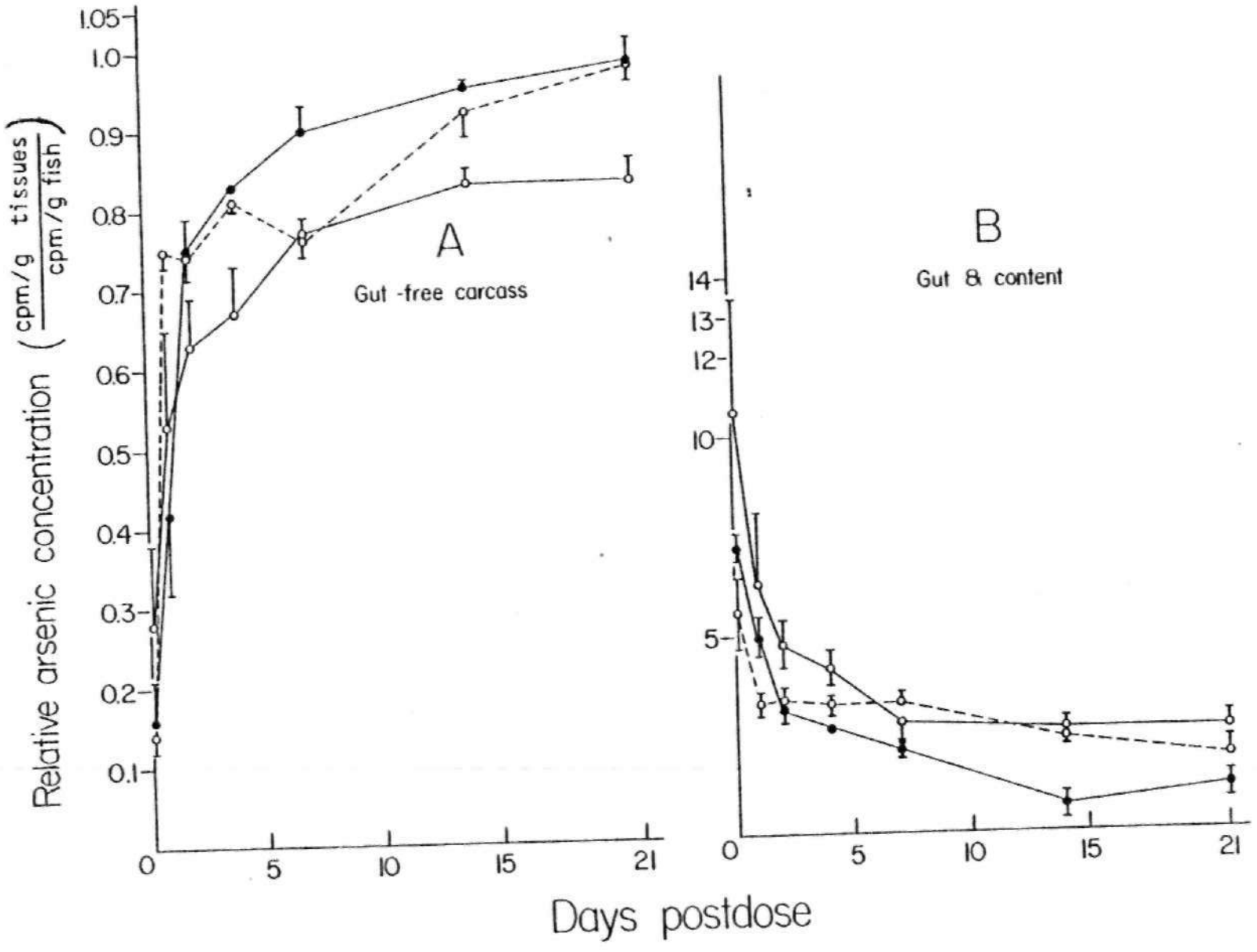
[b] Detectable level - 0.1 ppm. Assays carried out by Bondar-Clegg & Co. Ltd., Ottawa.

Tissue distribution of a pulse dose of ^{74}As in rainbow trout that were acclimated to sublethal doses of sodium arsenite for two weeks

In obtaining the results of adaptation to arsenic in the diet, based on tissue distribution patterns, fish were exposed to dietary arsenic for 14 days and then given a tracer-labelled dose of ^{74}As with their regular daily dietary arsenic. At various times postdose, the distribution of ^{74}As in various tissues was measured.

The results reported in Fig. 8A show that uptake of arsenic was rapid after ingestion of a 'pulse dose' of ^{74}As-labelled arsenic. The data in Fig. 8-11 are reported as relative concentration units, being the ratio of two values for concentration of radioactivity, one for the specific tissues and the other for whole body as previously explained. As such, changes in this number with time indicates the time course of distribution and redistribution of arsenic after its ingestion and assimilation. Tissue distribution of arsenic in specific tissues is also expressed as a proportion (%) of the total in whole fish tissue at time of sacrifice. Generally, preexposure did not seem to affect the pattern of distribution or the time at which peak concentrations were reached in any tissues. The data on tissue distribution (Fig. 8-11) suggest that certain tissues of preexposed fish accumulated higher levels of the element than those for the non-preexposed. However, an analysis of variance (ANOVA) on the data indicated that only the concentrations of arsenic in liver and bile of preexposed fish were significantly higher ($P<0.05$) than those for the control.

The pattern of tissue distribution is also interesting

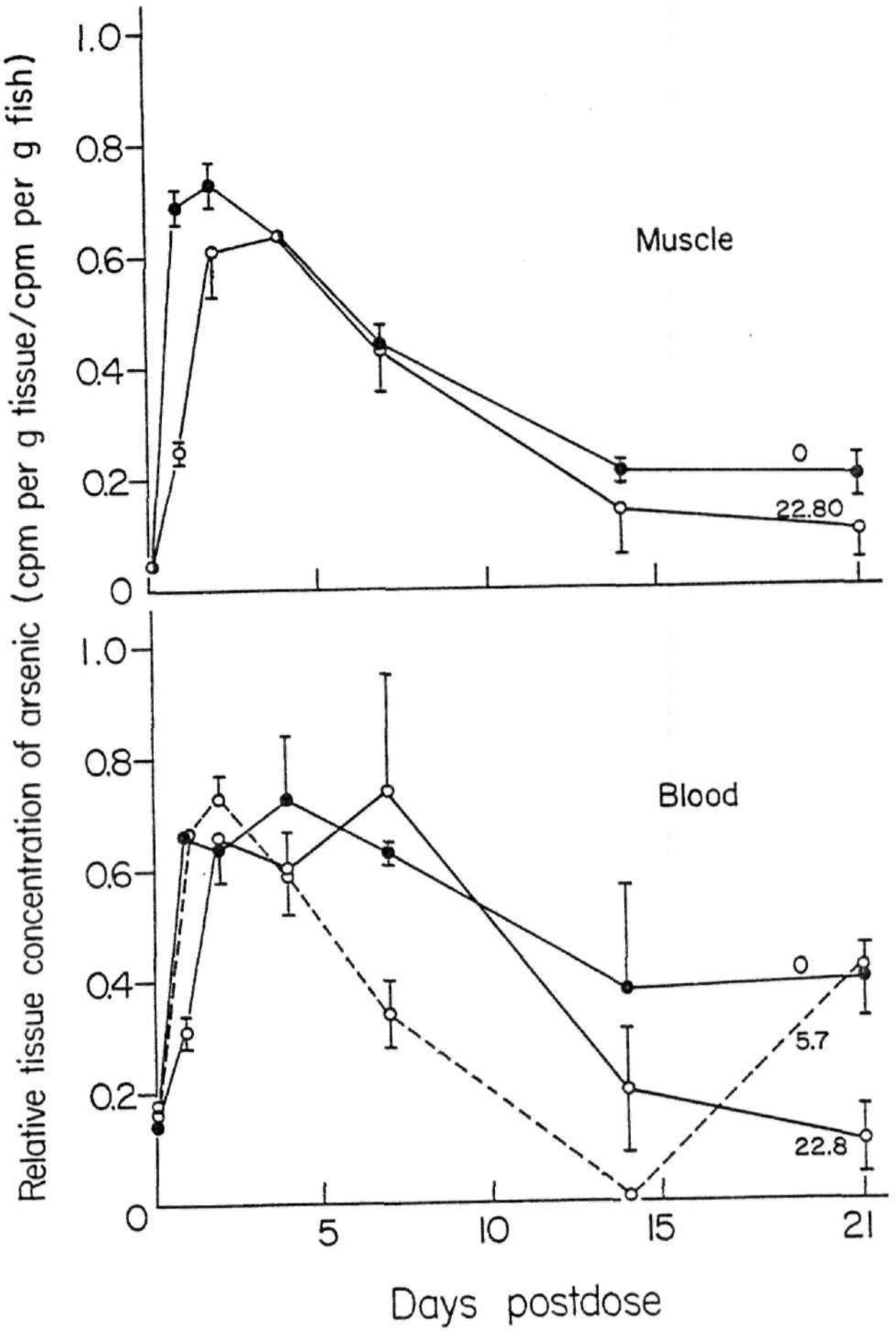

Relative arsenic concentration (cpm/g tissues / cpm/g fish)
1.05
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
A
Gut -free carcass
B
Gut & content
14
13
12
10
5
0
5
10
15
21
Days postdose

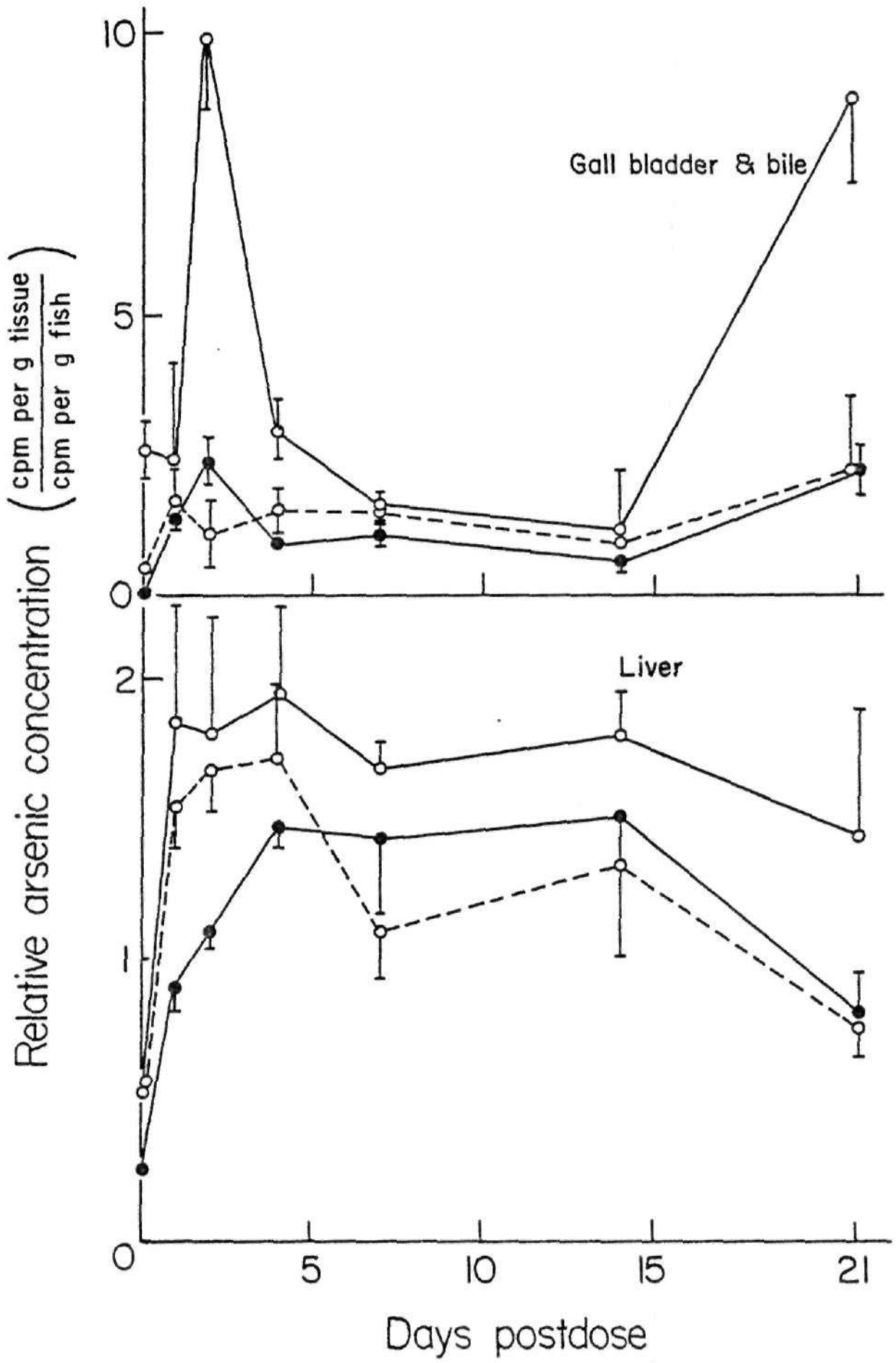
when looked at in detail. In all three groups, the blood and liver had their peak arsenic concentration at between 1-3 days and at 2 days respectively after the pulse dose of tracer arsenic (Fig. 9 and 10). Kidney and liver concentrations of tracer arsenic did not seem to show any decline up until 14 days when the kidney dropped a little in the control (Fig. 11). On the whole, tissue of fish that were preexposed to the highest concentration of arsenic (22.8 $mgAskg^{-1}$ diet) before pulse dose of tracer ^{74}As consistently exhibited highest levels of radiolabelled arsenic while the controls were lowest. The exception to this were the blood and muscle in which the preexposed contained less arsenic than the control (Fig. 9).

The total amount of arsenic in each tissue of control and preexposed fish is presented in Table 19. Like the non-preexposed fish mentioned previously (Table 13), the muscle and the skin (in that decreasing order) contained the highest proportion of arsenic. The skin accumulated arsenic continuously and did not seem to show any regression by the time the experiment was terminated. Similarly, the gastrointestinal tract tissue contained very high proportions ranging from 2-31% of the body burden (Table 19). Arsenic levels in the gills also accounted for relatively high proportions of the body burden of arsenic up to 9% in spite of it comprising only 3.6% of the body weight.

Binding of arsenic to liver protein

Data was obtained on arsenic binding to proteins of liver cytosol of control (non preexposed) fish given only a single dose of tracer-labelled ^{74}As in the diet and sacrificed 24 and 48 hours


Relative tissue concentration of arsenic (cpm per g tissue/cpm per g fish)
1.0
0.8
0.6
0.4
0.2
0
Muscle
0
22.80
Blood
0
5.7
22.8
5
10
15
21
Days postdose


Relative arsenic concentration (cpm per g tissue / cpm per g fish)
10
5
0
2
1
0
Gall bladder & bile
Liver
5
10
15
21
Days postdose

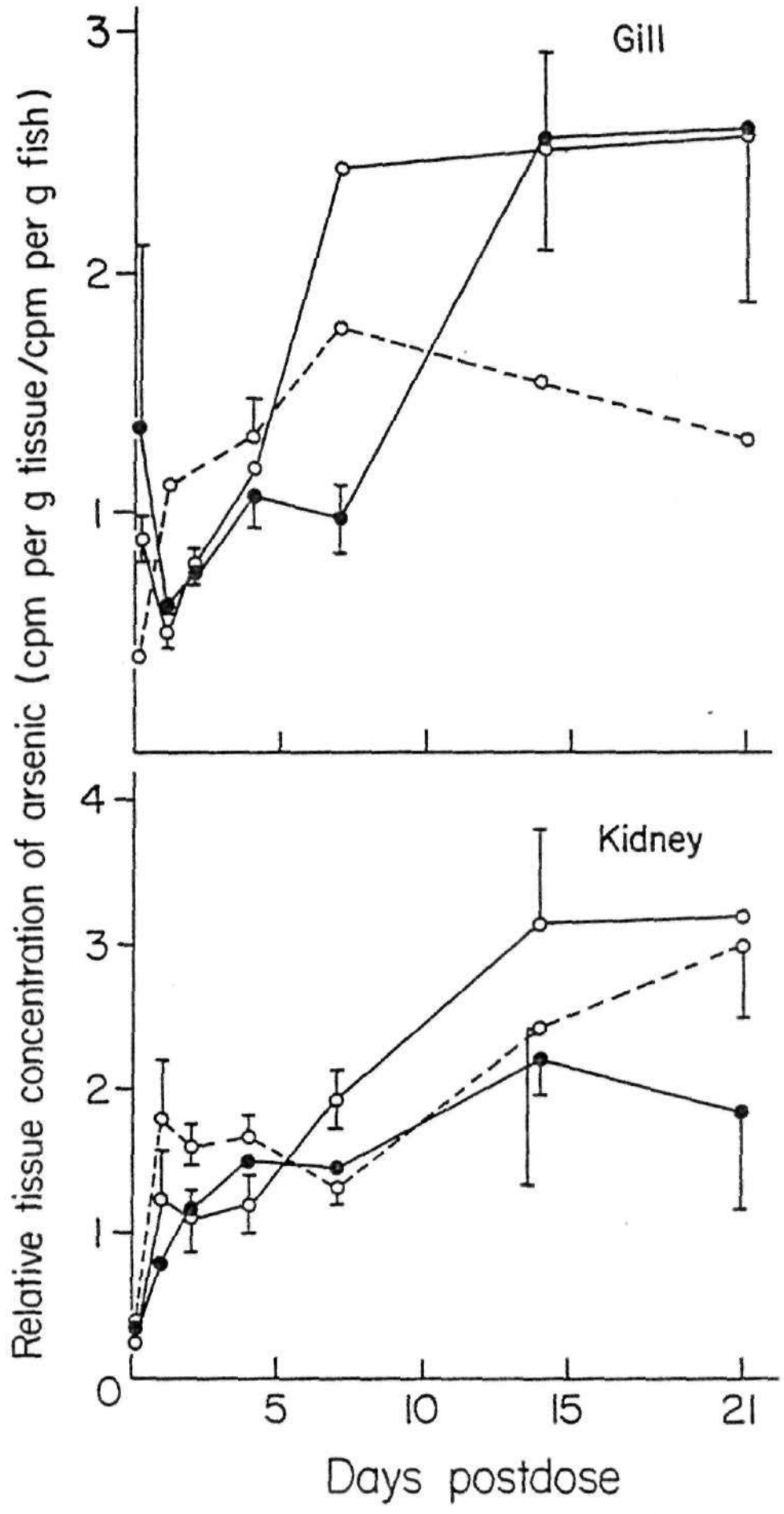

Gill
Kidney
Relative tissue concentration of arsenic (cpm per g tissue/cpm per g fish)
0
1
2
3
4
5
10
15
21
Days postdose

Table 19. The effect of preexposure to various levels of dietary arsenic[a] on the distribution[b] of a subsequent dose (10 ng ^{74}As $100g^{-1}$ fish) in tissues of rainbow trout, Salmo gairdneri held at 10°±1°C.

Tissue[c]	Preexposure Levels mg As Kg^{-1} diet	Time postdose, hours (h) or days (d)						
		2h	1d	2d	4d	7d	14d	21d
	0	0.4±0.1	2.1±0.3	2.0±0.2	2.6±0.2	2.0±0.0	1.2±0.6	1.3±0.2
Blood	5.7	0.6±0.1	1.8±0.3	2.3±0.1	1.8±0.2	1.1±0.2	0.0±0.0	2.3±0.5
	22.8	0.5±0.1	0.9±0.1	2.1±0.2	1.9±0.2	2.3±1.1	0.9±0.5	0.5±0.3
	0	0.0±0.0	0.4±0.0	0.5±0.1	0.9±0.1	0.8±0.3	0.2±0.1	0.0±0.0
Brain	5.70	0.1±0.0	0.4±0.1	0.4±0.0	0.5±0.1	0.7±0.2	0.0±0.0	0.3±0.3
	22.8	0.1±0.0	0.1±0.0	0.4±0.0	0.4±0.0	0.3±0.1	1.1±1.0	2.7±0.7
GI	0	85.6±4.4[e]	50.7±3.0	33.2±4.0	24.8±0.5	17.5±3.3	13.9±0.7	10.2±1.8
Tissue	5.7	30.1±3.0	15.0±4.0	27.0±2.5	22.0±1.2	27.0±2.4	14.0±3.0	11.0±2.0
	22.8	31.0±4.0	37.5±4.5	29.5±5.5	25.0±4.2	22.2±1.8	21.0±2.0	17.0±2.5
GI	0	-	-	-	-	-	-	-
Content	5.7	55.2±4.0	17.0±2.0	6.5±1.2	5.0±1.2	4.8±1.0	3.8±1.0	2.1±0.8
	22.8	52.2±5.3	33.3±5.0	13.2±2.5	11.2±2.5	8.0±1.3	4.0±1.0	8.0±1.3
	0	4.4±2.6	2.2±0.2	2.8±0.2	3.8±0.6	3.4±0.2	8.5±1.1	9.1±3.0
Gill	5.7	1.5±0.4	3.2±0.2	3.4±0.3	4.4±0.3	5.8±0.1	6.5±2.1	8.2±1.4
	22.8	2.8±0.7	1.8±0.3	2.5±0.3	3.7±0.9	6.9±1.2	7.2±1.1	7.8±1.8
	0	0.3±0.1	1.1±0.1	1.3±0.1	1.7±0.2	1.2±0.2	1.6±0.2	0.8±0.1
Liver	5.7	1.4±0.7	2.2±0.4	2.1±0.2	2.1±0.1	1.5±0.2	2.1±0.4	0.4±0.1
	22.8	0.8±0.1	2.8±0.7	2.4±0.4	2.9±0.5	2.4±0.2	2.7±1.2	1.3±0.5
	0	0.1±0.0	0.7±0.1	1.0±0.1	1.2±0.6	1.2±0.2	1.8±0.2	1.5±0.6
Kidney	5.7	0.2±0.1	1.3±0.2	1.3±0.1	1.4±0.1	1.1±0.1	3.4±1.4	4.4±1.1
	22.8	0.2±0.0	0.7±0.1	0.6±0.1	1.0±0.1	1.9±0.2	4.6±1.8	2.6±1.0
	0	3.1±1.2[d]	24.2±0.6	35.3±3.4	37.5±2.1	34.7±4.4	28.7±2.2	27.1±2.1
Muscle	5.7	3.4±0.7	31.5±1.5	33.2±2.0	29.3±0.3	20.0±1.3	10.0±0.8	9.2±1.7
	22.8	2.0±0.7	11.2±0.7	27.5±3.6	28.7±3.1	19.7±2.6	6.2±3.7	12.2±5.0
	0	-	-	-	-	-	-	-
Skin	5.7	1.8±0.5	6.8±0.3	6.0±1.2	7.7±0.6	9.8±0.5	21.2±1.2	19.9±3.5
	22.8	3.8±0.4	4.0±0.5	5.7±0.3	5.2±1.4	7.7±1.1	18.7±1.5	18.8±4.5
Gall Bladder	0	0.0±0.0	0.5±0.1	0.5±0.1	0.3±0.2	0.9±0.4	0.1±0.1	0.6±0.2
& Bile	5.7	0.1±0.0	0.2±0.1	0.1±0.0	0.3±0.2	0.2±0.1	0.0±0.0	0.8±0.5
	22.8	0.4±0.2	0.8±0.5	0.7±0.3	0.3±0.1	0.2±0.0	0.0±0.0	2.6±0.7

Table 19 (Cont'd)

Tissue	Preexposure Levels mg As Kg^{-1} diet	Time postdose, hours (h) or days (d)						
		2h	1d	2d	4d	7d	14d	21d
	0	0.0±0.0	0.1±0.0	0.2±0.0	0.1±0.0	0.3±0.2	0.0±0.0	0.0±0.0
Spleen	5.7	0.0±0.0	0.2±0.0	1.0±0.0	0.1±0.0	0.1±0.1	0.0±0.0	1.0±0.5
	22.8	0.1±0.0	0.2±0.1	0.2±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.9±0.5
Remaining	0	5.4±1.8	18.3±2.0	23.4±1.9	27.1±2.3	38.7±1.8	42.9±2.1	57.2±0.5
Carcass	5.7	4.1±1.0	20.2±1.0	17.2±1.5	24.6±1.7	27.9±2.1	36.6±2.6	38.4±4.9
	22.8	6.5±1.4	8.7±1.0	14.8±1.9	16.9±2.1	28.8±2.3	30.5±4.9	24.8±5.9

[a] Fish were preexposed to sodium arsenite for 2 weeks.

[b] Expressed as mean percentage ±S.E. of total body burden of arsenic based on the amount of radioactivity in tissues of 4 rainbow trout.

[c] For estimate of biomass in these tissues, see Table 2.

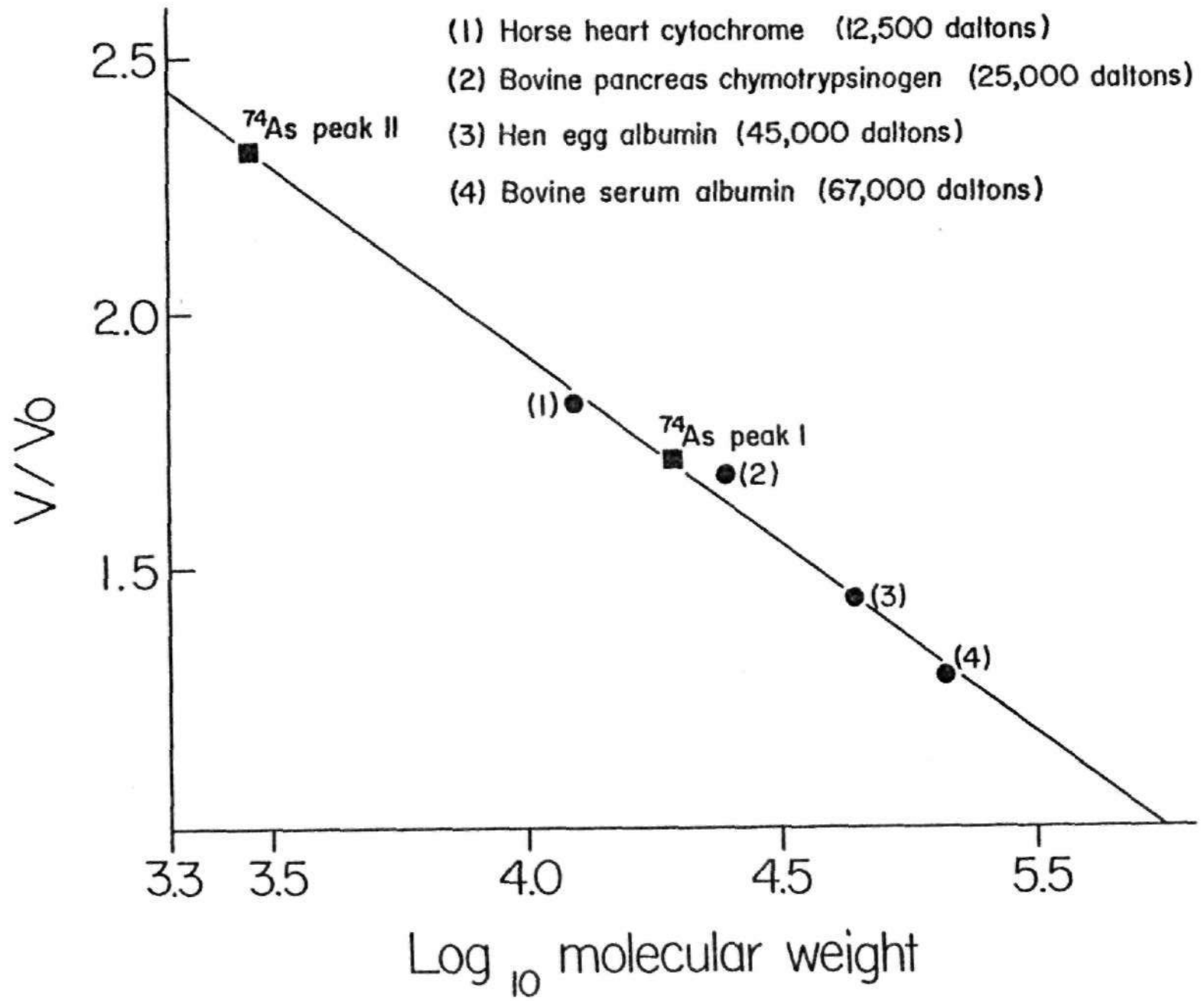
[d] Values are for skin and muscle combined.

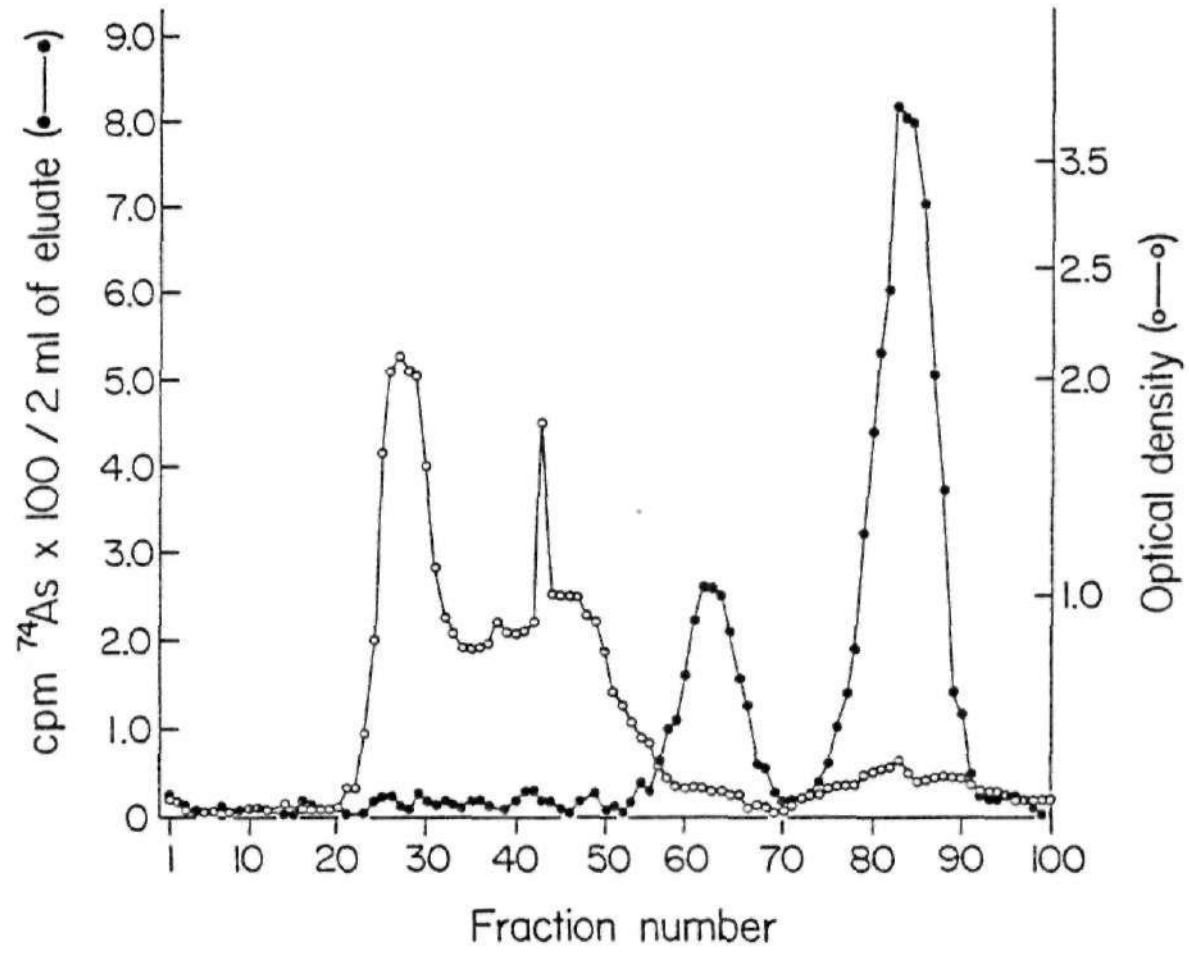
[e] Values for gastrointestinal (GI) tract and content.

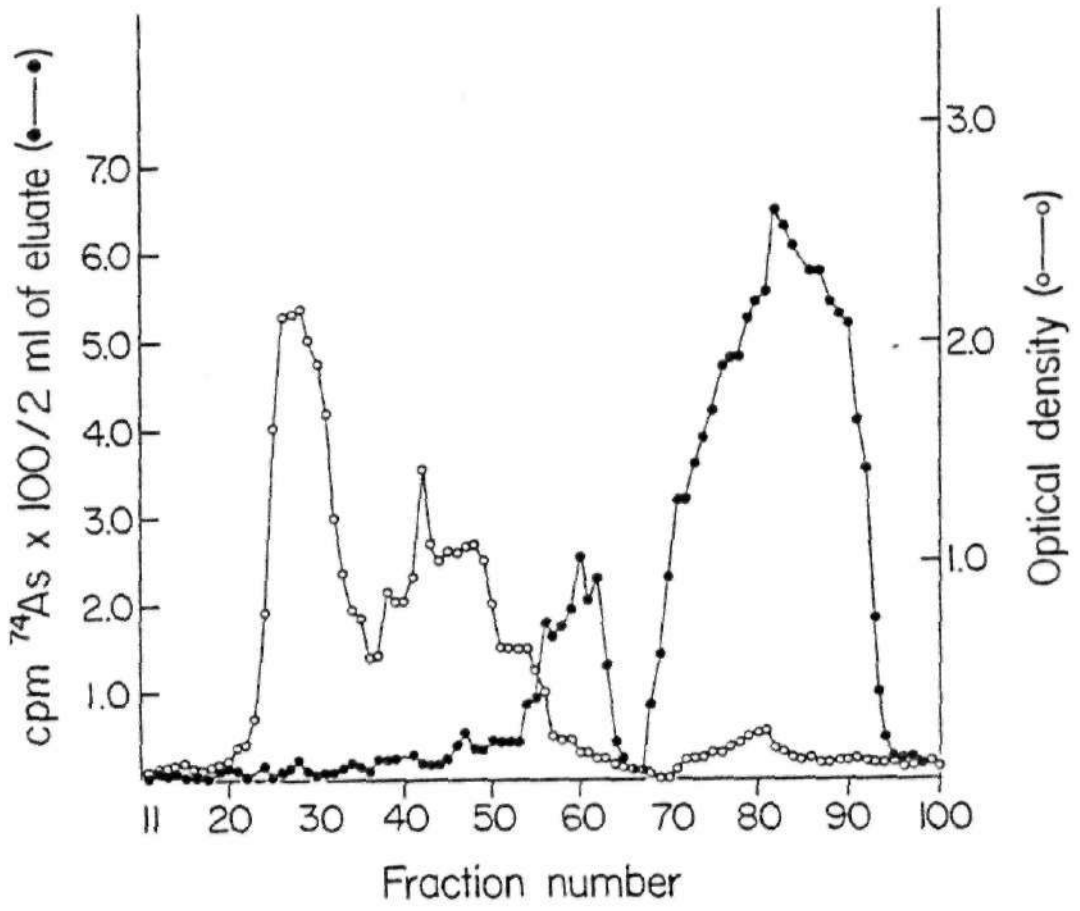
postdose. Test groups were similarly treated with a single dose of dietary ^{74}As after being fed a diet containing 5.7 and 22.8 mg As kg^{-1} for two weeks. The proteins in liver cytosol of the control and treated groups were fractionated by gel chromatography using Sepahcryl S-200 superfine. The sephacryl S-200 was calibrated with horseheart cytochrome c(12,500 daltons), bovine pancreas chymotrip-sinogen a(25,000 daltons), hen egg albumin (45,000 daltons) and bovine serum albumin (67,000 daltons) as illustrated in Fig. 12. Proteins from liver cytosol were partially resolved into two peaks (based on OD at 280 nm) as illustrated in Fig. 13-16. A third small peak was eluted after the first two main peaks.

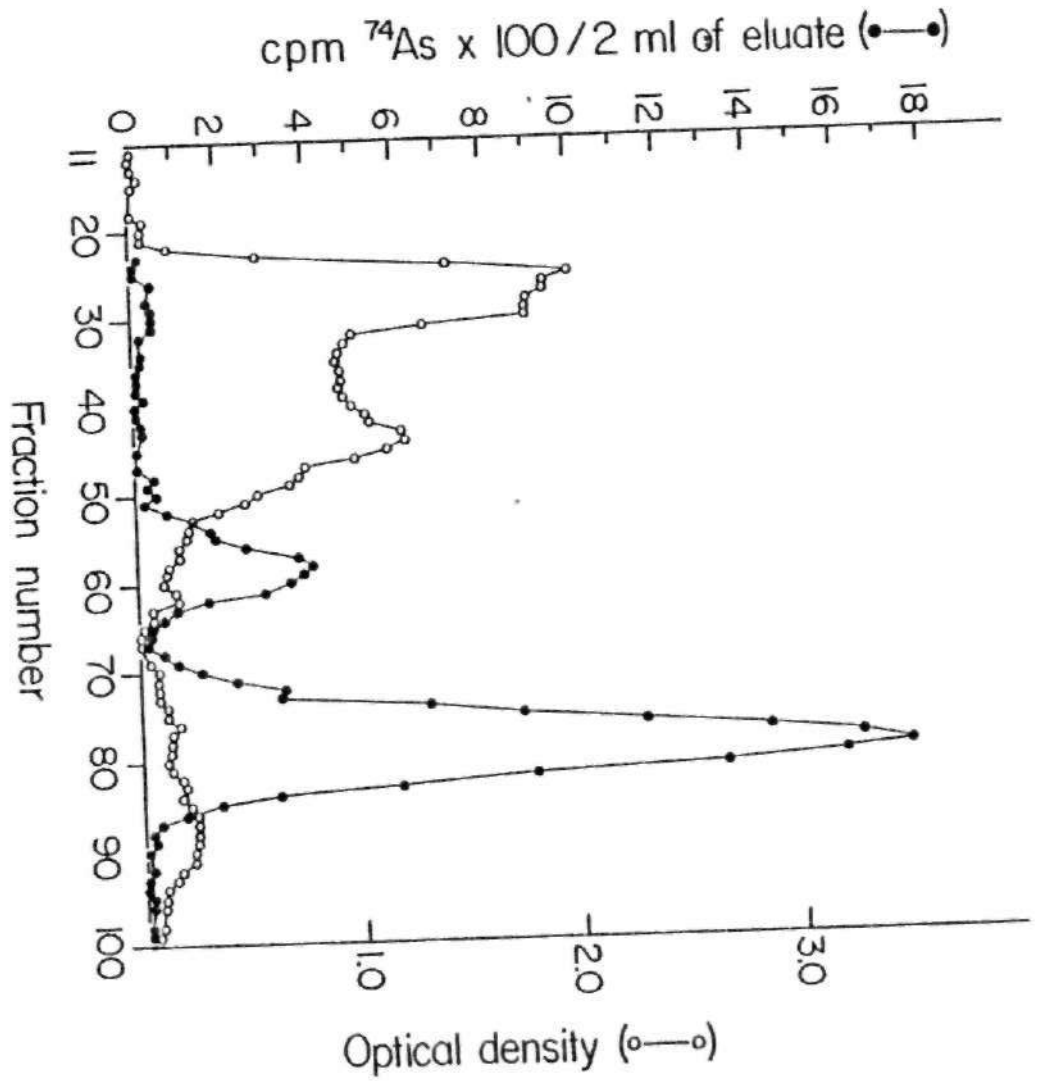
The elusion of ^{74}As was followed. The elusion pattern of ^{74}As showed 2 peaks of arsenic, the first (Peak I) of which coincided with the tailof the protein peak eluted in fractions 40-70 as shown in Fig. 13-16. On testing the fractions corresponding to the peaks for the presence of protein using trichloroacetic acid, only peak I, the fraction associated with ^{74}As (elution fractions 40-50) formed precipitates. The second peak of arsenic eluted in fractions 75-95.

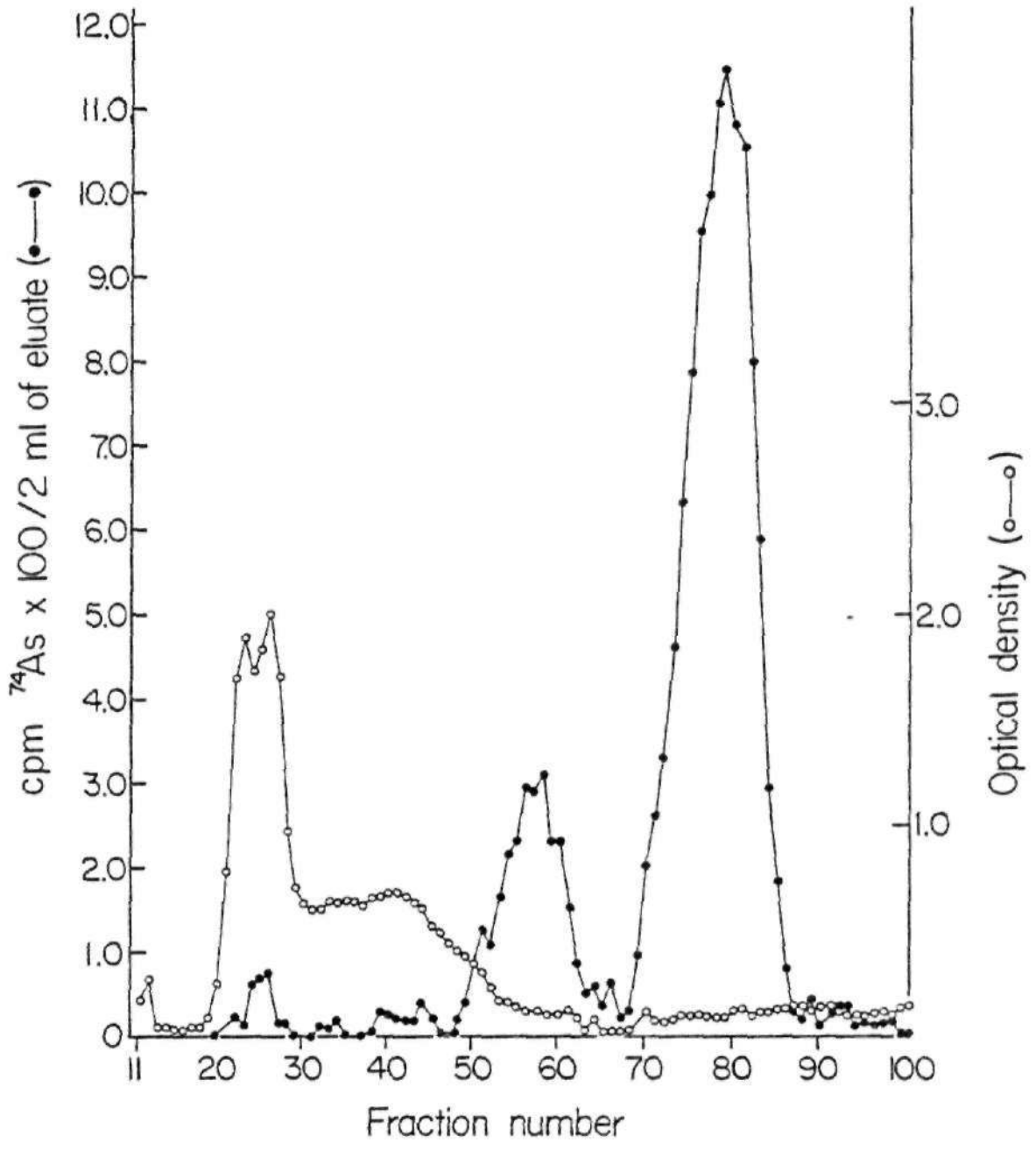
Molecular weight estimates based on the calibration shown in Fig. 12 indicated that the peak I protein-arsenic complex (eluate fractions 40-50) corresponded to molecular size of 19000 daltons; and peak II to molecular size 3,000 daltons containing a bigger proportion (75-85%) of arsenic applied to the column could have been elemental arsenic and arsenic bound to peptides.


(1) Horse heart cytochrome (12,500 daltons)
(2) Bovine pancreas chymotrypsinogen (25,000 daltons)
(3) Hen egg albumin (45,000 daltons)
(4) Bovine serum albumin (67,000 daltons)
74As peak II
74As peak I
(1)
(2)
(3)
(4)
2.5
2.0
1.5
Ve/Vo
3.3
3.5
4.0
4.5
5.5
Log 10 molecular weight


cpm ^{74}As x 100 / 2 ml of eluate (•—•)
Optical density (o—o)
Fraction number
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
1.0
2.0
2.5
3.5
1
10
20
30
40
50
60
70
80
90
100


cpm ^{74}As x 100/2 ml of eluate (•—•)
Optical density (○—○)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
3.0
2.0
1.0
11
20
30
40
50
60
70
80
90
100
Fraction number


cpm ^{74}As x 100 / 2 ml of eluate (•—•)
0
2
4
6
8
10
12
14
16
18
Fraction number
10
20
30
40
50
60
70
80
90
100
1.0
2.0
3.0
Optical density (o—o)


cpm ^{74}As x 100/2 ml of eluate (•—•)
Optical density (o—o)
Fraction number
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
1.0
2.0
3.0
11
20
30
40
50
60
70
80
90
100

DISCUSSION

Uptake of arsenic from water and its subsequent clearance

The data on uptake of arsenic from this study show that the accumulation kinetics of arsenic in rainbow trout can be described adequately by a two-compartment model. Both fast and slow compartments contribute to the total arsenic body burden, the fast making up to 70-83% of the total arsenic uptake with the slow-clearing compartment accounting for the remainder (Table 3). However, the turnover rate of arsenic in the fast compartment is so much faster than in the slow-clearing compartment, that the steady state arsenic content of the fast compartment is small and achieved quickly relative to that of the slow compartment. After a long time of exposure to arsenic in water, the fast-clearing compartment makes up a relatively smaller proportion (<20%) of the body burden of arsenic. For short term exposures, both compartments are important in contributing to the body burden.

The body burden of arsenic in fish was shown to increase with increase in the concentration of water-borne arsenic. Similarly, Sorensen (1976) found arsenic accumulation by green sunfish (Lepomis cyanellus) to be proportional to water arsenate concentration. Mean arsenic accumulation was 33.4, 541.2 and 581.6 ppm arsenic for fish exposed to levels of 100, 500 and 1000 ppm arsenic respectively.

The bioconcentration ratio (calculated at steady state) of 0.3 - 0.7 (Table 7) obtained for rainbow trout in the present study appears low when compared to values of 3-30 reported for fish species from various fresh-waters (Woolson, 1975). However, our values agree with those obtained in model ecosystem studies (Isensee et al. 1973; Schuth and Isensee (1974) in which arsenic as cacodylic acid (dimethylarsinic acid) had low concentration ratios of <1-130 in fish.

The total transfer coefficients of 7-19 x 10^{-3} ml $g^{-1}h^{-1}$ reported in this study for uptake of arsenic from water are low when compared to 2.03 h^{-1} reported by deFreitas and Hart (1975) for methylmercury in fish of similar size. Calculations made from the results of Schuth and Isensee, 1974, yielded a transfer coefficient of 1.6 x 10^{-3} ml g^{-1} h^{-1} from a static ecosystem for catfish (size not indicated) exposed to arsenic compounds for 2 days. The low transfer coefficents corroborate previous reports (Lunde, 1972; Woolson, 1975; Sephar et al. 1980) which indicated that bioaccumulation of arsenic in freshwater fish is generally poor and lower than that of other elements. Fish have been know to accumulate significantly higher amounts of cadmium (Spehar, 1976; Benoit et al. 1976) and lead (Holcombe et al. 1976) than arsenic.

Uptake of arsenic from food and its subsequent clearance

Uptake of arsenic from food as compared to water was more efficient. Tissue distribution data indicated that as high as 36% of the dose was already in the various tissues two hours postdose (Fig. 6). Curve analysis was difficult since the fast compartment corresponding to the voidance of unassimilated arsenic and the slow compartment are not well defined(Fig. 5). The net assimilation of 25% obtained for arsenic by extrapolation of the curve for the slow compartment back to zero time in this study must therefore represent an underestimate resulting from the fast excretion of arsenic. A more realistic assimilation efficiency was calculated by examining the tissue distribution. As has been calculated in the results section, the gross assimilation efficiency can be assigned a minimal value of 50% of the ingested dose, on the basis of the tissue distribution data. The results for whole body clearance (Fig. 5), however, indicate that 25% of the ingested arsenic had been eliminated from the fish by 12 hours postdose. Since voidance time of ingested material in rainbow trout exceeds 12 h, (Windell, 1969) the 25% of the ingested dose cleared by 12h postdose must have been assimilated. The gross assimilation efficiency was therefore approximately 75%.

The assimilation efficiency of 75% for ingested arsenic observed in this study is low when compared to 88% obtained for marine shrimp following uptake of inorganic ^{74}As from food at $13^{o}C$ (Fowler and Unlu, 1978). Arsenic was introduced into Purina Trout Chow in the present study, whereas the shrimps were fed radiolabelled natural live food, Artemia, which probably metabolised the inorganic arsenic to the organic

form. The disparity might have been due to the chemical form of the arsenic ingested as more than 90% of the total arsenic in the shrimp Pandalus borealis was found to be present in the organic form (Lunde, 1973). Moreover, Unlu (1979) indicated that the assimilation efficiency of organic arsenic ingested with the mussel Mytilus galloprovincialis by the crab Carcinus maenas was 65% whereas that for inorganic arsenic injected into the soft tissue was 14%.

The data for the retention of arsenic by the rainbow trout showed that the bulk of the arsenic was rapidly eliminated with less than 2% of the ingested or accumulated dose retained 21 days postdose. The half-life for whole body burden of arsenic was approximately 1 day, while the slow compartment which comprised from 17-25% of the body burden had a half-life in the order of 4.5 - 6 days. Similar rapid excretion of arsenic by mammals has been documented. Mealey et al. (1959) reported that following intravenous injection of ^{74}As in humans, the half-life of its elimination was 7 h. Hollins et al. (1979) indicated that the half-life of elimination of ^{74}As administered orally and intravenously in adult Beagle dog ranged from 2-10 h. This may be an indication that mammals excrete arsenic faster than the rainbow trout.

The retention and elimination of other metals by fish have been studied using radioisotopes. Elimination rates have been shown to vary with the route of exposure, species and a number of environmental factors. Ruohtula and Miettinen (1975) reported half-lives for methylmercury ranging from 202 - 516 days in rainbow

trout, while Jarvenpaa et al, 1970 demonstrated half-life of 1020 days in eels for the same compound. It was also demonstrated that orally administered cadmium (Cd^{109}) was rapidly eliminated by rainbow trout. After 42 days, about 1% of the administered dose was retained in the whole body (Jaakkola et al. 1972). Compared to these metals, it appears that arsenic is eliminated much faster.

The route of administration, whether through the diet or directly from the water via the gills seemed to have only a minor effect on the elimination of arsenic from the slow-clearing compartment. The fractional clearance rates and corresponding biological half-lives for the slow phase in the rainbow trout following uptake from both water and food (Tables 5 and 6) are not significantly different ($P<0.05$). On the contrary, deFreitas et al. (1974) reported that clearance times of mercuric chloride $HgCl_2$ from yellow perch, Perca flavescens, at 15-16^{o}C following uptake from water were longer than those established following uptake from food.

From the result of this study, we estimated a value of 75% as the gross assimilation efficiency of arsenic from the gastrointestinal tract of trout. On the basis of this assimilation efficiency from food and the transfer coefficient for uptake of arsenic from water, the magnitude of the water and food vector in contributing to the body burden of arsenic of fish under long-term exposure to equilibrium conditions can be calculated using values from field data. Table 20 shows the arsenic content in water, invertebrates and fish from freshwater lakes. The locations presented in this table represent three classes of arsenic-polluted bodies of

freshwater; Lake Superior, Lake Michigan, Lakes in the vicinity of Yellowknife (NWT, Canada) contain low, moderately high and very high levels of arsenic respectively (Table 20). For the purpose of this study we shall take Lake Michigan into consideration. Lake Michigan water and plankton were reported to contain 0.50-2.40 (mean 1.60) ppb and 4.10 - 7.90 (mean 6.00) ppm arsenic respectively (Seydel, 1972). At an ingestion rate of 5% body weight per day and an assimilation efficiency from the gastrointestinal tract of 75%, uptake of arsenic via the food vector for a 50 g fish from Lake Michigan will be approximately 11.3 µg/fish/day. A continuous uptake at a constant rate of 11.3 µg/fish/day will equal loss rate (steady state) from the fish when its body burden of arsenic is 94 µg/fish or 1.9 $\mu g/g^{-1}$ based on a fractional clearance of 12% per day obtained in this study (Fig. 5).

At the concentration of arsenic in Lake Michigan water, (2.4 $\mu g\ L^{-1}$) and a mean transfer coefficient (Tc) of 0.25/day (means of the three exposure groups - Table 3), rainbow trout would accumulate 30 ng As/fish/day at 10°C by direct uptake from water. At an estimated loss rate of 12% per day calculated in this study (slow compartment), the rate of uptake from water will equal loss rate of 12% per day when the fish body burden of arsenic is 2.5 µg/fish. Under these conditions the food vector would account for over 90% of the total uptake of arsenic. It seems that the water vector makes only a minor contribution to the body burden of arsenic, therefore subsequent exposure to arsenic in this study were made through the food vector. The

Table 20. Arsenic content of freshwater, invertebrates and fish from North American Lakes.

Sources	Concentrations of arsenic				
	Water µg L^{-1}	Invertebrates µg g^{-1}		Fish µg g^{-1}	References
Lake Michigan	0.5-2.4	4.1-7.9	(zooplankton)	-	Seydel 1972
Lake Superior		4.0	(zooplankton)	-	Seydel 1972
Yellowknife Bay, North West Territory, Canada	61.0	49-80	(snails)	0.3 (northern pike muscle) 0.2 (whitefish muscle) 0.3 (sucker muscle)	Falk et al. 1973
Lakes in NWT, Canada		700-2400	(zooplankton)	14.5±10.0 (Sculpin)	Wagemann et al. 1978
		1-1300	(other inverts)	149.0±53.0 (Sculpin)	

benthic community which accumulates relatively high levels of arsenic since they are closely associated with the sediment (Seydel), 1972; Wagemann et al. (1978) may constitute an important source of arsenic. One can therefore conclude that most of the arsenic in the higher order of predators is most likely transferred from the primary to the secondary consumers. However, arsenic does not appear to biomagnify in the trophic levels, as omnivores (fish) in certain lakes of Yellowknife (North west Territory, Canada) contained in most cases lower levels of arsenic than herbivores in the same lake (Wagemann et al. 1978). Therefore it seems that the tissue levels of arsenic in nature may depend primarily on the longevity of the fish as well as their growth rate rather than the position in the food chain.

Routes of Excretion of Ingested Arsenic

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 10) 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.

Taking into consideration the proportion of arsenic in the posterior gut content and the bile (assuming minimal reabsorption), it appears that about 35% of the ingested dose must have been eliminated in the feces. From the foregoing, it is clear that urinary and fecal excretion account for approximately 50-55% of the arsenic ingested by rainbow trout or 20-30% of assimilated arsenic. Other routes must exist, of which the gill may be important.

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 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 µg O_2 min^{-1}. $g^{-0.8}$ for the first and 8.0 µg O_2 $min^{-1} g^{-0.8}$ for the second set of fish. These values were considered high (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 µg 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.

In summary, the results of the present study show that approximately 15% of the ingested arsenic was excreted via the urine. Fecal and gill excretion might be of equal magnitude, accounting for as much as 80% of the administered arsenic. These values represent 11.3, 15 and 30% of the assimilated arsenic being eliminated via urine, feces and gill respectively during the 7-day period. The high proportion of arsenic excretion through the gill has ecological implications on the interaction effects of multiple toxicants such as copper, and arsenic which is a component of the insecticide, Paris green (copper acetoarsenite) and mining effluents. Some of the effects of copper levels above trace amounts on freshwater vertebrates have been documented (Sprague, 1964; Baker, 1969). Histological examination of gills of winter flounder (Pseudopleuronectes americanus) exposed to 3.2 mg/litre Cu++ (as copper sulphate) for periods ranging from 1-4 days showed extensive cellular damage and fusion of adjacent gill lamellae (Baker, 1969). Although the effects on the gas and ion exchange function of the gill was not determined it is very likely that damage could have affected exchange through the epithelial cells. The presence of other elements such as copper in water containing arsenic may therefore affect arsenic elimination through the gills and its resulting toxicity.

Tissue distribution and whole body clearance ^{74}As in rainbow trout preexposed to arsenic.

The ability of animals to adapt to stress situations is well documented. The response of aquatic organisms to toxicants

may vary with prior exposure to the toxicant or other environmental factors (Affleck, 1952; Goodman, 1951; Paul, 1952; Skidmore, 1964). Several authors have reported adaptive response to toxic metals in terrestrial (Norberg et al. 1971) as well as aquatic species (Sinley et al. 1974 and Beattie and Pascoe, 1978). Mice were reported (Norberg and Lind, 1971) to adapt to sublethal levels of cadmium, while rainbow trout adapted to zinc (Sinley et al. 1974) and cadmium (Beattie and Pascoe, 1978). Eels exposed to sublethal concentrations of mercuric chloride ($HgCl_2$) exhibited increased resistance by surviving subsequent exposure to usually lethal concentrations (Bouquegneau et al. 1975). These data, like the results of the present study, show that adaptation has given these organisms some degree of protection against subsequent exposure.

Some early ingestigators, Schwartze (1922) and Kuhs et al. (1939) reported that rats, dogs and rabbits were unable to adapt to the toxic effects of inorganic arsenic compounds, including sodium arsenite, although Kuhs et al. 1939 demonstrated adaptation of these animals to a number of organic compounds. Kuhs et al. (1939) observed that dogs that received an initial, near lethal dose of carbasone showed toxic symptons. The animals upon recovery, tolerated the same or even greater doses without the symptoms. In spite of the result of the study by Kuhs et al. (1939), Bencko and Symon (1969) demonstrated that preexposure of hairless mice to 50 ppm of arsenic trioxide in drinking water (8 - 10 mg As kg^{-1} per day) for 15 weeks increased the LD50 of a subsequent subcutaneous dose from 10.96 in the control to 13.98 mg kg^{-1} in the pretreated group. An additional

evidence of adaptation was provided by the fact that the growth of the experimental group was slower during the first week of experimentation, but showed compensation in growth thereafter, approaching that for the control at the end of 15 weeks.

Similarly, the results of this study showed that trout that were preexposed to 22,80 mg As kg^{-1} diet for 14 days demonstrated a significantly faster clearance relative to the control (Fig. 7). The lower, though insignificant ($P<0.05$), weight gain and lower blood hemoglobin level observed in the pretreated fish after 2 weeks of exposure and the subsequent recovery at 4 weeks may be regarded as an added evidence of some adaptive mechanism. Until recently, adaptation to arsenic has not been clearly demonstrated for fish. However, Dixon (1979) reported that rainbow trout (Salmo gairdneri) fingerlings preexposed to a sublethal level of 2950 µg L^{-1} As in water (arsenite oxidation state +3) for 21 days demonstrated a 47% increase (relative to the control) in the 144 hour arsenic LC50 of a subsequent arsenic as arsenic trioxide in water. Similar to the results of this study, Dixon further reported that preexposed fish showed a significantly higher increase in body burden of arsenic during the first 7 days of exposure to arsenic trioxide. After 14 days the body burden had already decreased relative to the 7 day level, suggesting that the increase tolerance observed was due to increased excretion of the subsequent dose of arsenic. Our observations agree with Dixon's. inspite of the differences in size of fish, route of administration and the temperature, which was $10^{o}C$ in this study and $15^{o}C$ for

Dixon's. Coincidentally, both studies employed arsenite for preexposure. The similarity in the results confirms that rainbow trouts can and did adapt to arsenic.

The mechanism of adaptation to chemical toxicants is not yet understood . The enhanced elimination rate of arsenic by pretreated fish observed in this study may have been due to increased elimination or reduced assimilation into the gut wall. Although no histopathology of the intestinal wall was carried out, the exposed fish grew as much as the control during the 4 weeks of exposure, suggesting the assimilation of the diet, and hence arsenic, was not impaired. It is therefore suggested that the enhanced elimination must have been brought about through other mechanisms besides decreased absorption. The higher levels of arsenic in the bile, liver and kidney of the preexposed fish suggest these organs must have been connected with the enhanced elimination (Fig. 7).

The tissue distribution data indicated that accumulation of arsenic in liver, bile with gallbladder and kidney was influenced by preexposure, suggesting some adaptation mechanism similar to the formation of metallothionein as has been shown for some group 2B elements (e.g. Cu, Ni, Zn and Cd) of the periodic table (Olafson and Thompson, 1974; Coombs, 1975). The detoxification of these metals have been shown to be by synthesis of metallothionein. One mechanism of metallothionein-mediated tolerance to heavy metals is the synthesis of excess of this protein when fish were preexposed. The excess metallothionein is then used to bind and detoxify metals acquired during subsequent exposure (Brown and Parsons, 1978; Pruell

and Engelhardt, 1979). Since the liver and kidney have been identified as the sites for the synthesis of metallothionein, binding of these metals have been associated with corresponding increase in their concentration in these tissues. For instance, Olson et al. 1977 indicated that the liver of rainbow trout S. gairdneri, that were exposed to mercury contained the major proportion of the Hg-thionein, suggesting the detoxification of mercury by synthesis of metallothionein. Contrary to our initial suggestion, that the arsenic was bound to thionein in the liver and the kidney, further investigation indicated that it was not. The chromatography of the soluble liver fraction showed that none of the peaks corresponded to the molecular weight range of 6,000 - 12,000 daltons as has been reported previously in fish (Olafson and Thompson, 1974, Coombs, 1975) for the group 2B elements. One may therefore conclude that it is possible that arsenic does not induce synthesis of thionein in the liver of rainbow trout. However, it still remains a fact that, similar to previous studies on mammals, the rainbow trout did show some adaptation possibly by fast metabolism of the inorganic into the organic form and subsequent excretion of the metabolite.

The two compartment clearance demonstrated for arsenic in this study has also been shown in the marine shrimp, Lysmata seticaudata (Fowler and Unlu, 1978). On the other hand studies by Mealey et al. (1959) with radioactive arsenic in man identified three compartments, having urinary clearance of 25, 2.5 and 0.3% h^{-1} respectively. Lunde (1973), Woolson et al. (1974) and Penrose (1975)

have shown that arsenic is present in fish as both water-soluble and lipid-soluble arseno-organic compounds. Unlu (1979) demonstrated that the flux of arsenic ingested with food by the crab, Carcinus maenas depended to a high degree on the chemical form. The inorganic form was cleared fast, while the organic was eliminated at a much slower rate. Similarly, the elimination kinetics of arsenic observed in the rainbow trout during this study may be related to the turnover of the chemical forms of arsenic. One major question about arsenic accumulation that is yet unanswered is the chemical nature of the metabolites after it is incorporated into the different tissues. This question is addressed in the next section of this study.

Chapter 2

Metabolism of inorganic arsenic to organoarsenicals in rainbow trout, *Salmo gairdneri*

INTRODUCTION

The toxicity of arsenicals has been shown to vary with chemical form (Penrose, 1974). As early as 1945, Challenger reported biological methylation of arsenic by fungi, McBride and Wolfe (1971) later found that laboratory bacterial cultures methylated inorganic arsenic to dimethylarsine. Benthic organisms, algae, zooplankton and phytoplankton have been reported to convert inorganic arsenic accumulated from the environment to organic arsenic compounds (Lunde, 1969, 1973). Fish may acquire their body burden of organoarsenical compounds by consuming the algae and plankton as well as by synthesizing them in their own tissues. Lunde (1969) suggested the existence of organoarsenic compounds in fish based on the ion exchange behavior of extracted arsenic compounds. Rapid transformation of inorganic arsenic to organic arsenic has been reported for freshwater and marine fish (Penrose 1975, Penrose et al. 1977). Recently, Edmonds et al. (1977) identified an organic form of arsenic in Australian rock lobster (Panulirus longipes sygnus) as arsenobetaine and Cooney et al. (1978) has shown that O-phosphatidyl trimethylarsoniumlactic acid, found in marine algae can be chemically degraded to arsenobetaine.

Ingestion of fish containing high levels of arsenic represents a potentially important vector for various forms of arsenic to predators including man. Interest in determining the levels of the different compounds of arsenic formed in fish arises from the recognition of their potential toxicity and possible carcinogenicity (Fowler, 1977). It is obvious that measurements

of total arsenic does not provide adequate information on toxicity of an ingested dose, since the chemical specific dose response relation will depend on the acquired body burden of arsenic. To estimate body burden, pharmacokinetics information is required on assimilation efficiency and retention or whole body clearance of each form of arsenic present in the ingested dose. The rate of elimination of arsenic in animals is influenced by the physical and chemical nature of the compound as well as species specific factors. Several studies have demonstrated differences in retention of ingested inorganic and organic arsenic. Coulson et al. (1935) noted that arsenic in marine crustaceans (presumably organic) was rapidly and completely eliminated by human consumers, whereas inorganic arsenic consumed by the same subjects was retained indefinitely. However, excretion of inorganic arsenic ingested as arsenite and arsenate by human was reported to be faster than that for organic arsenicals ingested with crab (Crecelius, 1977). Similarly, Charbonneau et al. 1978 demonstrated that inorganic arsenic ingested by monkeys as arsenic trioxide (As +3) was excreted in urine at a faster rate than the organic arsenicals ingested with fish. It seems therefore that any meaningful assessment of the environmental hazard of arsenic depends on the chemical characterization and toxicity of arsenic metabolites.

The present study was undertaken to examine the change in tissue distribution, chemical speciation and urinary excretion of an oral dose of pentavalent inorganic arsenic (^{74}As) in rainbow trout. The chemical speciation of arsenic was examined by cation exchange column chromatography of tissue extracts and thin layer chromatography of the eluates from the columns.

MATERIALS AND METHODS

Dosing of the fish with arsenic

After three weeks of acclimation, the fish were anaesthetized with MS222 and weighed (160-211g). Each fish was dosed by being forcefed a No 4 gelatin capsule containing 20 µCi ^{74}As (1 mCi/µg As in the form of arsenic acid, Amersham Searle, Arlington Hts, Ill.) added to approximately 500 mg pulverized trout chow.

Whole body clearance of arsenic

At 0.5, 6, 12, 24, 96 h postdose, each fish was rinsed for 10 minutes in uncontaminated water and put in a polyethylene bag. Radioactivity in live fish was measured with the single channel gamma counter equiped with a 3 x 3" sodium iodide detector crystal described earlier.

Tissue collection and treatment

Three or four fish were killed with MS222 at 6, 12, 24 and 96 h postdose and tissue samples collected for analysis. Blood was collected from the caudal vein in heparinized syringe. A portion was transferred to capillary hematocrit tube for hematocrit determination. The remaining blood was put in a centrifuge tube weighed, assayed for ^{74}As radioactivity and centrifuged at 3000 g for 30 minutes to separate plasma from erythrocytes. Weighed portions of the plasma and erythrocytes were also assayed for radioactivity.

The gall bladder was ligatured prior to removal and bile

was collected by puncturing the bladder. The gall bladder tissue was washed with distilled water, and the washings added to the bile which was then made up to 1 ml with distilled water. The total 1 ml was radioassayed and retained for chromatography.

Muscle filets from the lateral sides between the dorsal fin and lateral line were removed. One to two grams sub-samples of muscle were counted for radioactivity and homogenized twice at room temperature in 4 volumes of 0.15 M ammonium acetate. The homogenate was centrifuged at 27,000 g for 30 minutes in a Sorval centrifuge. The supernatant was concentrated to 1.0 ml by air evaporation at room temperature and ambient pressure. Losses from evaporation were insignificant (<2.5%). It was then centrifuged to remove excess ammonium acetate and the supernatant retained for chromatography. This supernatant contained about 78-82% of the total arsenic in the muscle.

The above method of extraction was not used for the liver and kidney since a preliminary study carried out using ammonium acetate resulted in a lower recovery. Therefore in the procedure used for liver or kidney, the tissue was assayed for radioactivity freeze-dried and extracted twice for 24 h each by Soxhlet extraction using methanol. Approximately 80% of the tissue arsenic was recovered in the methanol extract. The extract was evaporated to dryness at room temperature and ambient pressure, dissolved in 1 ml distilled water, centrifuged and the supernatant containing 65-70% of the total arsenic in the tissue retained for chromatography.

Urine collection was carried out by insertion of a

polyethylene tube (PE90) through the urinary papilla of lightly anaesthetized fish as previously described in Chapter 1. Urine samples were evaporated to 1.0 ml at room temperature and ambient pressure, assayed for total radioactivity, and retained for chromatography.

Ion exchange chromatography

Tissue extracts prepared as described above were fractionated by the method of Tam et al. (1978). One ml of each of the aqueous extracts was added to the column (0.85 x 16.5 cm) containing 10 ml of cation exchange resin AG 50W-X8 100-200 mesh (Bio-Rad Laboratories). The column was eluted successively with (1) 10 ml 0.5 N HCl (2) 15 ml H_2O, (3) 15 ml 5% (v/v) NH_4OH and (4) 15 ml 20% (v/v) NH_4OH solution. The ^{74}As content of 2 ml samples for each eluted portion was used to estimate the total arsenic in the various portions. The successive eluates from the column are designated fractions I, II, III and IV corresponding to fractions containing inorganic arsenic, monomethylated arsenic, unidentified arsenic #1 and unidentified organic arsenic #2 respectively. Dimethylarsinic acid was collected in the #IV fraction by Tam et al. (1978).

Recovery of arsenic compounds

Arsenic standard solutions were made by dissolving analytical reagent grade sodium arsenite, sodium arsenate, sodium methylarsenate, sodium methylarsonate and dimethylarsinic acid in deionised water. Each solution contained 10 mg of compound/ml. A mixture of the above standards was prepared by mixing 10.0 ml of

each of the above arsenic standard solutions. The final solution contained 2 mg of each arsenic compound/ml. One ml of the mixture of standard solution was applied to the top of the column and eluted as previously described. The eluates were collected in 2 ml fractions and submitted to x-ray counting as described by Tam et al. 1978. The recovery of these compounds ranged from 88-96%.

Thin layer chromatography (TLC)

Only eluate fractions I and IV from the cation exchange columns were examined by TLC as radioassays of the other two eluting fractions indicated they accounted for only a small proportion of the total arsenic applied to the column. These two eluates were neutralized with 0.5 N HCl, evaporated to dryness, dissolved in 1 ml methanol and centrifuged. The supernatant was radioassayed and spotted on TLC plates (20 x 20 cellulose sheets- Kodak). The solvent system MeOH: 1N NH_4OH (80:20) was allowed to travel 17 cm from origin. Arsenic-containing compounds were located by spraying with 1N $AgNO_3$ in 5% (v/v) NH_4OH solution or iodine vapour. The distribution of ^{74}As on the chromatogram was detected by radioassays of 1 cm wide strips of the cellulose sheets.

RESULTS

Arsenic was detected in the blood first sampled at 6 h postdose. At 96 h the total arsenic radioactivity of the blood was about 40% of its 24 h value (Fig. 1). The plasma to erythrocyte arsenic concentration ratio decreased from a value of 2.2 at 6 h to 1.2 at 12 h. The decreasing value of the ratio coincided with an increasing proportion of organic arsenic (Table 1). At 24 and 96 h the ratio were quite similar to the 12 h value of 1.2 as illustrated in Fig. 1. At 6 h postdose, about 50% of the arsenic present in the erythrocytes and plasma was inorganic decreasing to between 8 and 5% respectively by 96 h with a corresponding increase in the organic fraction. Arsenic (^{74}As) radioactivity in both plasma and erythrocytes was present mainly as inorganic arsenic at 6 h and primarily as the unidentified organic compound #2 by 96 h postdose (Table 1).

The ratio of total organic to inorganic arsenic increased with time in muscle, gill, kidney and bile with the organic arsenic fraction accounting for 30-64% at 6 h and over 86 after 24 h (Table 1). However, considerably higher concentration of organic arsenic (~75%) was detected in liver extract at 6 h. At 96 h postdose the organic arsenic fraction #IV accounted for a similar proportion of the total arsenic content of all tissues. This organic arsenic fraction #IV was shown by TLC to contain a major component with the same Rf as ethyl arsenobetaine (Table 2). Furthermore, only a small proportion of ^{74}As was recovered in the #II and #III fraction from the ion exchange column. No further attempt was made to characterize the compounds.

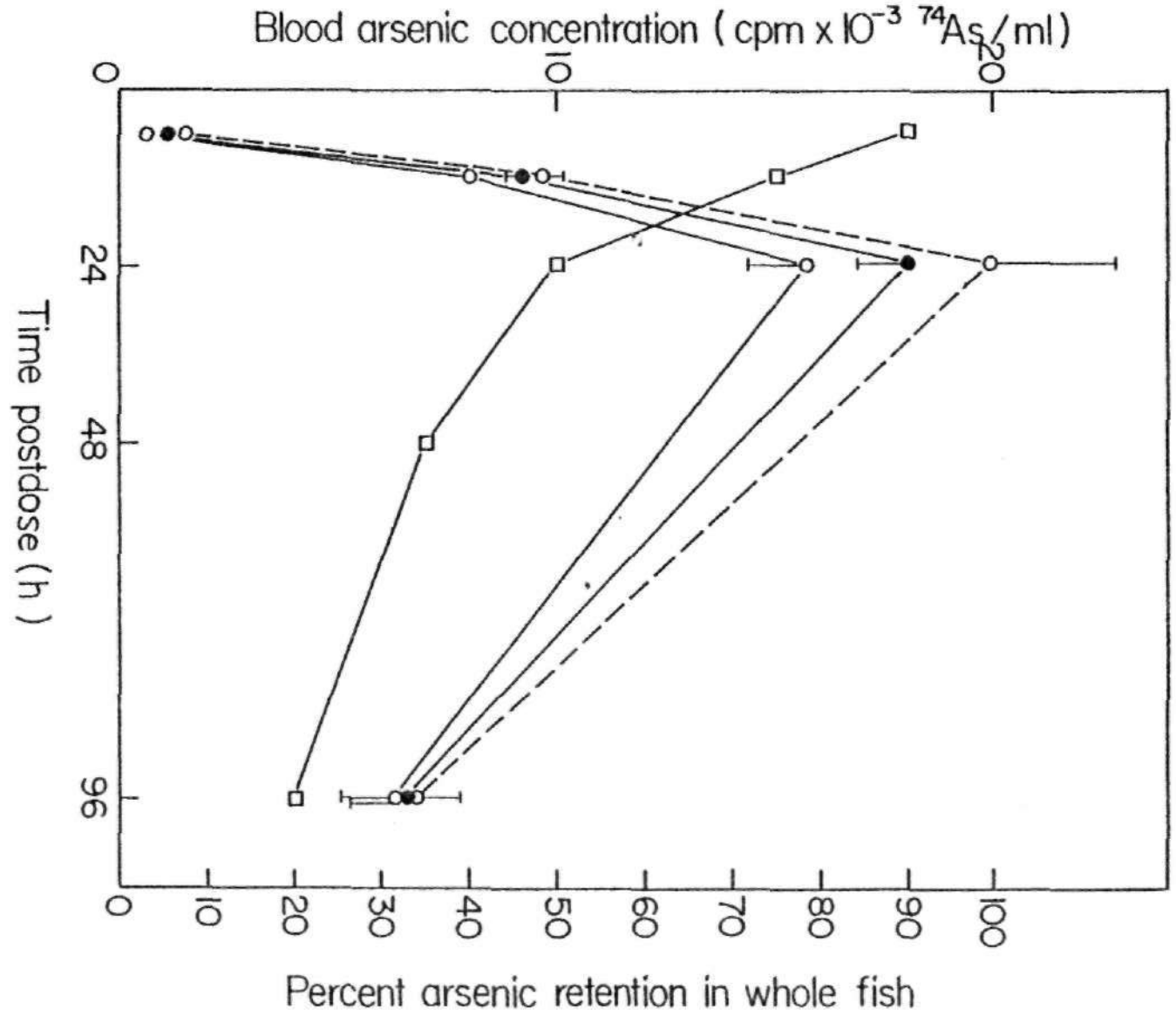

Blood arsenic concentration (cpm x 10^{-3} ^{74}As / ml)
0
10
20
Time postdose (h)
24
48
96
0
10
20
30
40
50
60
70
80
90
100
Percent arsenic retention in whole fish

Table 1. Percent[a] of total radioactivity present as various forms of arsenic in tissues of rainbow trout, Salmo gairdneri, following an oral dose of 20 µCi ^{74}As (arsenic acid) in 500 mg of diet.

Eluate Fractions	I	II	III	IV	I	II	III	IV
Time after dosing (h)	Inorganic arsenic (arsenate + arsenite)	Mono-methylated arsenic	Unidentified arsenic #1	Unidentified organic arsenic #2	Inorganic arsenic (arsenate + arsenite)	Mono-methylated arsenic	Unidentified arsenic #1	Unidentified organic arsenic #2
	ERYTHROCYTES				PLASMA			
6	44.8±5.7	10.7±1.6	8.3±1.8	36.3±9.2	58.4±9.3	1.2±0.7	1.6±0.8	38.9±10.2
12	29.9±9.2	2.6±1.4	1.3±0.9	66.1±9.2	46.9±7.3	1.1±0.3	0.3±0.3	51.7± 7.6
24	14.8±1.7	2.8±1.1	0.0±0.0	82.4±2.6	26.2±4.3	0.0±0.0	0.0±0.0	73.8± 4.3
96	7.8±3.1	2.9±1.7	0.0±0.0	89.3±1.7	5.3±1.0	2.5±2.2	0.0±0.0	92.3± 2.1
	LIVER				BILE			
6	17.2±7.5	7.2±5.2	1.1±1.1	74.5±8.3	25.4±8.3	5.4±0.8	6.4±3.5	63.3± 6.9
12	4.5±1.3	5.3±2.0	0.6±0.6	89.6±2.1	9.1±1.1	2.9±0.6	9.7±1.8	78.4± 1.9
24	13.2±4.5	7.4±3.0	3.7±2.7	74.6±1.9	3.7±1.5	0.8±0.4	2.3±1.0	93.4± 2.2
96	3.1±0.4	5.2±1.1	0.0±0.0	91.7±1.5	1.3±0.8	6.4±2.9	7.8±1.9	84.5± 5.5

Table 1 (Cont'd)

Eluate Fractions	I	II	III	IV	I	II	III	IV
Time after dosing (h)	Inorganic arsenic (arsenate + arsenite)	Mono-methylated arsenic	Unidentified arsenic #1	Unidentified organic arsenic #2	Inorganic arsenic (arsenate + arsenite)	Mono-methylated arsenic	Unidentified arsenic #2	Unidentified organic arsenic #2
	KIDNEY				MUSCLE			
6	30.1±3.5	7.7±3.5	5.0±2.9	57.3±7.1	49.1±8.2	16.9±3.4	0.0±0.0	34.0±5.4
12	29.7±4.0	11.8±4.0	5.6±3.5	52.9±3.2	19.9±7.3	5.2±1.8	2.9±2.9	71.9±5.6
24	6.9±1.9	15.2±2.4	0.0±0.0	75.7±2.0	6.8±0.8	1.0±0.2	0.0±0.0	92.2±0.8
96	6.1±3.1	6.9±2.6	2.9±1.4	84.0±6.7	0.0±0.0	0.0±0.0	0.0±0.0	98.5±1.5
	GILL							
6	69.0±7.7	20.1±3.5	1.1±0.7	9.4±4.2				
12[b]	61.1	23.8	0.9	14.2				
24	13.4±2.1	4.3±2.0	1.0±1.0	81.4±4.6				
96	3.8±1.4	1.5±1.5	0.0±0.0	96.0±2.2				

[a] Values represent means ± S.E. for 4 fish except at 6 h when 3 were used.

[b] Means of two fish.

Table 2. TLC[a] of arsenic compounds

Arsenic Compounds	Rf	
Sodium arsenate	0.24	
Sodium arsenite	0.59	
Sodium methyl arsonate	0.36	
Sodium methyl arsenate	0.36	
Dimethylarsinic acid	0.66	
Arsenobetaine	0.74	
Ethyl arsenobetaine	0.82	
Tetramethylarsonium iodide	0.86	
Kidney (12h)		
1st fraction from ion exchange	0.24	0.59
4th fraction from ion exchange	0.82	
Urine (72-80h)		
1st fraction from ion exchange	0.24	0.59
4th fraction from ion exchange	0.82	
Liver (24h)		
1st fraction from ion exchange	0.24	0.59
4th fraction from ion exchange	0.82	

[a]Chromatographed ascendingly on cellulose thin layer sheets.

Solvent system MEOH: 1N NH_4OH 80:20 (v/v), solvent front 17.0 cm.

Table 3. Urinary excretion of total arsenic and the metabolites in rainbow trout, Salmo gairdneri, following an oral dose of 5 µCi As (30ng arsenic[a]) in 500 mg of diet.

Eluate fractions				I	II	III	IV
Collection period	Urine Volume	Total ^{74}As in urine	Cumulative excretion	Inorganic arsenic (arsenate + arsenite)	Monomethylated arsenic	Unidentified arsenic #1	Unidentified organic arsenic #2
h	ml	dpm	% of dose	%	%	%	%
0 - 12							
Fish A	7.3	2222	0.02	71.0	8.2	6.0	14.7
Fish B	4.7	1705	0.02	75.8	4.0	7.0	13.1
45 - 52							
Fish A	9.4	86838	0.81	12.0	2.0	0.1	85.9
Fish B	10.2	127876	1.2	10.4	1.4	0.3	87.9
72 - 80							
Fish A	8.0	355000	4.0	14.8	2.9	0.2	82.1
Fish B	10.1	433894	5.1	9.3	4.5	0.1	86.1
96 - 111							
Fish A	10.0	124404	5.2	1.9	1.8	0.2	96.5
Fish B	10.3	82236	5.8	1.5	1.0	0.0	97.6
119 - 130							
Fish A	10.0	223456	7.2	1.3	0.6	0.1	98.0
Fish B	9.0	153538	7.2	0.0	0.5	0.0	99.5
216 - 224							
Fish A	6.4	88816	8.0	0.8	0.8	0.6	97.8
Fish B	9.0	98000	8.1	0.9	1.2	0.4	97.5
288 - 296							
Fish A	10.2	50776	8.5	4.2	1.7	0.6	94.1
Fish B	4.0	63068	8.7	0.0	0.1	0.6	99.3

[a] specific activity = 0.1 mCi per 0.6 µg As

Fractionation of arsenic in urine on the ion exchange column showed that more than 70% of the arsenic excreted 12 h postdose was found in fraction #I, and hence was inorganic, the rest was in fractions II, III & IV and therefore considered organic (Table 3). However, by 96 h essentially all (>90%) of the urinary arsenic was organic arsenic collected in column eluate fraction IV. Thin layer chromatographic separation of this fraction #IV in urine, liver and kidney extracts (Table 2) showed that a majority (60% or more) of the arsenic was located with an Rf of 0.82 and no significant amount was detected with an Rf of 0.66.

DISCUSSION

The plasma/erythrocyte arsenic ratio of approximately 1:1 observed in this study was similar to the plasma/erythrocyte ratio of arsenic observed in the dog (Charbonneau et al. 1978) and in man (Mealey et al. 1959). Our data indicate that arsenic is not bound exclusively to the red blood cells of rainbow trout as has been reported in the rat. Hunter et al. (1942) showed with albino rats that 97% of the arsenic in blood was in erythrocytes 72 h postdose, but there was no information on its chemical form. At this time postdose most of the arsenic in rat blood would have been dimethylarsinic acid since Charbonneau et al. (1979) have demonstrated this to be the case with dogs.

The results of the present study show that in vivo transformation of an oral dose of inorganic arsenic to organic forms occurs in rainbow trout but not as fast as reported for the dog (Charbonneau et al. 1979). However, similar to the results of Charbonneau et al. (1979) with dogs, and Braman and Foreback (1973), with monkeys monomethylated arsenic present in the tissues represented a relatively small proportion of the organic arsenic metabolites. The relatively small amount and transient nature of monomethylated arsenic in the liver and other tissues does not preclude this compound from being an intermediate in the synthesis of the major metabolites of arsenic. In mammals, dimethylarsinic acid was the major metabolite (Crecelius, 1977; Charbonneau et al. 1979), but this does not seem to be the case in trout and other aquatic species (Penrose, 1975).

Studies in man (Crecelius, 1977) and dog (Charbonneau et al. 1979) have shown that orally ingested inorganic arsenic was excreted mainly as dimethylarsinic acid and to a lesser extent as monomethylated arsenic compounds. Following oral ingestion of inorganic by dogs, it was rapidly methylated to dimethylarsinic acid which was detected in plasma, erythrocytes and urine within 50 minutes after ingestion (Charbonneau et al. 1979). The authors further indicated that only an insignificant amount of monomethylated compound was detected in either blood or urine. Results from the present study with fish, unlike mammals, demonstrate that the major metabolite in trout was not dimethylarsinic acid, since its Rf value (Thin layer Chromatography) coincided with that for ethylarsenobetaine and not dimethylarsinic acid.

The large difference in the Rf values of ethyl arsenobetaine (0.82) and dimethylarsinic acid (0.66) clearly separates these two compounds, and on this basis we concluded that most of the arsenic in fish urine was not dimethylarsinic acid. The coincidence in Rf value between ethyl arsenobetaine and the arsenic in fish urine and in all other tissues examined suggests that the major organoarsenic compound in fish may have a structure related to arsenobetaine.

The site and mechanism of arsenic methylation is not yet defined. Transformation of an oral dose of inorganic arsenic by the brown trout was shown to be faster than that for arsenic administered intramuscularly (Penrose, 1975). He suggested that methylation may occur in the gut prior to absorption into the blood. Charbonneau

et al. 1979 noted that an intravenous injection of ^{74}As as arsenic acid to dogs appeared in the erythrocytes as dimethylarsinic acid as early as 10 minutes after dosing. The authors suggested that methylation might have taken place in the erythrocytes as well as in the liver, but this may not necessarily follow.

The results of the present study have shown that the liver ontained higher concentrations of arsenobetaine much earlier than other tissues. These findings suggest that the liver may be the major site for the conversion of inorganic arsenic in fish. However, the intestinal epithelium might be an important site for arsenic metabolism, possibly leading to monomethylated arsenic production, with the major synthesis of arsenobetaine being mediated by the liver.

The mechanism of arsenic methylation is not understood and as such requires further investigation. The elimination kinetics of arsenic is known to be influenced by the physical and chemical form of the arsenic. Coulson et al.(1935) studying arsenic elimination in rats reported that inorganic arsenic ingested as arsenious trioxide was excreted at a slower rate than arsenic ingested with shrimp. More than 98% of the arsenic fed with shrimp was excreted within 4 days of feeding, whereas only 21% of the arsenious trioxide fed was excreted. When humans ate shrimp containing arsenic, all of the arsenic was excreted in 4 days. Similarly, Crecelius (1977) reported biological half-lives of elimination of 10 and 30 h for inorganic arsenic ingested with wine and organic arsenic ingested by human with crab respectively. On the contrary, Charbonneau et al. (1978) noted that inorganic

arsenic ingested by monkeys as arsenic trioxide was excreted in the urine at a faster rate than organic arsenicals ingested with fish. One day after ingestion of fish containing "organic" arsenic, mean total excretion by each of the four monkeys was 40% of the ingested dose. Excretion of ingested inorganic arsenic given to the same monkeys after the fish arsenic was allowed to return to baseline level, was 60% of the ingested dose for the same period. The variation between the results of Coulson et al. (1935) and Charbonneau et al. (1978) could have been due to the difference in chemical form of arsenic in fish and shrimp. However, the clearance data from this study and the results of other studies (Schwartze 1922; Kuhs et al. (1939); Dixon (1979)) have indicated that animals are able to adapt to arsenic. The enhanced excretion of the inorganic arsenic observed by Charbonneau et al. (1978) could have been partially due to a gradual induction of the metabolic processes required to detoxify and excrete arsenic as well as to a natural capacity to eliminate the inorganic arsenic more rapidly.

In conclusion, the result of this study demonstrate that arsenic is metabolized very rapidly by the rainbow trout. It appears the elimination kinetics of arsenic observed in this fish is a function of the turnover of the metabolites, with the fast and slow-clearing compartments representing two independent pools of arsenic compounds.

Chapter 3

Long-term effects of arsenic accumulation in rainbow trout, *Salmo gairdneri*

INTRODUCTION

The toxicity of arsenic and its compounds to aquatic organisms is well documented. Acute exposure has been shown to result in reduced growth or increased mortality in aquatic organisms. Acute toxicity levels have been established for several species of fish. Minnows exposed to 20 ppm As (sodium arsenite -$NaAsO_2$) and 250 ppm arsenic, as sodium arsenate in water survived for a maximum of 36 and 16 hours respectively (Grindley, 1946). Gilderhus (1966) obtained a 96 h LC50 value for sodium arsenite at 12°C as 25.6 ppm for rainbow trout, Salmo gairdneri, 34 ppm for bluegills (Lepomis macrochirus). A number of studies have been carried out on the effects on fish of long-term exposure to sublethal concentrations of various forms of arsenical herbicides. The "safe" concentrations recommended for one of the earliest common aquatic herbicide, sodium arsenite, varied from 2-10 ppm (Surber, 1931; Mackenthum, 1955). It was reported that these levels were not toxic in fish.

The above-mentioned studies indicate that the rainbow trout (Gilderhus, 1966; Hale, 1977) and other fish species (Sorensen, 1976) had very high 96 h LC50 values, suggesting that fish are relatively tolerant of arsenic. However, very little has been done on the long-term effects or toxicity of arsenic ingested with the diet in spite of many reports (Woolson, 1975) that indicated a contamination of aquatic food chain by arsenic.

Among the few studies on the long-term effects of arsenic are those of Lawrence (1958) and Gilderhus (1966) who

reported that 4 ppm sodium arsenite in water reduced the survival and growth of bluegills, Lepomis macrochirus. Speyer (1975) and Speyer and Leduc (1975) noted a decrease in body and fat weight in yearling rainbow trout exposed to different concentrations of arsenic trioxide in water for 21 days. Sublethal concentration of arsenic as low as 0.1 ppm in water resulted in the alternation of conditioned response in goldfish, Carassius auratus (Weir and Hine, 1970). These authors reported that exposure to 0.1 ppm As in water for 48 hours caused up to 48% impairment in the avoidance reaction of goldfish to light and electric shock.

The primary purpose of this study was to empirically determine the validity of the kinetic parameter values established for arsenic for the short-term studies reported in Chapter 1. Growth and other physiological parameters such as hematology and histopathology were examined with a view to correlating them to observed tissue levels of arsenic

MATERIALS AND METHODS

Preparation of the diet

The diet used for this study was Purina Trout Chow. Sodium arsenite ($NaAsO_2$) solution was added dropwise to pellets of the chow from an automatic pipette as described in the materials and methods for chapter one. The diets consisted of the control which contained no arsenic, and three others containing 10,20 and 30 mg As kg^{-1} diet. These levels are equivalent to a daily dose of 0.2,0.4 and 0.6 mg As kg^{-1} fish wet weight respectively at a feeding rate of 2% of fish body weight per day.

Experimental procedure

All test fish were fed the control diet and acclimated to experimental conditons for at least two weeks prior to the start of the experiment. A day prior to the start of the experiment, 5 fish were randomly selected and sacrificed for establishing baseline blood hemoglobin, hematocrit and tissue arsenic residue levels. At the beginning of the experiment, 96 rainbow trout of 29.2 ± 0.1 g wet weight were selected and randomly alloted to 4 groups of 24 in 120-litre aquaria. One group served as the control and the other three were to be exposed to 10,20 and 30 mgAs kg^{-1} of diet. A flow rate of approximately 12 litres h^{-1} was maintained in each aquarium. Following the allocation to the test groups, each fish was anaesthetized in MS222 and tagged as previously described, blotted dry and weighed. Subsequent weighings were carried out at 2-week intervals and the fish ration adjusted accordingly.

After 2,4 and 6 weeks of exposure, 4 fish were sacrificed (all the fish in each aquarium having been weighed) from each of the four test groups for blood hemoglobin (Hb) hematocrit (PCV) and tissue arsenic residue. Blood hemoglobin (cyanmethemoglobin method) and hematocrit were determined separately for each fish, using standard techniques (Blaxhall, 1972). Arsenic residue analysis was also carried out separately on muscle from each fish, and on pooled samples of gill, skin and liver.

At the end of the experiment, all surviving fish in each test group were weighed. Blood hemoglobin content was measured on 5 fish from the surviving fish in each test group. All the remaining fish were frozen pending tissue arsenic residue analysis.

Determination of residual arsenic

The four fish that were sacrificed from each test group after 2,4,6, and 8 weeks of exposure were thawed out for analysis. Arsenic analysis was carried out on freeze-dried muscle. An 8 to 10-gram muscle fillet was weighed out from each fish, homogenized with its own weight of water, and freeze-dried to constant weight. Liver, gill and skin were freeze-dried and later ground up using a mortar and pestle. After drying, samples were stored in dessicators ($CaCl_2$) until used. Arsenic residue analysis was carried out by Professor J.G. Dick, Dept. of Chemistry, Concordia University, Montreal. The method used was the flame atomic absorption technique involving the generation of arsine and its injection by argon gas entrainment into a hydrogen-air flame (Dick et al. 1976). Arsine was generated from wet-ashed samples

by treatment with hydrochloric acid and sodium borohydride. This technique overcomes the arsenic absorbance inhibition known to result from phosphate interference when the carbon or graphite furnace methods were used (Dick et al. 1976). The general conditions maintained during the various analytical procedures were as outlined by Dick et al. (1976).

RESULTS

Effect of arsenic on growth

The initial mean wet weight of the test groups were 29.19 ± 1.13 for the control and 29.58 ± 1.03, 29.07 ± 1.03 and 29.22 ± 1.04 for the 10, 20 and 30 mg As kg^{-1} diet groups respectively. Growth was calculated from the sum of the individual wet weight of the remaining fish at 2, 4, 6 and 8 weeks and expressed as percent wet weight gain. The results show that the percent wet weight gains in the control group increased from 21.6% at 2 weeks to 79.3% at 8 weeks (Fig. 1). The growth of fish that were exposed to 10 and 20 mg As kg^{-1} diet were not different from those for the control. However, the weight gains of fish exposed to 30 mg As kg^{-1} diet were significantly less than those for the control throughout the duration of exposure ($P < 0.05$).

Effects of arsenic on blood hemoglobin levels

Hemoglobin and hematocrit for the control group did not deviate much from the baseline values throughout the test period (Table 1). Blood of fish exposed to 10 mg As kg^{-1} diet exhibited no loss in hemoglobin content up until 8 weeks of exposure when the level dropped to a significantly low level of 9.30 g/100 ml blood ($P < 0.05$). Similarly, the 20 and 30 mg As kg^{-1} diet exposure group showed slight deviations from the baseline level up until the sixth week. After 8 weeks of exposure, the hemoglobin levels showed a significant ($P < 0.05$) reduction of 20.5% for the 20 mg As kg^{-1} diet group and 29% for the 30 mg group.

Fish exposed to 10 mg As kg^{-1} diet showed a decrease in

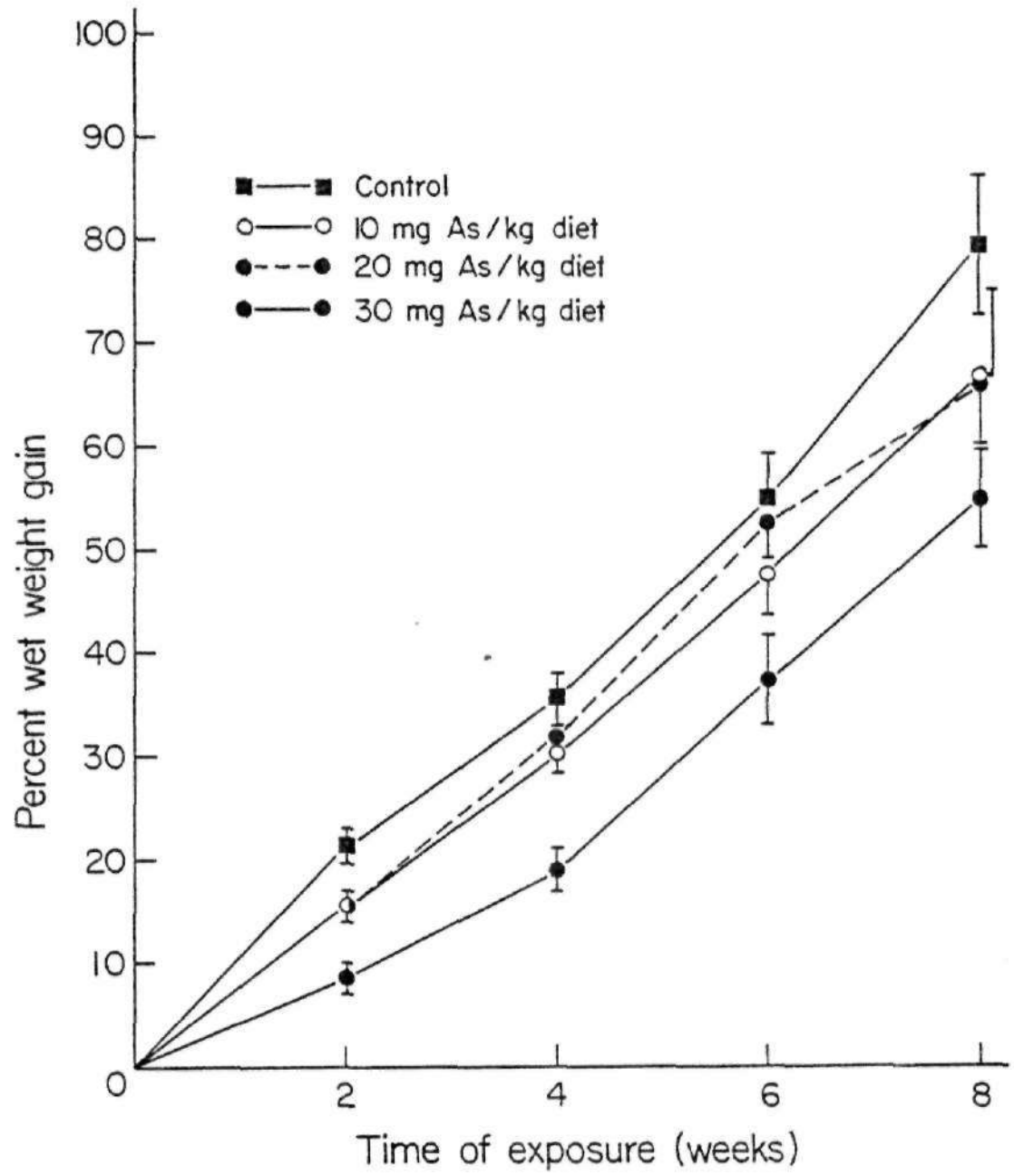

Control
10 mg As/kg diet
20 mg As/kg diet
30 mg As/kg diet
Percent wet weight gain
0
10
20
30
40
50
60
70
80
90
100
2
4
6
8
Time of exposure (weeks)

Table 1. Mean hemoglobin (g/100 ml blood), hematocrit and mean corpuscular hemoglobin concentration (MCHC) of rainbow trout exposed to arsenic for 2 to 8 weeks at 10 ± 1°C.

Dose of Arsenic mg Kg^{-1} diet	Weeks of Exposure 2	4	6	8
		Hemoglobin (Hb)[a]		
0	10.55 ± 0.22	10.92 ± 0.23	10.35 ± 0.98	10.75 ± 0.22
10	11.72 ± 0.64	10.32 ± 0.39	9.93 ± 0.84	9.30* ± 0.62
20	11.20 ± 0.43	11.08 ± 0.70	10.71 ± 0.15	8.43* ± 0.23
30	10.73 ± 0.45	10.66 ± 0.56	9.83 ± 1.00	7.56* ± 0.53
		Hematocrit (Hct)		
0	37.68 ± 1.22	35.40 ± 0.51	33.60 ± 1.21	37.60 ± 1.63
10	37.25 ± 2.01	34.60 ± 1.32	31.40* ± 2.43	38.40 ± 1.57
20	36.00 ± 1.78	35.00 ± 1.05	32.75* ± 0.48	35.25 ± 1.55
30	32.25 ± 2.10	31.80* ± 1.49	32.80* ± 1.65	33.50* ± 1.26
		MCHC[b]-Hb/Hct x 100		
0	28.00 ± 1.00	31.00 ± 1.00	31.00 ± 2.00	29.00 ± 1.00
10	31.00 ± 1.00	30.00 ± 2.00	32.00 ± 2.00	24.00* ± 1.00
20	32.00 ± 1.00	35.00 ± 3.00	33.00 ± 1.00	25.00* ± 1.00
30	34.00 ± 2.00	34.00 ± 2.00	30.00 ± 2.00	22.00* ± 2.00

[a] Baseline values determined before fish were alloted to the exposure groups were 10.60 ± 0.44, 36.45 ± 0.81 and 29.00 for hemoglobin, hematocrit and MCHC respectively.

[b] MCHC = Mean Corpuscular Hemoglobin concentration.

* Values significantly different from the baseline levels at $P < 0.05$ - Student-t test.

hematocrit after 4 and 6 weeks of exposure, followed by an increase at 8 weeks. The 30 mg As kg^{-1} diet group had consistently lower hematocrit.

The baseline mean corpuscular hemoglobin concentration (MCHC) was 29%. The control group did not show any significant difference from the baseline mean throughout the duration of the experiment. However, the MCHC was significantly reduced in all treated groups after 8 weeks of exposure to arsenic. The values were 25%, 25 and 22 for 10, 20 and 30 mg As kg^{-1} diet groups respectively.

Arsenic residue in tissues

In general, the levels of arsenic accumulated in tissues were directly related to levels of arsenic to which the fish were exposed (Table 2). Among the tissues analysed, the liver accumulated the highest concentration of arsenic during the 8 weeks of exposure. The trend is not quite clear as to the relationship between the amount of arsenic accumulated and duration of exposure, since the liver for each of the test groups attained its highest concentration of arsenic at different times.

The skin had the next highest level among the tissues examined. The concentrations of arsenic in the skin of the 10 mg kg^{-1} diet group reached 1.05 µg g^{-1} after two weeks of exposure and showed only an insignificant ($P < 0.05$) deviation from this value at other time periods. The 20 mg As kg^{-1} diet group increased to 1.54 µg g^{-1} at 4 weeks after which it declined. The skin of fish exposed to 30 mg kg^{-1} diet, like the liver, contained higher levels

Table 2. Residue[a] of arsenic in tissues (µg g^{-1} dry wt) of rainbow trout, Salmo gairdneri, exposed[b] to 10, 20 and 30 mg As Kg^{-1} diet for 8 weeks at 10 ± 1°C. Means ± S.E. of 4 samples for muscle and single pooled samples for other tissues are shown.

Exposure groups mg Kg^{-1} diet	Weeks of Exposure 2	4	6	8
		Muscle		
10	1.35 ± 0.12	1.40 ± 0.13	1.24 ± 0.05	1.28 ± 0.15
20	1.45 ± 0.20	1.59 ± 0.16	1.26 ± 0.15	1.28 ± 0.12
30	2.40 ± 0.44	1.60 ± 0.10	1.49 ± 0.14	1.53 ± 0.07
		Liver		
10	1.31	1.18	1.77	1.55
20	1.99	2.60	2.45	3.41
30	6.24	6.04	4.98	5.21
		Gills		
10	2.80	0.68	0.89	0.84
20	0.90	0.98	0.68	1.71
30	3.37	1.43	1.84	1.88
		Skin		
10	1.05	0.95	1.18	1.21
20	1.26	1.54	1.43	1.45
30	2.47	1.56	1.94	1.98

[a] No arsenic was detected in control tissues

[b] The exposure levels of 10, 20 and 30 mg/kg diet are equivalent to 0.2, 0.4 and 0.6 mg/kg fish wet weight per day.

of arsenic than the other two groups.

The residue level in the gills of fish exposed to 10 and 30 mg As kg^{-1} diet increased to a maximum at two weeks and showed a decline thereafter. However, the 20 mg group seemed to show a different trend. This difference could have been due to an unusually high level in one of the tissues that made up the pooled sample.

The muscle of fish exposed to the lowest level of arsenic attained maximum concentration of arsenic of 1.35 $\mu g\ g^{-1}$ after 4 weeks of exposure. The levels in the muscles of fish from this group analysed at subsequent time periods were not significantly different ($P < 0.05$). Arsenic levels in the 20 mg As kg^{-1} diet group increased to 1.59 g g^{-1} after 4 weeks and declined. Similarly, muscle of fish from the highest exposure group contained $2.40 \pm 0.44\ \mu g\ g^{-1}$ arsenic after two weeks of exposure, but decreased to significantly lower levels ($P < 0.05$) for the remaining duration of exposure.

DISCUSSION

This section discusses the long-term accumulation of arsenic in the tissues of rainbow trout, and its effects on growth (increase in wet weight) and other physiological parameters.

Arsenic fed in the diet to rainbow trout was shown to impair growth, primarily at high concentrations. As can be seen from Fig. 1, growth of fish exposed to 10 mg As kg^{-1} diet for 8 weeks was not impaired, but those exposed to 20 and 30 mg As kg^{-1} diet for the same period showed significantly reduced growth relative to the control, suggesting a dose-dependent effect. A number of studies have demonstrated a somewhat similar toxic effect of arsenic on other fish species. Lawrence (1958) reported that two applications of 4.0 ppm sodium arsenite made two months apart in experimental outdoor ponds reduced bluegill (Lepomis macrochirus) production by about 42% compared to the control. Benthic invertebrates were also reduced by 34%. Two applications of 8.0 ppm applied one month apart reduced the number of fish by an average of 65%. A later study by Gilderhus (1966) noted a similar effect on adult and immature bluegills (L. macrochirus). The marked reduction in the production of bluegills in the above studies must have been due to the fact that the fish accumulated arsenic from water directly via the gills as well as by consuming contaminated invertebrates. Recently, Speyer (1975) reported a decrease in the growth of yearling rainbow trout that were exposed to 6.0 mg As/litre (as arsenic trioxide) for 21 days. Similar to the results of the present study, Speyer observed a growth impairment that was dose-

dependent, with most marked effect at the highest concentrations.

The reduced growth observed in this study may have been due to the shortage of energy resulting from arsenic interference with the energy-yeilding metabolic processes.

As mentinoned previously in the general introduction, the mode of arsenic action is the uncoupling of oxidative phosphorylation (arsenate) and inhibition of certain enzymes (As + 3) (Lehninger, 1972). Since no high energy phosphate (ATP) is generated when arsenates substitute for phosphate, shortage of energy may be created in the tissues. In order to understand how environmental conditions, including arsenic, influence growth we must first consider the fates of the energy derived from the food consumed. The availability of energy for growth is dependent on the distribution of energy among other possible fates. First and foremost, the energy an organism obtains from its food is used to sustain the activities essential for survival and reproduction. It is after these requirement are met that growth can take place. Warren (1971) suggested the term "Scope for growth" for the difference between the energy of the food consumed and the energy value of all other uses and losses of food besides growth.

Data for rainbow trout have indicated that orally ingested arsenic caused a significant decrease in hemoglobin levels that was inversely related to duration of exposure. The mean corpuscular hemoglobin concentration (MCHC) was also reduced after 8 weeks, suggesting that the primary effect of the arsenic on blood was a

decrease in the hemoglobin in the red blood cells and not hemolysis of the cells. Similarly, Zharkova (1971) and Speyer (1975) noted that arsenic caused a reduction in blood hemoglobin in rats and rainbow trout respectively. The growth impairment observed in the rats and trout in this study could have been due to inhibition of enzymes as well as to a reduced oxygen-carrying capacity of blood. This could have resulted in an inefficient utilization of assimilated food.

In the present study arsenic accumulation in rainbow trout tissues increased with increasing levels of arsenic in the diet. The liver, which has always been identified as a target organ in arsenic poisoning (Gilderhus, 1966; Bencko and Symon, 1970), contained the highest levels of arsenic. Gilderhus (1966), in the previously mentioned study on bluegills reported a high level of 11.6 ppm arsenic in the liver of fish exposed to 4 ppm arsenic for 16 weeks. This level is much higher than the 6.24 ppm maximum for the present study. The marked difference may be due to the fact that the bluegills were exposed to arsenic via both food and water for a longer duration whereas the trout were exposed to arsenic in the food for a shorter duration. High levels of arsenic in the liver have been associated with functional changes. Oxygen consumption in homogenized liver tissue of hairlessmice exposed to 50 mg As/litre in drinking water for 256 days was markedly suppressed compared to the control (Bencko and Symon, 1969). Similarly, the high levels of arsenic observed in the liver of rainbow trout exposed to arsenic in this study may have caused histological changes resulting in impairment of liver functions.

Residue levels of arsenic in the skin were next highest to those for the liver. The keratin of hair and nails is rich in disulphide, and it has been postulated that the arsenic is incorporated into the growing portion of the hair root and the nail base (Shapiro, 1967). Similarly, arsenic could have been bound to sulfhydryl groups in the skin and scale of fish, resulting in the observed high levels.

Muscle tissue sampled in the present study did not accumulate as much arsenic as the liver or skin. Gilderhus (1966) analysed the muscle of the bluegills and also found arsenic residues in muscle (1.3 $\mu g\ g^{-1}$) to be relatively low compared to the liver.

The gills did not seem to accumulate any substantial amount of arsenic during the continuous exposure. This observation might be due to the fast elimination of arsenic via the gill as has been shown in the first chapter of this study.

Generally, there is a similarity between the pattern of tissue distribution of arsenic observed following the pulse dose of radiolabelled arsenic as described in Chapter 1 and during the long-term exposure. In both cases, the liver contained much higher concentrations than other tissues, further strengthening the suggestion of its probable role in metabolism and excretion of arsenic.

In summary, inorganic arsenic is accumulated by fish, though to a lesser extent than the group 2B metals (Holcombe et al. 1978, Spehar et al. 1980). High levels in tissues such as the liver resulted in impairment in the growth of the fish.

Summary and Conclusions

The results of arsenic kinetics and metabolism presented in this thesis define the bioaccumulation potential of arsenic in rainbow trout in terms of 1) efficiency of uptake from food 2) efficiency of uptake from water; and 3) elimination or fractional clearance from whole fish, with emphasis on the routes of elimination. The effect of adaptation to arsenic on whole body elimination and tissue distribution of arsenic was also examined. The results and conclusions are summarised below:

1) Arsenic absorbed from water was accumulated into two compartments which were distinguishable by differences in their fractional clearance rates. Uptake of arsenic from water by trout was poor, with bioaccumulation ratio of less than 1. This is in agreement with results of previous studies for uptake of arsenic from water by trout. The uptake rates of arsenic were directly related to the concentrations in water. Clearance of arsenic was rapid with biological half-lives of 0.6 and 5 days for the fast and slow-clearing compartments respectively.

2) Arsenic accumulation from food was more efficient with an assimilation efficiency of approximately 50% of the ingested dose, based on tissue distribution data. Clearance of arsenic occurred in two phases, similar to that for water. The fast compartment, which comprised about 25% of the ingested dose, had a half-life of less than one day, while the slow clearing compartment had a half-life of 6.4 days. The high assimilation efficiency of arsenic from food would favour accumulation of arsenic by trout from its diet. However, there was no observed biomagnification through the food vector.

3) There was no effect of route of exposure on the relative size of the fast or slow-clearing phases of arsenic.

4) The results on tissue distribution of arsenic following a pulse dose of radiolabelled arsenic showed higher levels of arsenic in bile, liver and kidney than in other tissues, the bile containing the highest concentration. The high concentrations in these tissues may be related to their role in the excretion of arsenic.

5) The data on the routes of arsenic excretion by rainbow trout demonstrate that fecal and gill excretion might be of equal magnitude and could account for 80% or more of the total arsenic loss, urinary excretion accounting for 20% or less. This present demonstration of gill excretion has important implications on the interactive effects of arsenic with other toxicants such as copper which is known to affect gill function.

The estimate of fecal excretion in rainbow trout made from this study is much higher than in mammals, in which fecal excretion is minimised by reabsorption of arsenic excreted into the gastro-intestinal tract, before it is voided in the feces. Urinary excretion has been shown to be the primary route of arsenic elimination in mammals, whereas in this study, urinary excretion has been shown to be comparatively less important.

6) Characterization of the metabolites in the different tissues was carried out to find out if there is any correlation between the phases of clearance and chemical speciation. The results of the present study show that the initial fast-clearing phase coincided with the postdose period when the inorganic arsenic content of

the tissues were still high. Subsequently, the organic arsenic content of the tissues increased as methylation took place. Since at 96 h postdose, the major organic metabolite represented over 80% of the arsenic in the tissues examined, one can conclude that the elimination pattern observed for the slow-clearing compartment was determined primarily by the major organic metabolite.

7) Thin layer chromatography (TLC) of this metabolite showed it had the same Rf value as ethyl arsenobetaine standard (0.82), separating it clearly from dimethylarsinic acid, which had an Rf value of 0.66. On the basis of the wide difference in Rf value, it may be concluded that the major metabolite of inorganic arsenic in trout is not dimethylarsinic acid, as has been shown for mammals.

8) The ability of trout to acclimate to arsenic was examined:

Fish that were acclimated to a sublethal dose of 22.8 mg As kg^{-1} diet for two weeks, demonstrated a significantly faster clearance of the subsequent single dose of radiolabelled arsenic (^{74}As). Fractional clearance rate of ^{74}As was 0.22 and 0.11 d^{-1} for the acclimated and control fish respectively.

Tissue distribution of ^{74}As in the control and fish that were acclimated as previously mentioned showed that the relative concentrations of arsenic (^{74}As) in liver and bile of fish pre-exposed to 22.8 mg As kg^{-1} for two weeks, were signficantly higher than that for the control. These results suggest that preexposure could have affected the binding of arsenic to liver proteins.

There was no difference in the arsenic binding pattern to proteins of liver cytosol in control fish given only a single dose of tracer-labelled arsenic in the diet and test groups dosed

with ^{74}As after two-week acclimation to non-radioactive arsenic. In both the control and treated, gel chromatography of liver cytosol protein showed that elution of arsenic was in two peaks. One of the peaks corresponded to molecular size 19,000 daltons and the other corresponding to molecular size 3,000 daltons could have been elemental arsenic and arsenic bound to amino acids. Since none of these peaks corresponded to molecular weight range of 6,000-12,000 daltons that have been reported for metallothionein in fish, it can be concluded that arsenic may not induce the synthesis of thionein-type proteins in fish.

The specific mechanism involved in the enhanced elimination and tolerance to arsenic is not known. However, the high concentration of arsenic in liver and bile suggests the enhanced excretion might have been mediated via the liver. The observed adaptation has an ecological significance not only to the organism, but to the entire community. Due to this type of adaptation marked difference in bioassay results carried out on two populations of the same species may result from differences in their recent environmental history. Chemically induced adaptation of fish could be a major obstacle preventing accurate application of laboratory established toxicity results to field situations. Luoma (1977) proposed the use of resistance testing to indicate the contaminants of primary concern within an aquatic ecosystem. Also, Dixon (1979) indicated that acclimation dose-response curves and application factor could be developed for arsenic in rainbow trout. A knowledge of the biologically available concentrations of contaminants at which resistance develops will help in understanding toxicity of

contaminants in the environment. Usually, estimates of application factor is based on the concentrations of the contaminant in water. However, for some species, accumulation of contaminants through the diet is of a greater magnitude than direct uptake from water; this is particularly true for arsenic. I therefore suggest that resistance testing, as proposed by Luoma, should not be based only on the level of the contaminant in water, but also on the body burden of the contaminant in the organism in which resistance is developed.

9) Exposure of trout to arsenic in food for a long period of eight weeks resulted in high levels of arsenic in the liver and skin compared to other tissues. Heavy accumulation of arsenic in liver and other tissues could cause functional changes which could reduce the organisms chance of survival. This could be particularly true when it inhibits enzyme involved in the intermediary metabolic pathways, creating a shortage of energy required for sustaining the biological functions of the organism.

Many gaps still exist in our understanding of the kinetics and metabolism of arsenic in fish, some of which are outlined below:

(i) the identification of the organic metabolites formed from inorganic arsenic.

(ii) the identification of the organs where the metabolism of arsenic is carried out.

(iii) the exact mechanism whereby adaptation to arsenic is effected needs to be investigated.

(iv) as already mentioned in the discussion, the toxicity of arsenic to mammals has been known to be modified by the presence of other toxicants. The investigation of this aspect in fish is of ecological significance since it is very unlikely that arsenic will occur singly (in the absence of other toxicants) in the aquatic envrionment.

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