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ACUTE TOXICITY, UPTAKE, DEPURATION AND TISSUE DISTRIBUTION OF TRI-N-BUTYLTIN IN RAINBOW TROUT, SALMO GAIRDNERI

by

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EXECUTIVE SUMMARY

Tributyltin was demonstrated to be extremely toxic to rainbow trout and lake trout. The 96-h LC_{50} values are as low as 1.4 µg Sn/L. Partitioning into a wide variety of tissues was demonstrated. Depuration is relatively slow, mainly by hepatic dealkylation and biliary-fecal excretion.

MANAGEMENT IMPLICATIONS

This article demonstrates that current levels of tributyltin in Canadian fresh waters may be placing native fish populations at risk, particularly in harbours, marinas and shipping channels.

RÉSUMÉ A L'INTENTION DE LA DIRECTION

On a montré que le tributylétain est extrêmement toxique pour la truite arc-en-ciel et le touladi. La CL_{50} après 96 h ne dépasse guère 1,4 ug de Sn/L. On a montré que le composé se distribue dans une grande variété de tissus. L'élimination est relativement lente et se fait principalement par désalkylation hépatique et excrétion biliaire dans les fèces.

PERSPECTIVE-GESTION

Cet article montre que les concentration actuelles de tributylétain dans les eaux douces canadiennes peuvent présenter des risques pour les populations indigènes de poissons, tout particulièrement dans les ports, les marinas et les voies navigables.

ABSTRACT

The tri-n-butyltin cation (Bu_3Sn^+), the active ingredient in organotin-containing antifouling paints, was highly toxic to rainbow trout (mean weight, 1.5 g); the 96-h LC50 was 1.41 µg Sn L^{-1}. Lake trout (mean weight, 5.9 g) were more tolerant with a 96-h LC50 of 5.21 µg Sn L^{-1}. Rainbow trout concentrated significant levels of Bu_3Sn^+ during a 64 d exposure at 0.21 µg Sn L^{-1}, with bioconcentration factors of 406 and 570 (based on Bu_3Sn^+ and total Sn, respectively). Rainbow trout depurated Sn slowly on transfer to Bu_3Sn^+-free water. During the 32 d period following transfer, the whole-body concentration of Bu_3Sn^+ fell by 25% while the total Sn concentration was reduced by 17%. The total Sn concentrations in tissues of rainbow trout at the end of a 15 d exposure to 0.42 µg Sn L^{-1} indicated that Bu_3Sn^+ partitions into trout on the basis of a three compartment model. Peritoneal fat (mean concentration, 9.18 mg Sn kg^{-1}) constituted one compartment; kidney, liver and gall bladder/bile (mean concentration range, 3.07 to 3.72 mg Sn kg^{-1}) a second; and all other tissues (mean concentration range, 0.49 to 1.53 mg Sn kg^{-1}) a third. After 15 d of depuration, the system had simplified; only two compartments (liver plus gall bladder/bile and all other tissues) were apparent. Varying proportions of Bu_3Sn^+ and its metabolites di-n-butyltin, n-butyltin and inorganic tin were present in all tissues sampled. The percent of metabolites in liver (74) and gall bladder/bile (89) were, however, significantly higher than the levels in all other tissues, which ranged from 10 to 43. This suggests hepatic dealkylation and biliary-fecal excretion.

Key words: tri-n-butyltin, rainbow trout, acute toxicity, bioconcentration, metabolism, excretion.

RÉSUMÉ

Le cation tri-n-butylétain (Bu_3Sn^+), la matière active des peintures antisalissures à base d'organo-étain, est extrêmement toxique pour la truite arc-en-ciel (poids moyen de 1,5 g); la CL_{50} après 96 h est de 1,41 ug de Sn/L. Le touladi (poids moyen de 5,9 g) est plus tolérant (CL_{50} après 96 h de 5,21 ug de Sn/L). La truite arc-en-ciel a concentré des quantités importantes de Bu_3Sn^+ au cours d'une exposition de 64 jours à 0,21 ug de Sn/L. Les facteurs de bioconcentration étaient de 406 pour le Bu_3Sn^+ et de 570 pour le Sn total. La truite arc-en-ciel a éliminé le Sn lentement lorsqu'on l'atransféré dans une eau dépourvue de Bu_3Sn^+. Au cours de la période de 32 jours qui a suivi le transfert, la concentration de Bu_3Sn^+ dans l'organisme a diminué de 25% alors que la concentration totale de Sn a diminué de 17%. Les concentrations totales de Sn observées dans les tissus de la truite arc-en-ciel au bout d'une exposition de 15 jours à 0,42 ug de Sn/L ont indiqué que le cation Bu_3Sn^+ se répartit dans la truite selon un modèle à trois compartiments. Les graisses péritonéales (concentration moyenne de 9,18 mg de Sn/kg) constituent un des compartiment, les reins, le foie et la vésicule biliaire/bile (concentration moyenne de 3,07 à 3,72 mg de Sn/kg) en constituent un second et tous les autres tissus (concentration moyenne de 0,49 à 1,53 mg de Sn/kg) forment le troisième compartiment. Après une élimination de 15 jours, le système s'est simplifié; il ne reste plus que deux compartiments: le foie et la vésicule biliaire/bile d'une part et les autres tissus d'autre part. On a découvert des proportions variables de Bu_3Sn^+ et de ses métabolites, le di-n-butylétain, le n-butylétain et l'étain inorganique dans tous les tissus examinés. Les métabolites étaient toutefois en proportions significativement plus élevées dans le foie (74) et dans la vésicule biliaire/bile (89) que dans tous les autres tissus (10-43). On peut penser que les voies d'élimination sont la désalkylation hépatique et l'excrétion biliaire dans les fèces.

Mots-clés: tri-n-butylétain, truite arc-en-ciel, toxicité aiguë, bioconcentration, métabolisme, excrétion.

INTRODUCTION

Organotin compounds are used as thermal stabilizers for polyvinyl chloride, as catalysts in the production of polyurethane foams and as biocides (Thompson et al., 1985). The toxicity of tin compounds to humans (US EPA, 1975), terrestrial animals (Luijten, 1972) and phytoplankton (Wong et al., 1982) has been extensively studied. Organotin compounds are generally more toxic than inorganic tin compounds. Progressive introduction of organic groups to the tin atom in any $R_nSn^{(4-n)+}$ series produces maximal biological activity against all species when n=3 (triorganotin compounds) (Davies and Smith, 1980).

The first biocidal application of organotin compounds was described in 1950. Bis(tri-n-butyltin) oxide (TBTO) proved to be both highly effective against the majority of fouling organisms and more durable than the traditional copper-based hull coatings (Beaumont and Budd, 1984). During the 1970s, from 1.4 to 1.8 million kg of organotins were produced in the United States annually, approximately 0.91 million kg of which were TBTO (Zuckerman et al., 1978). Although TBTO is incorporated into the paint matrix before application to boat and ship hulls, tri-n-butyltin compounds in dilute aqueous solution probably dissociate to form the hydrated tri-n-butyltin cation (Bu_3Sn^+) (Maguire et al., 1983). The toxicity of tri-n-butyltin compounds is essentially independent of the nature of the counter ion (Polster and Halacka, 1971; Davies and Smith, 1980).

Concern over the impact of tri-n-butyltin compounds on aquatic organisms initially arose over problems encountered with the commercial cultivation of the Pacific oyster (Crassostrea gigas) in France and England (Abel et al., 1986). Oysters grown in areas of heavy boating activity exhibited shell malformations, poor growth, reduced reproductive capacity and elevated body burdens of tin (Waldock and Thain, 1983). The tri-n-butyltin species has been detected in water and sediments taken from various locations in Canada, predominantly from harbours and other areas of heavy boat and ship traffic (Maguire et al., 1982, 1986; Maguire, 1984, 1986). In some of these locations, concentrations were sufficiently high to cause concern regarding impact on aquatic organisms. Contamination of fish was also a concern, particularly since salmon (Oncorhynchus tshawytscha) reared in marine net-pens treated with tri-n-butyltin compounds to retard the growth of fouling organisms accumulated levels of tri-n-butyltin sufficient to cause mortality (Short and Thrower, 1986).

This study was initiated to assess the acute toxicity and accumulation of Bu_3Sn^+ in representative freshwater fish. The 96-h LC50 of Bu_3Sn^+ was determined for both rainbow trout (Salmo gairdneri Richardson) and lake trout (Salvelinus namaycush Walbaum). The uptake and depuration of Bu_3Sn^+ by rainbow trout were determined to estimate the potential for bioconcentration. Finally, the concentrations of Bu_3Sn^+ and its metabolites (di-n-butyltin, Bu_2Sn^{2+}; n-butyltin, $BuSn^{3+}$; inorganic tin) were determined in thirteen tissues of rainbow trout to assess tissue distribution and metabolism.

MATERIALS AND METHODS

Acute Toxicity Tests

The acute toxicity of Bu_3Sn^+ to rainbow trout (Salmo gairdneri), with a mean (S.D.) weight of 1.47 (0.22) g, was assessed with a lethality test which exposed groups of 10 fish to nominal Bu_3Sn^+ concentrations of 0, 0.48, 0.86, 1.54, 2.69 or 4.80 µg Sn L^{-1}. The test was carried out in triplicate for a total of 18 treatments. The fish were not fed during the test, which continued for 96 h. Photoperiod was maintained at 12 h light: 12 h dark. Mortality observations were recorded on a logarithmic time series (Sprague, 1973).

The test system consisted of eighteen 35 L glass aquaria. Each received 325 mL min^{-1} of filtered Lake Ontario water (total hardness, 135 mg L^{-1} as $CaCO_3$) at 15°C; the 95% replacement time was 5 h (Sprague, 1973). Toxicant solutions were added to the water supplies by a peristaltic pump. The solutions were prepared with bis(tri-n-butyltin) oxide (97% pure, Ventron, Danvers, MA) which had been purified by passage through an activated silica gel column with benzene as eluent. All toxicant solutions, including the control, contained 4.80 mg L^{-1} ethanol as a toxicant carrier. During subsequent tests, the exposure system operated for a minimum of 2 wk before the fish were introduced. This period of "seasoning", as well as continuous agitation of the toxicant stock solutions and the rapid

turnover rates of test solutions in the aquaria, substantially reduced the variability of assayed Bu_3Sn^+ concentrations.

Temperature, dissolved oxygen and pH were recorded every 12 h from a different (randomly selected) tank. Mean (S.D.) values were: temperature, 15.5 (0.8)°C; dissolved oxygen, 8.93 (0.51) mg L^{-1} and pH, 7.76 (0.06). Every 12 h a 1.0 L water sample was drawn from each tank, acidified with 10 mL HCl, and stored in glass for analysis of Bu_3Sn^+ content. Mean concentrations ranged from 73 to 156% of nominal with coefficients of variation $\leq$24%.

The acute toxicity of Bu_3Sn^+ to lake trout (Salvelinus namaycush), with a mean (S.D.) weight of 5.94 (2.54) g, was determined as outlined for rainbow trout with two exceptions. Only one replicate was undertaken and the flow of water to each tank was increased to 925 mL min^{-1} to allow for the biomass of the larger fish and for a 95% replacement time of 2 h. Water characteristics did not differ significantly from those given for the rainbow trout bioassay. Mean Bu_3Sn^+ concentrations ranged from 108 to 171% of nominal with coefficients of variation $\leq$14%.

Kinetics and Bioconcentration

To assess uptake, depuration and bioconcentration potential, 60 rainbow trout, with an initial mean (S.D.) weight of 13.8 (1.8) g, were exposed to Bu_3Sn^+ at a nominal concentration of 0.36 µg Sn L^{-1} for 64 d. After 1, 2, 4, 8, 16, 32 and 64 d, three fish were sampled,

weighed, individually wrapped in acetone-washed aluminum foil and frozen at -10°C for determination of both Bu_3Sn^+ and total Sn content. On day 64 the remaining fish (39) were transferred to toxicant-free water for a 32 d depuration period. On days 1, 2, 4, 8, 16, and 32 three fish were sampled as outlined for the uptake phase.

Six of the tanks and the exposure protocol outlined for the toxicity tests were used for the bioconcentration study. To accommodate the increased biomass, the flow of water to each tank was increased to 1700 mL min^{-1} for a 95% replacement time of 1 h. The fish were fed with a nutritionally complete commercial diet (Martin Feed Mills, Elmira, Ont.) at a ration of 2% of their wet weight each day. Temperature, pH and dissolved oxygen content, determined on sampling days, did not differ significantly from the values reported for the toxicity tests. Water samples (1.0 L acidified) for determination of Bu_3Sn^+ were taken on each fish-sampling day. The mean (S.D.) concentration during the uptake phase, 0.21 (0.18) µg Sn L^{-1}, represented 58% of nominal. All water samples taken during the depuration phase contained no detectable Bu_3Sn^+.

Tissue Distribution and Metabolism

The effects of Bu_3Sn^+ exposure on the tissue distribution of both Bu_3Sn^+ and its metabolites di-n-butyltin (Bu_2Sn^+), n-butyltin ($BuSn^{3+}$) and inorganic tin were determined in a 30 d study. A total of 20 rainbow trout (10 per treatment), with an initial mean (S.D.) weight

of 32.7 (3.3) g, were exposed to nominal Bu_3Sn^+ concentrations of 0 or 0.50 µg Sn L^{-1}. Five exposed and one control fish were sampled after 15 d. For each fish, thirteen tissues (blood, brain, gall bladder/bile, gills, gonads (M and F), gut, gut contents, kidney, liver, muscle, peritoneal fat and remaining carcass) were excised, weighed and frozen (-20°C) pending analysis. The remaining fish were transferred to toxicant-free water for an additional 15 d, at which time they were also sampled. Analysis of the control fish to establish background levels of tin revealed that, with two exceptions, total tin concentrations were less than 0.20 mg kg^{-1}. The exceptions were peritoneal fat (0.56 mg kg^{-1}) and gall bladder/bile (1.90 mg kg^{-1}). In all of the control tissues tested, the largest proportion of tin ($\geq$84%) was present as inorganic tin.

The metabolism study used two of the tanks and the exposure protocol described for the toxicity tests. The flow of water to each tank was maintained at 2.5 L min^{-1} for a 95% replacement time of 0.8 h. The fish were fed daily, as outlined for the bioconcentration study. Measurements of dissolved oxygen, temperature and pH, recorded every second day during the 30 d period, did not differ significantly from those observed during the toxicity tests. Water samples (1.0 L, acidified) for analysis of Sn content were collected every second day during the uptake phase. With the exception of days 4 and 6 when levels of $Bu_2Sn^{2+} \leq$ 0.04 µg Sn L^{-1}, only Bu_3Sn^+ was present. The mean (S.D.) assayed Bu_3Sn^+ concentration of 0.42 (0.10) µg Sn L^{-1} represented 84% of nominal.

Tin Analysis

The concentrations of Bu_3Sn^+, Bu_2Sn^{2+}, $BuSn^{3+}$ and inorganic tin were determined in either water or fish tissue by a method which involved extraction of the analytes from acidified aqueous solution with benzene containing the complexing agent tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one), pentylation of the extract to produce mixed butylpentyltin derivatives, purification and concentration of the derivatized extract, and determination of the concentration of the derivatives by gas chromatography-quartz tube furnace atomic absorption spectrophotometry.

The method for water (Maguire et al., 1982) required modification when applied to fish tissue. Whole fish were homogenized and the homogenate dispersed in 12 N HCl (10 mL g^{-1} homogenate). After 2 h of digestion at 21°C with magnetic stirring, the digest was diluted 5-fold with doubly-distilled water and extracted three times (1 h each with magnetic stirring) with 5% (w/v) tropolone-benzene solution. The combined extracts were dried by passage through anhydrous sodium sulphate and the volume reduced to 300 mL by rotary evaporation.

The butylpentyltin derivatives were prepared by refluxing the extracts for 2 h with 10 mL of Grignard reagent (2 M n-pentylmagnesium bromide in diethyl ether). After the derivatized extract had cooled, excess Grignard reagent was removed by washing with 100 mL of 1 N H_2SO4. The dried extract was reduced to 2 mL by rotary evaporation and cleaned by passage through a 3% water-deactivated

silica gel column (2.5 cm i.d. column with 20 cm of silica gel above 3 cm of anhydrous sodium sulphate). The column was pre-washed with hexane and the extract eluted with 2 mL of benzene followed by 300 mL of hexane (5 mL min^{-1}). The extract was reduced to 1 mL (rotary evaporation) and the concentration of the butylpentyltin derivatives determined by gas chromatography-quartz tube furnace atomic absorption spectrophotometry (Maguire and Tkacz, 1983).

Mean (S.E.) recoveries of Bu_3Sn^+, Bu_2Sn^+ and $BuSn^{3+}$ from water samples spiked with from 1 to 10 mg Sn L^{-1} varied from 96 (4) to 103 (8)% (Maguire and Huneault, 1981). Recoveries of inorganic tin from water at pH 5 to 8 were poor at 35 (23) %, probably because of the formation of unextractable SnO_2 (Maguire et al., 1983). For lake trout (Salvelinus namaycush) spiked with each species at 0.02 to 0.10 mg Sn kg^{-1} wet weight, recoveries of Bu_3Sn^+ were 94 (14) to 104 (12) %; of Bu_2Sn^{2+}, 66 (11) to 83 (4) %; of $BuSn^{3+}$, 55 (10) to 63 (5) %; and of Sn(IV), 21 (11) to 97 (2) % (Maguire et al., 1986). Although the method described above was developed for lake trout, it is reasonable to assume that it would be equally as effective for other fish.

The concentrations of butyltin species and inorganic tin in water and fish reported in this article have not been corrected for recovery. Although Sn(IV) was the only inorganic tin species for which recoveries were determined, the tin present in our water and fish samples is reported as total inorganic tin, since any Sn(II) which might have been present would likely have been oxidized to Sn(IV) during pentylation.

Statistics

For the bioassays, 96-h LC50s were calculated by probit analysis (Finney, 1971) using mortalities and mean assayed Bu_3Sn^+ concentrations. Since no control mortalities occurred, correction was not necessary. The 96-h LC50s were compared on the basis of relative potency (Finney, 1971). Data for the uptake and depuration study were subjected to either linear or Gauss-Newton nonlinear regression analysis (Zar, 1984) to determine the rate constants for uptake (k_1) and depuration (k_2). Bioconcentration factors (BCF, concentration in tissue/concentration in water) were calculated for each fish sampled during the uptake period using the mean assayed analyte concentration in all water samples taken up to that point in the exposure (Spacie and Hamelink, 1985). The significance of changes in BCF with time were assessed by analysis of variance. Results for the tissue distribution/metabolism study were analyzed by factorial analysis of variance and differences were determined by Tukey's comparison of means (Zar, 1984). Prior to analysis, all data were tested for homogeneity of variance (Bartlett's test) (Zar, 1984). When nonconformity was encountered, log transformations were made before analysis. The nontransformed data are reported here to facilitate biological understanding. The significance of all results was assessed at the 0.05 probability level.

RESULTS

The triplicate toxicity tests with rainbow trout yielded 96 h LC50 values (fiducial limits) of 1.49 (1.34 to 1.61), 1.28 (1.02 to 1.52), and 1.46 (1.16 to 1.84) µg Sn L^{-1} for a mean (S.D.) of 1.41 (0.11) µg Sn L^{-1}. The 96 h LC50 for lake trout was 5.21 (4.84 to 5.60) µg Sn L^{-1}, significantly higher than any of the values obtained with rainbow trout.

In terms of both actual tissue concentration (Fig. 1a) and bioconcentration factor (BCF, Fig. 2a) rainbow trout accumulated Bu_3Sn^+ rapidly, effectively reaching equilibrium in 24 to 48 h. The rate constant for uptake (k_1) of Bu_3Sn^+ was 5.11 h^{-1}. Individual BCFs calculated for the period from 48 to 1536 h ranged from 210 to 550 and did not differ significantly from each other. As such, the grand mean (S.D.) for all times of 406 (98) represents the best estimate of BCF. Results for total Sn species paralleled those for Bu_3Sn^+ (Fig. 1b, 2b). The grand mean BCF (S.D.) based on total tin species was 570 (212), while k_1 was 8.38 h^{-1}.

Upon transfer to Bu_3Sn^+-free water, rainbow trout depurated tin slowly in terms of both Bu_3Sn^+ (Fig. 3a) and total tin species (Fig. 3b). During the 32 d period following transfer, the whole-body concentration of Bu_3Sn^+ fell by 25%, while the total tin concentration was reduced by 17%. The rate constants for depuration (k_2), based on either Bu_3Sn^+ or total tin, were 0.0110 h^{-1} and 0.0103 h^{-1} respectively.

The distribution and metabolism study showed that Bu_3Sn^+ was present in all tissues sampled, with the relative proportions of the dealkylated metabolites di-n-butyltin (Bu_2Sn^{2+}), n-butyltin ($BuSn^{3+}$) and inorganic tin varying considerably between tissues (Table 1, Fig. 4). At the end of the 15 d exposure period, and based on the sum of all tin species (Fig. 4), the mean tin concentration in peritoneal fat, 9.18 Sn mg kg^{-1}, was highest. The mean concentrations in kidney, liver and gall bladder/bile, which ranged from 3.07 to 3.77 mg Sn kg^{-1}, were not significantly different from each other, but significantly lower than the peritoneal fat concentration and significantly higher than the means shown by all other tissues. Tissue concentrations of the individual butyltin species and inorganic tin showed patterns of distribution somewhat different from the above (Table 1). The mean concentrations of Bu_3Sn^+ (mg Sn kg^{-1}) were highest in peritoneal fat (5.56), kidney (2.30), liver (1.21) and remaining carcass (1.38). Bu_2Sn^{2+} (mg Sn kg^{-1}) concentrations were also highest in peritoneal fat (2.73) followed by liver (1.04), kidney (0.96) and gall bladder/bile (0.90). The mean gall bladder/bile $BuSn^{3+}$ concentration of 1.30 mg Sn kg^{-1} was significantly higher than the mean concentrations in all other tissues, which ranged from 0.02 to 0.42. No significant differences in inorganic tin concentration were apparent among tissues.

A 15 d depuration period not only reduced overall tin levels but also altered the relative tissue distributions (Table 2, Fig. 4). Based on the sum of all analytes (Fig. 4), the mean concentrations

(mg Sn kg^{-1}) in liver (5.94) and gall bladder/bile (5.43) were significantly higher than the mean levels in all other tissues, which ranged from 0.51 to 3.32. The patterns of tissue distribution of the individual cations (Table 2) were substantially different from those apparent prior to the depuration period. There were no significant differences in mean Bu_3Sn^+ concentrations among tissues. Liver and gall bladder/bile mean Bu_2Sn^{2+} concentrations (1.27 and 1.93 mg Sn kg^{-1} respectively) were significantly higher than the levels apparent in all other tissues, which were not significantly different from each other. The same trend was evident for $BuSn^{3+}$ and inorganic tin concentrations.

DISCUSSION

The acute toxicity results reported here demonstrate that Bu_3Sn^+ is extremely toxic to salmonids, consistent with previous work (Seinen et al., 1981; Thompson et al., 1985; Short and Thrower, 1986). Although the elevated 96 h LC50 obtained with lake trout (5.21 µg Sn L^{-1}) relative to rainbow trout (1.41 µg Sn L^{-1}) could be due to interspecific variation in susceptibility, the deviation is more likely due to size differences (lake trout, 5.9 g; rainbow trout, 1.5 g). Over this size range, small fish are generally less tolerant of toxicants than larger fish, since they have a higher metabolic rate and hence assimilate waterborne toxicants more quickly (Sprague, 1985).

Rainbow trout accumulated Bu_3Sn^+ rapidly and cleared it slowly, consistent with what one would expect for a lipophilic chemical with a log octanol-water partition coefficient of 3.34 (Ward et al., 1981). Based on the Bu_3Sn^+ cation alone, a mean measured BCF of 406 was apparent. This compares favourably with the calculated BCF (k_1/k_2) of 465 based on our uptake and depuration constants, and with the value of 520 reported for sheepshead minnow (Cyprinodon variegatus) by Ward et al. (1981). When the sum of all tin species in the fish tissues was taken into consideration, respective measured and calculated BCFs of 570 and 813 were obtained. It is evident that care must be taken to specify whether calculations of BCF are based on the concentration of parent compound alone, or on the sum of the concentrations of the parent compound and its metabolites.

The total tin concentrations in tissues of rainbow trout at the end of the 15 d uptake period (Fig. 4a) suggest that Bu_3Sn^+ partitions into trout on the basis of a three compartment model. Peritoneal fat constitutes one compartment; kidney, liver and gall bladder/bile a second; and all other tissues the third. After an additional 15 d of depuration the system has simplified (Fig. 4b) in that only two compartments were evident; the concentrations in liver and gall bladder/bile, while not significantly different from each other, were significantly higher than the concentrations in all other tissues. The concentrations in peritoneal fat and kidney now fell into the second compartment.

At the end of the uptake period, liver and gall bladder/bile contained the highest proportions of Bu_3Sn^+ metabolites (74 and 89% respectively). All other tissues, except for the gut and the gut contents, contained metabolites at levels ranging from 10 to 43%. The metabolite proportions in the gut (60%) and the gut contents (81%) most probably reflect deposition via the bile. As would be expected, all tissues showed a higher proportion of metabolites at the end of the 15 d depuration period. The relative proportions from one tissue to another were, however, no different from the above.

The elevated proportions of metabolites in the liver, and particularly in the gall bladder, suggest that Bu_3Sn^+ is dealkylated in the liver and excreted via the bile. A similar phenomenon was observed by Krigman and Silverman (1984) who reported that butyltin compounds, when given intraperitoneally to rats, are excreted solely by the biliary-fecal route. A process by which Bu_3Sn^+ is dealkylated in the mammalian liver has been suggested by several workers. Kimmel et al. (1977) and Fish et al. (1976) reported that several organotin species, including Bu_3Sn^+, undergo alpha, beta, gamma, and delta-hydroxylation by a cytochrome P-450 dependent hepatic monooxygenase system. In the case of tributyltin, the alpha-OH and beta-OH metabolites are subjected to debutylation to form dibutyltin and finally monobutyltin (Bridges et al., 1967). Preliminary observations with spot (Leiostomus xanthurus), an estuarine fish, suggest that this metabolic pathway also occurs in fish (Lee, 1986).

Although high levels of total Sn were apparent in kidney at the end of the uptake period, there would appear to be little metabolism of Bu_3Sn^+ in this tissue. The proportion of parent compound to dealkylated metabolites was a reflection of values observed in blood. The role of the kidney in the actual excretion of Bu_3Sn^+ and its metabolites is unclear. The high levels of Sn observed in kidney are most likely a reflection of translocation from the liver by the blood and the affinity of kidney tissue for ionized electrolytes, and do not necessarily imply significant levels of urinary excretion. This premise is supported by the Sn levels present in kidney tissue at the end of the 15 d depuration period; levels were not significantly different from those in all tissues except liver and gall bladder/bile.

One of the most interesting aspects of the metabolism study was the rapid assimilation and depuration of Bu_3Sn^+ and its metabolites by peritoneal fat. The initially high levels are consistent with reports that hydrophobic contaminants will partition preferentially into the lipid fraction of an organism (Chiou, 1985). The rapid depuration most probably reflects translocation of the Bu_3Sn^+ to the liver for metabolism and excretion. It should be noted that relatively high proportions of inorganic tin (15 to 40%) were present in peritoneal fat, and that an appreciable concentration (0.56 mg Sn kg^{-1}) was present in the peritoneal fat of fish which had not been exposed to Bu_3Sn^+. Inorganic tin is an essential micronutrient in mammals, with the highest concentrations in adipose tissue (Schwartz, 1974). The

levels of inorganic Sn in peritoneal fat reported here, and to some extent the rapid changes in the concentrations of the butyltin species, may reflect normal Sn metabolism.

Although the sample sizes were too small to allow inclusion in the statistical analysis, appreciable quantities of Bu_3Sn^+ and its metabolites were present in brain and reproductive tissue of rainbow trout (Tables 1 and 2). Both inorganic and organic Sn compounds are demonstrated mammalian neurotoxicants (Reiter and Ruppert, 1984). The reproductive effects of organotins on aquatic organisms are unknown. Although any discussion of the behavioural and reproductive impacts of Bu_3Sn^+ and its metabolites at this time would be purely speculative, their presence in these tissues indicates that further work on reproductive and neurological impacts is warranted.

In conclusion, Bu_3Sn^+ is acutely toxic to rainbow trout with a 96-h LC50 of 1.41 µg Sn L^{-1}, is subject to bioconcentration with a BCF of 406, and partitions rapidly into all rainbow trout tissues. Bu_3Sn^+ is subject to hepatic dealkylation and biliary-fecal excretion. Since Bu_3Sn^+ appears to persist in fresh water with a half-life of six weeks to a few months (Maguire and Tkacz, 1985; Maguire, 1986), and since more than 0.2 µg Sn L^{-1} of Bu_3Sn^+ (the level used in this bioconcentration study) has been observed in Canadian fresh waters (Maguire *et al.*, 1986), tributyltin-containing antifouling agents may be placing native fish populations at risk, particularly in harbours, marinas and shipping channels.

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Table 1. Mean (N, S.D.) concentrations of butyltin species and inorganic tin in tissues of rainbow trout exposed to 0.42 µg Sn L^{-1} of Bu_3Sn^+ for 15 d. Within columns, means without a superscript letter in common are significantly different.

Tissue	Species concentration (mg Sn kg^{-1})				
	Bu_3Sn^+	Bu_2Sn^{2+}	$BuSn^{3+}$	Inorganic tin	Total
liver	1.21 (4, 0.40)^{bc}	1.04 (4, 0.39)^{ab}	0.42 (4, 0.36)^{b}	2.10 (3, 0.42)^{a}	4.77 (4, 0.93)^{b}
gall bladder/bile	0.34 (3, 0.11)^{c}	0.90 (3, 0.11)^{ab}	1.30 (3, 0.28)^{a}	0.53 (3, 0.13)^{a}	3.07 (3, 0.37)^{b}
kidney	2.30 (3, 0.62)^{b}	0.96 (3, 1.00)^{ab}	0.25 (3, 0.28)^{b}	0.15 (3, 0.05)^{a}	3.66 (3, 0.67)^{b}
carcass	1.38 (5, 0.35)^{bc}	0.04 (5, 0.02)^{b}	0.05 (5, 0.03)^{b}	0.08 (4, 0.04)^{a}	1.55 (5, 0.37)^{c}
peritoneal fat	5.56 (5, 1.46)^{a}	2.73 (5, 2.48)^{a}	0.14 (3, 0.01)^{b}	1.34 (3, 0.93)^{a}	9.77 (5, 4.31)^{a}
gill	1.04 (4, 0.50)^{c}	0.16 (4, 0.03)^{b}	0.05 (4, 0.03)^{b}	0.13 (4, 0.07)^{a}	1.38 (4, 0.49)^{c}
blood	0.67 (5, 0.11)^{c}	0.07 (5, 0.02)^{b}	0.04 (3, 0.01)^{b}	0.07 (5, 0.01)^{a}	0.85 (5, 0.12)^{c}
gut	0.50 (4, 0.16)^{c}	0.20 (4, 0.12)^{b}	0.17 (4, 0.09)^{b}	0.39 (4, 0.26)^{a}	1.27 (4, 0.52)^{c}
muscle	0.32 (5, 0.07)^{c}	0.11 (5, 0.02)^{b}	0.02 (5, 0.02)^{b}	0.04 (5, 0.04)^{a}	0.49 (5, 0.08)^{c}
gut contents[1]	2.50 (1, -)	6.50 (1, -)	3.80 (1, -)	0.42 (1, -)	13.20 (1, -)
brain[1]	2.20 (1, -)	0.15 (1, -)	0.11 (1, -)	0.50 (1, -)	2.96 (1, -)
gonad - M[1]	2.90 (1, -)	0.19 (1, -)	ND	1.20 (1, -)	4.29 (1, -)
gonad - F[1]	0.42 (2, 0.11)	0.07 (2, 0.02)	ND	0.05 (2, 0.00)	0.54 (2, 0.13)

[1] Excluded from anova; insufficient sample size

Table 2. Mean (N, S.D.) concentrations of butyltin species and inorganic tin in tissues of rainbow trout exposed to 0.42 µg Sn L^{-1} of Bu_3Sn^+ for 15 d followed by 15 d in toxicant-free water. Within columns, means without a superscript letter in common are significantly different.

Tissue	Species concentration (mg Sn kg^{-1})				
	Bu_3Sn^+	Bu_2Sn^{2+}	$BuSn^{3+}$	Inorganic tin	Total
liver	1.32 (4, 0.38)[a]	2.17 (4, 1.27)[a]	1.27 (4, 0.63)[ab]	1.18 (4, 0.50)[a]	5.94 (4, 2.67)[a]
gall bladder/bile	0.48 (3, 0.13)[a]	1.37 (3, 1.15)[b]	1.93 (3, 1.45)[a]	1.65 (3, 1.09)[a]	5.43 (3, 2.48)[a]
kidney	1.50 (4, 0.22)[a]	0.53 (4, 0.32)[c]	0.75 (4, 0.38)[bc]	0.45 (4, 0.30)[b]	3.23 (4, 0.65)[b]
carcass	2.25 (5, 2.76)[a]	0.07 (4, 0.04)[c]	0.07 (4, 0.06)[c]	0.18 (4, 0.06)[b]	2.57 (4, 2.70)[b]
peritoneal fat	0.73 (4, 0.32)[a]	0.15 (4, 0.13)[c]	0.04 (3, 0.02)[b]	0.68 (4, 0.29)[b]	1.60 (3, 0.73)[b]
gill	0.54 (5, 0.08)[a]	0.15 (5, 0.06)[c]	0.08 (5, 0.02)[c]	0.49 (5, 0.24)[b]	1.26 (5, 0.35)[b]
blood	0.41 (4, 0.28)[a]	0.09 (4, 0.02)[c]	0.06 (4, 0.04)[c]	0.46 (4, 0.22)[b]	1.01 (4, 0.31)[b]
gut	0.38 (5, 0.23)[a]	0.16 (5, 0.07)[c]	0.12 (5, 0.08)[c]	0.24 (5, 0.11)[b]	0.90 (5, 0.41)[b]
muscle	0.31 (5, 0.03)[a]	0.10 (5, 0.02)[c]	0.09 (5, 0.04)[c]	0.03 (4, 0.02)[b]	0.51 (4, 0.07)[b]
gut contents[1]	0.19 (1, -)	0.41 (1, -)	0.66 (1, -)	2.03 (1, -)	3.29 (1, -)
brain[1]	1.80 (1, -)	0.34 (1, -)	0.06 (1, -)	0.06 (1, -)	2.26 (1, -)
gonad - M[1]	0.34 (1, -)	0.13 (1, -)	0.05 (1, -)	0.19 (1, -)	0.71 (1, -)
gonad - F[1]	1.80 (2, 0.28)	0.35 (2, 0.06)	0.42 (2, 0.02)	2.85 (2, 2.61)	5.42 (2, 1.82)

[1] Excluded from anova; insufficient sample size

FIGURES

Fig. 1. Uptake of Sn by rainbow trout, in terms of either Bu_3Sn^+ (A) or total Sn (B), during a 64 d exposure to Bu_3Sn^+ (0.36 µg Sn L^{-1}). Each point represents the mean of three organisms.

Fig. 2. Bioconcentration factors (BCF) based on either Bu_3Sn^+ (A) or total Sn (B) for rainbow trout exposed to Bu_3Sn^+ (0.36 µg Sn L^{-1}) for 64 d. Each point represents the mean of three fish with 95% confidence intervals.

Fig 3. Depuration of Sn by rainbow trout, in terms of either Bu_3Sn^+ (A) or total Sn (B), during the 32 d period following a 64 d exposure to Bu_3Sn^+ (0.36 µg Sn L^{-1}). Each point represents the mean for three fish.

Fig. 4. Mean total Sn concentrations in muscle (M), blood (B), gut (GU), gill (GI), carcass (C), gall bladder/bile (GB), kidney (K), liver (L) and peritoneal fat (P) of rainbow trout either exposed to Bu_3Sn^+ (0.42 µg Sn L^{-1}) for 15 d (uptake) or subjected to exposure plus an additional 15 d in toxicant-free water (clearance). Within groups, columns which do not share a common tone are significantly

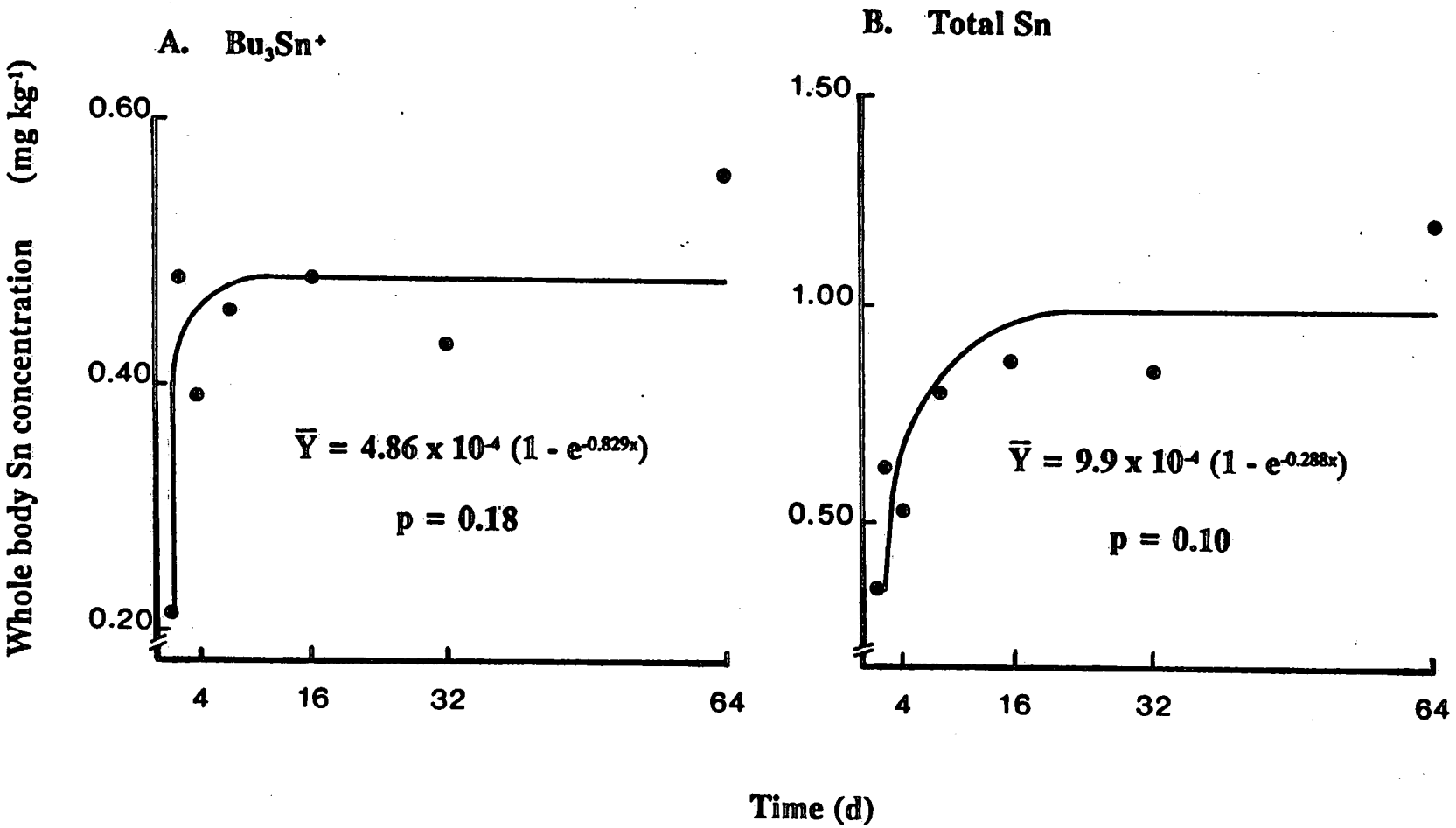

A. Bu_3Sn^+
B. Total Sn
Whole body Sn concentration (mg kg^{-1})
0.60
0.40
0.20
1.50
1.00
0.50
$\bar{Y} = 4.86 \times 10^{-4} (1 - e^{-0.829x})$
p = 0.18
$\bar{Y} = 9.9 \times 10^{-4} (1 - e^{-0.288x})$
p = 0.10
4
16
32
64
Time (d)

Fig. 1

Fig. 2

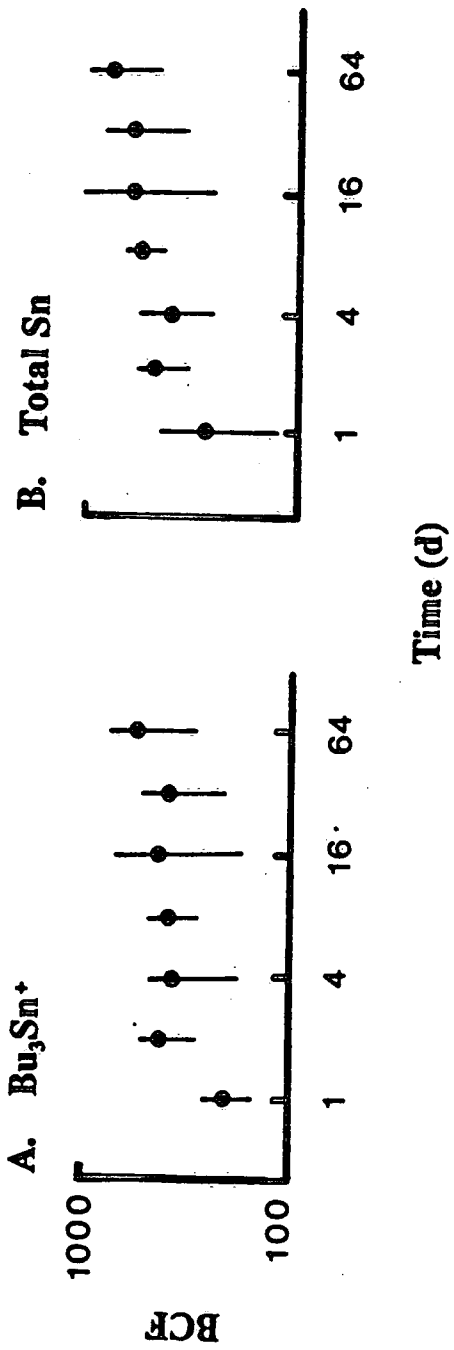
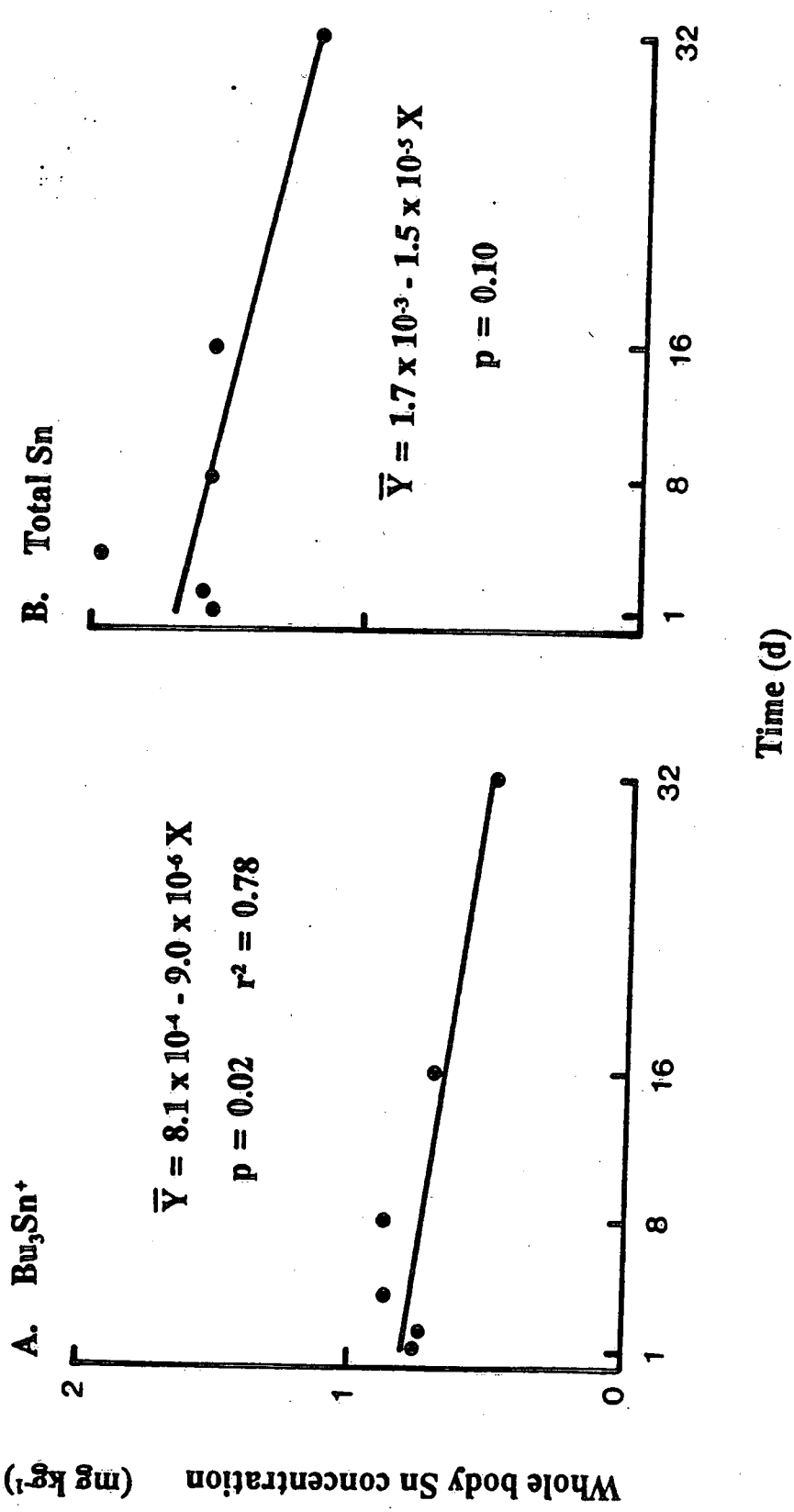


Fig. 3


A. Bu_3Sn^+
$\overline{Y} = 8.1 \times 10^{-4} - 9.0 \times 10^{-6} X$
p = 0.02 $r^2 = 0.78$
B. Total Sn
$\overline{Y} = 1.7 \times 10^{-3} - 1.5 \times 10^{-5} X$
p = 0.10
Whole body Sn concentration (mg kg^{-1})
Time (d)
0
1
2
1
8
16
32

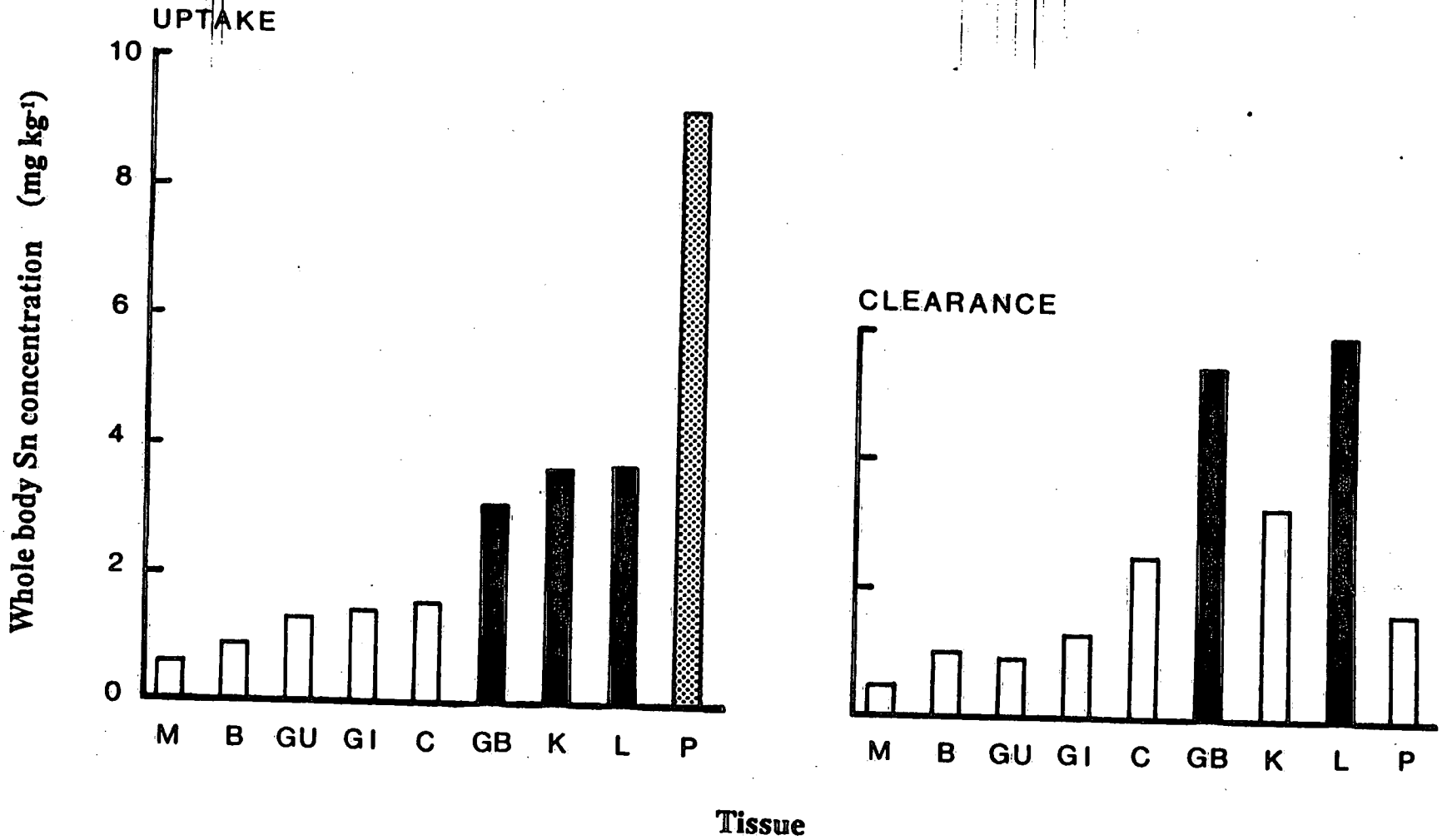

UPTAKE
Whole body Sn concentration (mg kg-1)
10
8
6
4
2
0
M
B
GU
GI
C
GB
K
L
P
CLEARANCE
M
B
GU
GI
C
GB
K
L
P
Tissue

Fig. 4