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Water chemistry at the gill surfaces of fish and the uptake of xenobiotics

Introduction

There is a massive production of chemicals synthesized to meet the demands of industry. Most of these chemicals are foreign to the body (xenobiotics). They enter the environment where they are available for uptake by organisms. Most animals possess enzymes (multi-function oxidases) which reduce the toxicity of the xenobiotics and facilitate their excretion. Many xenobiotics, however, are resistant to metabolic degradation, are accumulated in the body and can be extremely toxic.

Most organic chemicals must enter the body before they can exert their toxic effect(s). Uptake can occur through the food chain or by direct uptake from the environment across the respiratory surface and skin. The uptake of xenobiotics from water by fish is determined by numerous factors, the most important of which are the transfer capacity of the gills and the physio-chemical properties of the compound.

Exchange at the gills

In adult fish, the gills comprise the major surface area of the body, and constitute a thin but continuous barrier between the environment and the blood. Water flows over the gills counter to blood flow ensuring a constant partial pressure gradient of O_2 and CO_2 over the duration of blood transit through the gills. Conditions for diffusion of O_2 are further maximized by the presence of haemoglobin which binds molecular oxygen keeping the PO_2 in the blood low. The gills in fish are hyperventilated with water relative to blood to ensure adequate rates of oxygen uptake from the environment. The content of oxygen per unit volume in water is less than 10% of that in blood and consequently fish at rest maintain a gill ventilation : blood perfusion ratio between 10 and 15 (Cameron & Davis, 1970; Kiceniuk & Jones, 1977; Jones & Randall, 1978). This ensures that oxygen delivery to the gills by water is matched by the oxygen transport capacity of the blood. In general, conditions at the gills in fish are adjusted to meet the metabolic demand

for oxygen (Randall, 1990). During exercise ventilation rate, diffusing capacity and cardiac output all increase to maximize gas exchange across the gills (Kiceniuk & Jones, 1977; Randall, 1990). These characteristics which make the gills so efficient for gas exchange, also augment xenobiotic uptake. Xenobiotic uptake from the water is predominantly across the gills (Neely, 1979; Gobas, Opperhuizen & Hutzinger, 1986; Randall & Brauner, 1993) and this constitutes the major exchange site with the water.

Toxicant uptake across the gills can be limited at three sites: delivery to the gills (VENTILATION LIMITATIONS), diffusion across the gill epithelium (DIFFUSION LIMITATIONS) and removal by the blood from the gills (PERFUSION LIMITATIONS) (Fig. 1). Toxicant delivery to the gills is determined by the product of ventilation volume and the water solubility of the toxicant. Diffusion of a xenobiotic across

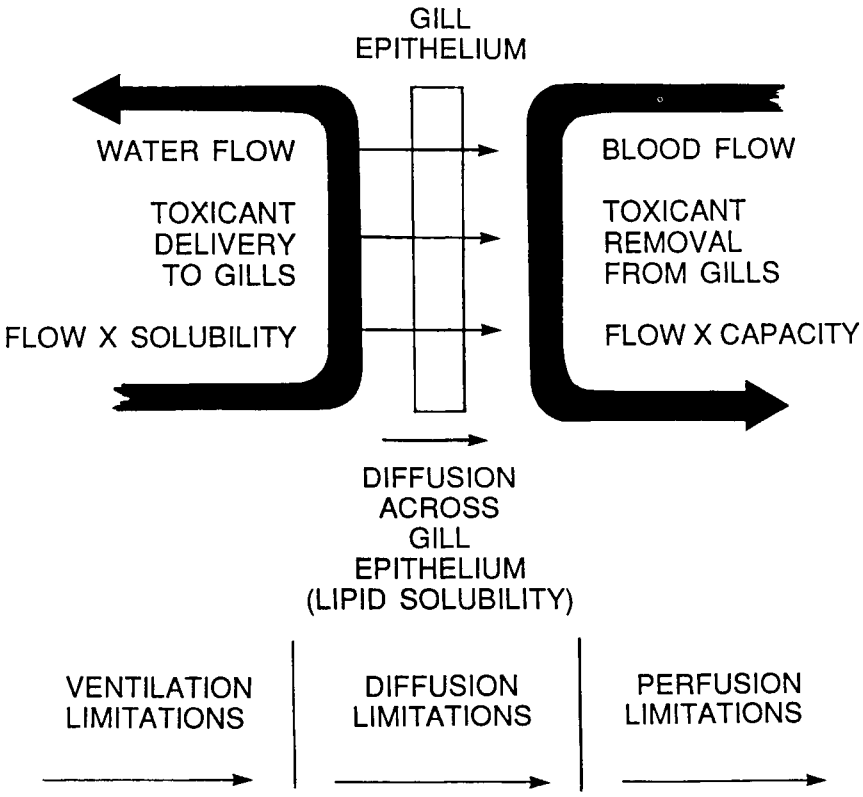

Fig. 1. Sites at which limitations to xenobiotic uptake exist at the gills.

the gill epithelium will depend largely on lipid solubility. Finally, removal of the toxicant by the blood will be determined by the product of blood flow and toxicant solubility in the blood. The amount of the toxicant that can be held in the blood will depend on the extent of binding of the chemical and the lipid solubility of the chemical and lipid content of the blood.

Octanol : water partition coefficient

Many organic substances are much more soluble in lipids than in water. These compounds enter animals because they are lipid soluble and then accumulate in the body fat of the animal. This BIOACCUMULATION of substances in animals due to the high fat solubility of compounds has long been recognized. The relative solubility of chemicals in water and lipids is often measured as the octanol : water partition coefficient (K_{ow}). Octanol was chosen to represent 'biological lipids' and K_{ow} became the standard for quantifying the partitioning characteristics of compounds (Connell, 1990; Niimi, 1991).

In resting fish exposed to numerous non-dissociated chemicals, log uptake rate constant increases approximately linearly over a range of log K_{ow} between 1 and 4, remains constant between log K_{ow} 4 and 6 (Fig. 2, McKim, Schmieder & Veith, 1985) and may decrease above log K_{ow} 6. Assuming the lipid content in fish blood is 5%, during initial exposure to a xenobiotic with log K_{ow} of 2 or higher almost all of the xenobiotic in the blood will be bound to lipids and proteins (Schmieder & Henry, 1988) and the ability of the blood to remove the toxicant will far exceed the ability to deliver it and thus will never be limiting. Thus, uptake of xenobiotics with log $K_{ow} > 2$ will either be ventilation or diffusion limited (Randall & Brauner, 1993). An increase in K_{ow} could result from an increase in lipid solubility or a decrease in water solubility. In general, for chemicals with a log K_{ow} of up to 6, it is a decrease in water solubility which is responsible for an increase in log K_{ow} (Dobbs & Williams, 1983; Verschueren, 1983; Chessells, Hawker & Connell, 1992). Thus, for high K_{ow} chemicals (above log K_{ow} 4) water solubility is very low and uptake may be ventilation limited while at lower K_{ow} (log K_{ow} between 1 and 4) water solubility is relatively high and uptake is likely diffusion limited (Randall & Brauner, 1993).

Gill micro-environment

Uncharged molecules diffuse across non-polar lipid membranes much more easily than charged molecules. This is of significance in the uptake of compounds which dissociate into weak acids because the membrane

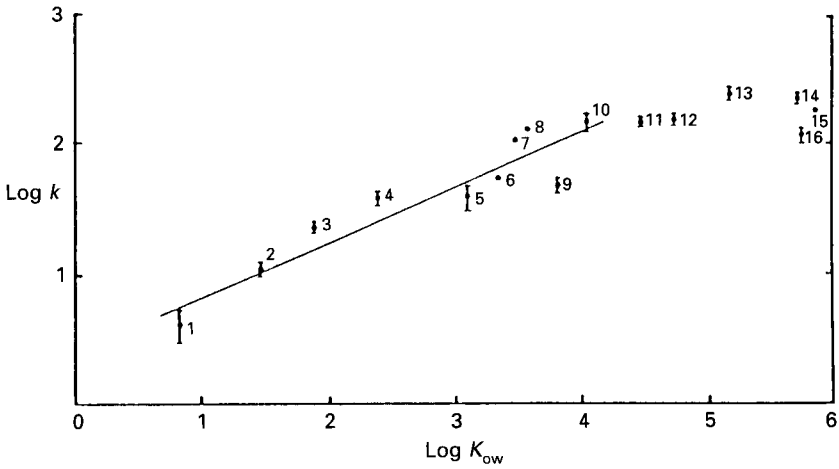

Fig. 2. Relationship between log uptake rate (where water pH< pK_a) and log K_{ow} in guppies. The numbers refer to compounds: 1=butyric acid; 2=phenol; 3=benzoic acid; 4=4-phenylbutyric acid; 5=2,4-dichloro-phenol; 6=2-sec-butyl-4,6-dinitrophenol; 7=3,4-dichlorobenzoic acid; 8=2,6-dibromo-4-nitrophenol; 9=2,4,5-trichlorophenol; 10=2,4,6-trichlorophenol; 11=2,3,4,6-tetrachlorophenol; 12=tetrachloroverathrol; 13=pentachlorophenol; 14=pentachloroanisol; 15=2,4,6-trichloro-5-phenylphenol; 16=DDT; 17=2,3,6-trichloro-4-nitrophenol. (From Saarikoski *et al.*, 1986.)

diffusivity of the dissociated form of the compound is very low relative to the non-dissociated form. The proportion of a compound which is in the dissociated form is dependent upon the pH. The pH of the bulk water in which a fish respires is easy to measure; however, it is the pH in the gill micro-environment which determines the concentration of the undissociated form in water next to the gill surface. The distance between the secondary lamellae is very small relative to their height and length and subsequently there is a large boundary effect on water flow through the channel. Fish excrete protons into this boundary layer (Lin & Randall, 1991) which acidifies expired water when bulk water pH is above 5.5 (Lin & Randall, 1990). The best estimate of gill micro-environment water pH is probably a mean of inspired and expired values.

Saarikoski *et al.* (1986) measured the uptake rate of a variety of weak acids over a range of water pH (Fig. 3). As bulk water pH approached and exceeded the pK_a of the xenobiotic, the concentration of the dissociated form increased and uptake rate decreased. Measured

uptake rates generally somewhat exceeded predictions based on the hypothesis that only the non-dissociated form was available for uptake. The concentration of the non-dissociated form over a range of water pH, however, was calculated from bulk water pH and pK_a. This difference between estimated and measured weak acid uptake may be due to the difference between bulk water and gill micro-environment pH. It may also indicate that there is some uptake of the dissociated form of a weak acid.

Modelling xenobiotic uptake across fish gills

There has been considerable effort to model the uptake of xenobiotics using a variety of models which range from simple, one compartment models to complex pharmaco-kinetic models (Barron, Stehly & Hayton,

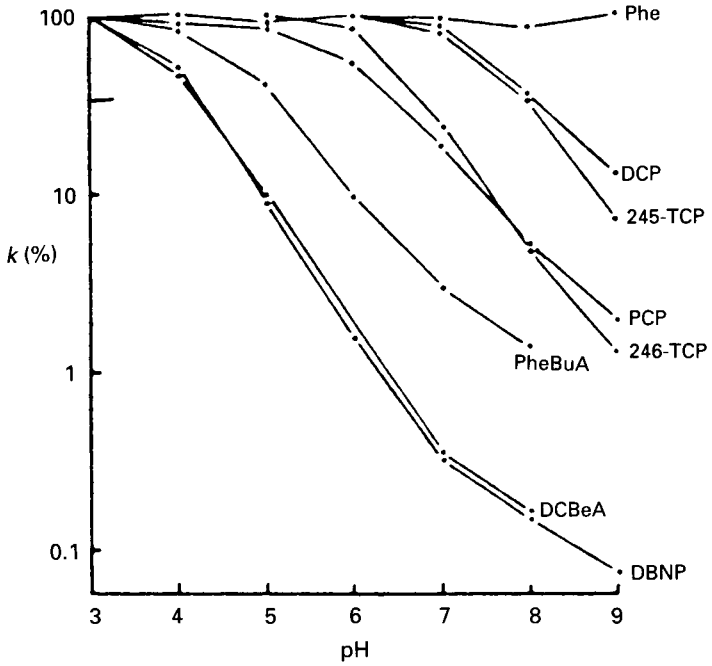

Fig. 3. Relationship between percentage rate of absorption (k%) of weak acids by guppies and water pH. Phe=phenol (pK_a=10.05), DCP=2,4-dichlorophenol (pK_a=7.85), 245-TCP=2,4,5-trichlorophenol (pK_a=7.07), 246-TCP=2,4,6-trichlorophenol (pK_a=6.22), PCP=pentachlorophenol (pK_a=4.71), PheBuA=4-phenylbutyric acid (pK_a=4.76), DCBeA=3,4-dichlorobenzoic acid (pK_a=3.7), DBNP=2,6-dibromo-4-nitrophenol (pK_a=3.7). (From Saarikoski *et al.*, 1986.)

1990). Hayton and Barron (1990) have recently proposed a physiological model to describe xenobiotic uptake across the gills in fish:

$$\text{Uptake rate} = (C_w - C_f)[d(D_m^{-1})\ A\ K_m) + (h(D_a^{-1})A) + (\dot{V}_b K_b^{-1}) + \dot{V}_w^{-1}]^{-1} \quad (1)$$

This model consists of three components: 1) the xenobiotic concentration gradient between the environment and the fish, where C_w and C_f are the concentration of the xenobiotic in the external water and the plasma water, respectively, 2) physiological and anatomical characteristics of the gills, where d is epithelial thickness, h is aqueous stagnant layer thickness, A is gill surface area, $\dot{V}_b$ is effective gill blood flow, $\dot{V}_w$ effective water flow, and 3) physical constants specific to the compound, where D_m is diffusion coefficient in epithelium, D_a is diffusion coefficient in water, K_m is epithelium/water distribution coefficient, and K_b is the blood/water distribution coefficient.

During initial exposure to a xenobiotic, the first component, the concentration gradient between the environment and the fish, will be equal to the aqueously dissolved concentration of the xenobiotic in the environment. This will persist for some time because, immediately after entering the blood, lipophilic compounds will dissolve in lipids and bind to proteins (Schmieder & Henry, 1988) keeping the aqueously dissolved concentration of the xenobiotic in the plasma very low. The hydrophobicity of many xenobiotics results in both micelle formation and a large degree of binding to organic matter in the inhalant water (Black & McCarthy, 1988). In both situations, the chemical is not aqueously dissolved and therefore not available for uptake by diffusion. When calculating the diffusion gradient for a xenobiotic, it is important to know the aqueously dissolved concentration rather than xenobiotic content in the inhalant water.

The second component describing the physiological and anatomical characteristics of the gills is not easy to quantify. For example, it is very difficult to estimate the surface area of the gill and even if an anatomical value is derived it probably does not reflect the functional surface area of the gills because in resting fish, only 60% of the gill is perfused with blood (Booth, 1978). The thickness of the respiratory epithelium ranges from 0.5 to 11 μm depending upon the species (Hughes, 1984) and the thickness is probably not constant from the proximal to distal portion of the secondary lamellae. To complicate matters further many model parameters are not constant and are influenced by the metabolic rate of the fish. For instance, during exercise, there is an increase in cardiac output and ventral aortic blood

pressure (Kiceniuk & Jones, 1977) which increases the perfused gill surface area and gill blood flow, and reduces the thickness of the respiratory epithelium (Randall, 1990). The thickness of the aqueous stagnant layer or boundary layer will also be greatly influenced by the velocity of water flow over the gills. Thus, all of these model parameters are difficult to measure, they are species specific but most importantly they are all adjusted to meet the metabolic demand of the animal to ensure adequate oxygen uptake.

Oxygen consumption rate can be measured easily and accurately. Thurston and Gehrke (1993) have compiled an oxygen data bank (OXYREF) which contains about 5000 measurements of oxygen consumption rate from over 300 species of fish exposed to different temperatures at rest and during exercise. Analyses of these data indicate that the main determinant of oxygen consumption rate over a broad range of fish species and temperatures is body weight. Oxygen is a lipid-soluble molecule which diffuses across the gill lamellae transcellularly as do most xenobiotics. Assuming conditions for oxygen transfer are indicative of those for toxicant transfer the development of a general coefficient (λ) describing xenobiotic uptake as a function of oxygen consumption rate can be used to replace the gill model constants in equation (1).

The third component in equation (1) has to do with characteristics specific to the xenobiotic to which the fish is exposed. As mentioned above, one of the most important physio-chemical characteristics which determines xenobiotic uptake rate is K_{ow}. Both K_m and K_b are dependent upon the K_{ow} of the xenobiotic and therefore a general K_{ow} term can be used in place of K_b and K_m. As described above, for a variety of compounds the logarithm of chemical uptake rate increases linearly with log K_{ow} between 1 and 4.3 and is constant between log K_{ow} 4.3 and 6 (McKim *et al.*, 1985). Thus, during initial exposure to a compound, equation (1) can be simplified to:

$$\text{Uptake} = (C_w)\ \lambda\ (K_{ow}\ s) \tag{2}$$

where λ is a coefficient describing xenobiotic uptake as a function of oxygen consumption rate, and $K_{ow}\ s$ is the coefficient used to account for uptake of chemicals of various log K_{ow} s. For xenobiotics with log K_{ow} between 4.3 and 6, this coefficient is 1, for those between 1 and 4.3 this coefficient is $10^{0.429 \log K_{ow} - 1.842}$ (Randall & Brauner, 1993) calculated from Saarikoski *et al.* (1986).

The diffusion coefficient of a chemical in water is inversely proportional to the square root of the molecular weight (Opperhuizen *et al.*, 1985) and so, for small molecules, diffusion coefficients are relatively

insensitive to differences in molecular weight. Saito *et al.* (1990) demonstrated that compounds greater than 2000 in molecular weight are not absorbed across the gills in carp while Zitko and Hutzinger (1976) have proposed that the upper molecular weight limit for xenobiotic uptake is approximately 600. Thus only small molecules cross the gills and, as a result, diffusivity parameters have been excluded from equation (2).

To predict λ, the uptake of xenobiotics in fish based upon $\dot{M}O_2$ values retrieved from OXYREF, we used 1,2,4,5-tetrachlorobenzene (TCB) because it does not dissociate, is not easily metabolized, has a log K_{ow} of 4.97 and there is no influence of TCB exposure on $\dot{M}O_2$ (Brauner *et al.*, 1994). In rainbow trout exposed to a large range of TCB concentrations there is no significant effect of toxicant exposure on oxygen consumption rate in either resting or active fish (Fig. 4(*a*) and 4(*b*)). This, and oxygen consumption rate in fish can be used to predict toxicant uptake.

The relationship between $\dot{M}O_2$ and TCB uptake rate was examined in five species of fish similar in size, and for several different weight classes of rainbow trout. Forced exercise was chosen as the means to influence oxygen consumption rate because during exercise, fish can increase $\dot{M}O_2$ relative to resting rates by up to 10 fold (Brett, 1964). In all tests fish were introduced into a 130 l Brett-type respirometer (described by Gehrke *et al.*, 1990) for two hours at a water velocity of 18 cm s^{-1}. Tetrachlorobenzene was added and a water sample was taken and fish were forced to swim for 2 h at either a low or a high water velocity (less than 80% of maximal swimming velocity) while $\dot{M}O_2$ was measured. At the end of the test, a water sample was taken and the fish were removed from the respirometer, killed and immediately frozen. The fish and water samples were kept frozen at −80 °C and then transported to Montana State University in dry ice for TCB analyses. The entire fish was homogenized, TCB from the tissue was concentrated in hexane by Soxhlet extraction and total body and water TCB concentration was measured by gas chromatography.

Five species of fish: goldfish (*Carrasius auratus*, 11.43 ± 0.99 g), large mouth bass (*Micropterus salmoides*, 4.8 ± 0.18 g), channel catfish (*Ictalurus punctatus*, 3.7 ± 0.06 g), fathead minnow (*Pimephales promelas*, 3.92 ± 0.34 g) and rainbow trout (*Oncorhynchus mykiss*, 5.02 ± 0.07 g) were exposed to water TCB concentrations of 260 or 780 μg l^{-1} at low or high swimming velocity with exception of the large mouth bass which were only capable of swimming at the low velocity. There is a significant correlation between TCB uptake rate and $\dot{M}O_2$ at both the low TCB concentration (r^2 = 0.29) and the high TCB

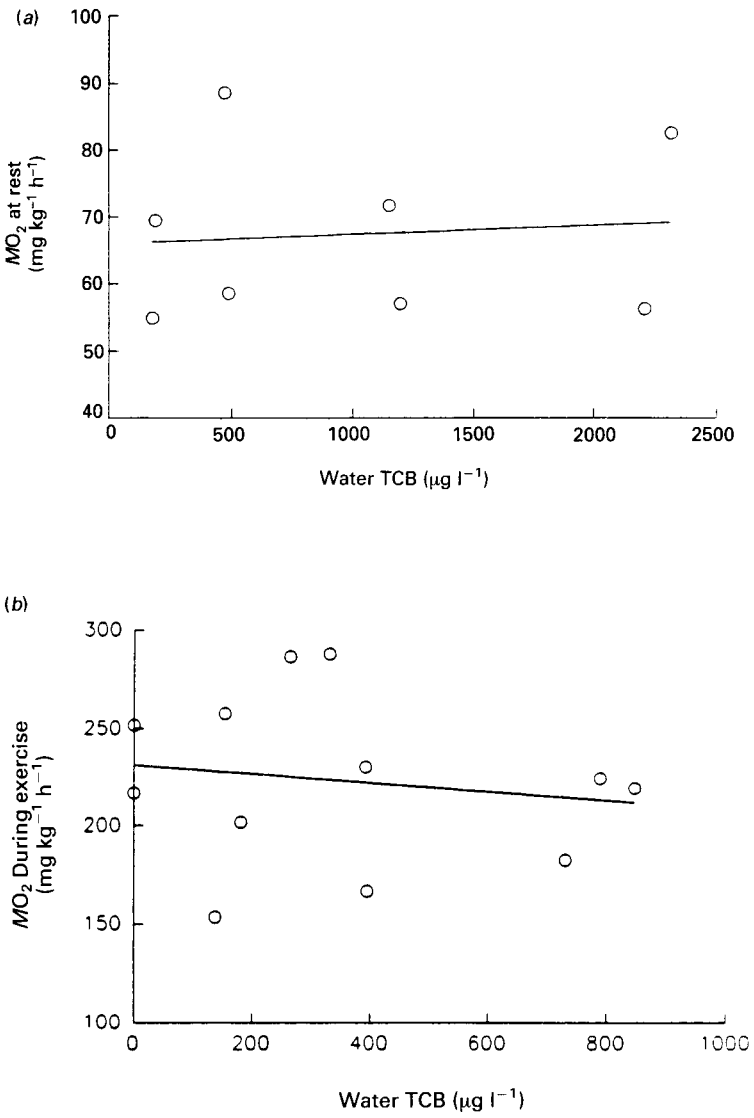

Fig. 4(*a*) The effect of water 1,2,4,5-tetrachlorobenzene (TCB) concentration on the oxygen consumption rate of adult rainbow trout at rest (r^2=0.01) or (*b*) swimming at 1.25 body lengths per second (Bl s^{-1}) (r^2=0.02).

concentration (r^2 = 0.79). The rate of toxicant uptake at a given oxygen consumption rate is dependent upon water TCB concentration.

During initial exposure to a toxicant, the gradient for toxicant uptake is equal to the toxicant concentration in the water and uptake is proportional to the external concentration (Spacie & Hamelink, 1982). In an attempt to standardize for external TCB concentration and generate one regression equation describing toxicant uptake relative to $\dot{M}O_2$, the measured TCB uptake rate was divided by the mean water TCB concentration over the exposure period. It is apparent, however, that two distinct relationships exist, one for each TCB exposure concentration. One explanation for this is that the measured TCB concentration in the water is an overestimation of the aqueous concentration. The high water TCB concentration (760 μg l^{-1}) is near to the maximal water solubility for TCB and it is possible that TCB is binding to organic matter or forming micelles. Thus, the aqueous TCB concentration in the high TCB treatment may be overestimated.

Lipid content in fish is important during long-term exposure to xenobiotics particularly as fish approach equilibrium with the environment (Connell, 1990; Geyer, Scheunert & Korte 1985), but the importance of lipid content to toxicant uptake during initial exposure is not known. The lipid content of the five species was measured and significant differences were found between goldfish and bass relative to channel catfish, fathead minnow and rainbow trout (Fig. 5). The inclusion of lipid in the relationship describing toxicant uptake as a function of $\dot{M}O_2$ significantly improved the coefficient of determination at the low TCB concentration (r^2=0.85) but marginally reduced the coefficient in the high TCB concentration (from 0.79 to 0.68) (Fig. 6). These results indicate that the body lipid content in fish may be an important determinant of toxicant uptake during initial as well as prolonged exposure to TCB; however this requires further investigation.

Medium (38.6 ± 0.96 g) and large (412.5 ± 6.9 g) rainbow trout were forced to swim at one of two water velocities during exposure to an aqueous TCB concentration of 260 μg l^{-1}. Toxicant uptake rate was divided by the mean TCB concentration during the exposure duration and when regressed against $\dot{M}O_2$ the coefficient of determination is significant (r^2=0.59). However, the slope of this regression line differs from that for the five species of small fish exposed to the low TCB concentration. The low coefficient of determination (r^2=0.32) for the five species of small fish exposed to the low TCB concentration is predominantly due to the low TCB uptake rate measured in goldfish and large mouth bass. The goldfish are more than twice the size of the other fish in this group and when the goldfish data are combined