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SERUM SORBITOL DEHYDROGENASE ACTIVITY AS AN INDICATOR OF CHEMICALLY-INDUCED LIVER DAMAGE IN RAINBOW TROUT

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Running head:

SSDH and liver damage in rainbow trout

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EXECUTIVE SUMMARY

SERUM SORBITOL DEHYDROGENASE ACTIVITY AS AN INDICATOR OF LIVER DAMAGE IN RAINBOW TROUT.

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This paper reports on an investigation of the enzyme serum corbital dehydrogenase (SSDH) as a fast response indicator of liver damage in trout when exposed to organic contaminants by either waterborne chemicals or intraperitoneal injection.

It was found that SSDH levels in blood could:

i) easily be measured;
ii) were unaffected by storage of serum for 30 days or more;
iii) were linearly correlated with toxicant dose (exposure) of fish;
iv) provide an early indication of liver damage;
v) may be useful to assess the impact of chemical contaminants on fish.

SOMMAIRE EXÉCUTIF

LA SORBITOL-DÉSHYDROGÉNASE SÉRIQUE - INDICATEUR DES DOMMAGES HÉPATIQUES CHEZ LA TRUITE ARC-EN-CIEL

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Le présent document porte sur une recherche effectuée au sujet de l'enzyme appelée sorbitol-déshydrogénase sérique (SSDH). La recherche visait à déterminer si la SSDH était un indicateur rapide des dommages hépatiques chez la truite lorsqu'elle était exposée à des contaminants organiques, soit par exposition de type hydrique, soit par injection intrapéritonéale.

On a découvert que les concentrations de SSDH dans le sang :

i) pouvaient se mesurer facilement;
ii) ne variaient pas en fonction de l'entreposage de sérum pendant au moins 30 jours;
iii) étaient liées de façon linéaire à la dose de substances toxiques administrée aux poissons;
iV) indiquaient rapidement les dommages hépatiques;
v) pouvaient servir à évaluer l'incidence des contaminants chimiques sur les poissons.

Abstract

The utility of serum sorbitol dehydrogenase activity (SSDH) to diagnose chemically-induced liver damage in fish was assessed with rainbow trout. The assay was both precise and repeatable and was unaffected by long-term sample storage in liquid nitrogen, by fish weight, by sex or by fasting. Exposure of trout to phenol, p-chlorophenol, p-phenoxyphenol, carbon tetrachloride or copper injected intraperitoneally (IP) or added to water, caused an exposure-dependent increase in SSDH activity. Peak activity occurred 48 h after the start of exposure. Cyanide had no effect on SSDH activity, consistent with previous observations of no hepatotoxicity. The response to toxic chemicals was unaffected by impaired liver function induced by pre-exposure to excessive levels of dietary carbohydrate. The carrier solution for injected chemcials may however, affect chemical responses. SSDH activity of control fish injected with corn or fish oil was higher then controls injected with an ethanol-saline solution. Nevertheless, good exposure-response relationships were established with oil carriers. Elevated SSDH activity was strongly correlated to decreased serum protein levels. Hepatic lesions visible at the light microscope level were observed with waterborne toxicant exposure; parenchymatous edema was closely associated with increased liver somatic index (LSI). With IP dosing, however, biochemical lesions preceded histopathology since no visible lesions or changes in LSI were associated with toxicant exposure. Therefore, SSDH activity provides a good, reliable indicator of sublethal hepatotoxicity in studies of chemical effects on fish.

Key Words: serum sorbitol dehydrogenase, liver, toxicity, rainbow trout, phenol, p-chlorophenol, p-methylphenol, p-phenoxyphenol, carbon tetrachloride, copper, hydrogen cyanide.

LA SORBITOL-DÉSHYDROGÉNASE SÉRIQUE - INDICATEUR DES DOMMAGES HÉPATIQUES CHEZ LA TRUITE ARC-EN-CIEL

D.G. Dixon, P.V. Hodson et K.L.E. Kaiser

Résumé

Une foule d'expériences ont permis de savoir s'il était utile de mesurer la fluctuation des effets de la sorbitol-déshydrogénase sérique (SSDH) dans le sang de la truite arc-en-ciel pour déterminer quantitativement les dommages hépatiques après une injection intrapéritonéale de substances toxiques (IP) ou une exposition de type hydrique à ces dernières. La SSDH avait complètement fait effet 48 heures après l'injection intrapéritonéale de paraméthylphénol contenu dans de l'éthanol salin (0,75 de la DL50) ou de benzène contenu dans de l'huile de maïs (0,50 de la DL50). Il y avait une importante corrélation linéaire entre l'effet de la SSDH et la dose de substances toxiques chez les poissons échantillonés 48 heures après l'injection IP de phénol, de parachlorophénol, de paraphénoxyphénol ou de tétrachlorure de carbone. L'exposition de type hydrique au paraméthylphénol (28 uM) et au cuivre (0,0021 uM) pendant 96 heures augmentait l'effet de la SSDH de 295 et de 85 p. 100 respectivement, par rapport aux témoins. L'exposition de type hydrique au cyanure d'hydrogène (0,5 uM) pendant 96 heures ne changeait pas les concentrations de SSDH. Les poissons auxquels on a imposé un régime digeste à forte teneur en glucides présentaient des concentrations de SSDH de 70 p. 100 supérieures à celles des poissons dont le régime était à basse teneur en glucides. L'alimentation n'empêchait pas la SSDH de réagir aux substances toxiques. Chez les poissons qui ont absorbé une quantité plus ou moins grande de glucides et qui ont reçu une injection intrapéritonéale de tétrachlorure de carbone (0,55 de la DL50), l'effet de la SSDH augmentait de 350 et de 420 p. 100 respectivement, par rapport aux témoins. L'augmentation de l'effet de

la SSDH était nettement liée à une réduction des concentrations de protéines sériques. On a observé au microscope des lésions hépatiques provoquées par l'exposition de type hydrique aux substances toxiques; l'oedème parenchymateux était fortement lié à une augmentation de l'indice somatique du foie (ISF). Cependant, on n'a observé aucune lésion ou variation de l'ISF provoqué par l'injection intrapéritonéale de substances toxiques. On a observé des lésions biochimiques avant toute preuve d'histopathologie. Le porteur de substances toxiques, la privation de nourriture pendant six semaines et le sexe des jeunes poissons n'ont pas influé sur les concentrations de SSDH. L'effet de la SSDH sur du sérum entreposé à -195^{o}C est resté stable pendant au maximum 30 jours.

Mots clés : sorbitol-déshydrogénase sérique, toxicologie du foie, truite arc-en-ciel, phénol, parachlorophénol, paraméthylphénol, paraphénoxyphénol, tétrachlorure de carbone, cuivre et cyanure d'hydrogène.

Introduction

Sorbitol dehydrogenase (SDH, 1.1.1.14) was first described in a mammalian system by Blakley (1951). It catalyzes the reversible interconversion of fructose and the polyhydric alcohol sorbitol, an oxidation-reduction reaction which occurs predominantly in the liver (Wolf et al., 1973). In fisheries research, hepatic isozymes of SDH have been used as markers to delineate populations of cyprinids (Engel and Faust, 1971) and salmonids (Khanna et al., 1975). A quantitative assay for SDH was applied to analyses of liver and serum of rainbow trout by d'Apollonia and Anderson (1980).

The diagnostic value of serum SDH (SSDH) in mammals was first recognized by Gerlach (1957); it is now used extensively to indicate mammalian liver damage (Alemu et al., 1977; Jaeger et al., 1974; Wolf et al., 1973; Yagminas and Villeneuve, 1977), particularly acute hepatitis (Barondess and Erle, 1960). Unlike serum or plasma concentrations of other liver enzymes, such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) or lactate dehydrogenase (LDH), SSDH is not elevated in other organ diseases, but is specific to liver damage (Asada and Galamos, 1963).

Although serum and plasma enzyme activities have been used to indicate chemical effects on fish (Bouck, 1980; Christiansen et al., 1977; Dixon et al., 1985; Gingerich, 1982; Gingerich and Weber, 1979), SSDH has not been evaluated. This research assesses the use of SSDH activity to monitor toxicant effects on liver tissue of rainbow trout (Salmo gairdneri) and defines modifying factors of the response.

Studies were undertaken with rainbow trout to identify whether SSDH would respond to exposure to hepatotoxic chemicals, whether analytical and chemical exposure methods would bias results, and whether non-chemical causes of liver damage would confound responses to chemicals. Specifically, we examined 1) the precision of SSDH analyses; 2) optimal serum storage temperatures, since all sample analyses cannot be completed simultaneously; 3) the effect of fasting, since fish are

not usually fed during toxicant exposures; 4) the effect of sex and weight; 5) the effect of chemical carriers (saline or oil) in fish injected intraperitoneally (IP-dosed); 6) the optimal sampling time for fish IP-dosed with p-methylphenol (PMP) or benzene (B); 7) dose-response curves for phenol (P), p-chlorophenol (CP), p-phenoxyphenol (PPP), and carbon tetrachloride (CCl_4); 8) the response to waterborne exposure of fish to PMP, hydrogen cyanide (HCN), and copper (Cu); and 9) the effects of impairment of liver function by dietary carbohydrate on SSDH activity, before and after exposure to PMP.

Materials and Methods

Source and maintenance of fish

Rainbow trout, purchased as required from Goossen's Trout Farm, Otterville, Ontario, were held in the laboratory for a minimum of three weeks at a temperature of 15^0C on a 12 h light : 12 h dark photoperiod before any tests. They were fed a commercial diet (Martin Feed Mills, Elmira, Ontario, formula MNR - 82G) at a rate of 2% wet weight per day. Tank volumes and water flow-rates conformed to the guidelines for bioassays of Sprague (1973). Experiments 5, 6 and 7 were completed at the Canada Centre for Inland Waters, Burlington, Ontario, where, during both holding and experiments, the water had the following mean (SD, n) characteristics: pH, 7.71 (0.09, 32); alkalinity, 91 (6, 32) $mg.L^{-1}$ as $CaCO_3$; total hardness, 138 (7, 32) $mg.L^{-1}$ as $CaCO_3$; and dissolved oxygen, 8.6 (0.6, 62) $mg.L^{-1}$ (85% saturation). All other work was completed at the University of Waterloo, Waterloo, Ontario, where the mean characteristics of the water were: pH, 7.91 (0.06, 21); alkalinity, 294 (4, 21) $mg.L^{-1}$ as $CaCO_3$; total hardness, 58 (6, 21) $mg.L^{-1}$ as $CaCO_3$; and dissolved oxygen, 8.9 (0.6, 71) $mg.L^{-1}$ (90% saturation).

Toxicant administration

Injection

Fish were anaesthetized with MS 222 before IP injection with p-methylphenol (PMP, Aldrich Chem. Co. lot 091227, purified by redistillation), p-chlorophenol (CP, BDH Chem. lot 2450950, purified by recrystalization from hexane), phenol (P, BDH Chem. lot 0754110), p-phenoxyphenol (PPP, Eastman Kodak Co. lot B8A, purified by recrystalization from methanol-water/hexane), benzene (B, Caledon Lab., HPLC grade) or carbon tetrachloride (CCl_4, Caledon Lab., HPLC grade).

The PMP, P, and CP were injected using 0.9% NaCl in 5% ethanol as a carrier solvent. Cod liver oil (Life Brand, local pharmacies) was used as a carrier for B and PPP. The concentrations of toxicants in the carriers were adjusted so that a 100 g fish received 1 mL of carrier from a 1 mL gas-tight syringe fitted with a 22 gauge needle. Control fish were injected

with carrier only. CCl_4 was injected without a carrier using a 100 uL gas-tight syringe fitted with a 26 gauge needle. Control fish were sham-injected. Following injection, the fish were placed in 40 L continuous-flow tanks (maximum, 10 per tank) until sampling.

Waterborne exposure

Exposure of fish to waterborne PMP, hydrogen cyanide (HCN) and copper (Cu) used 70 L continuous-flow tanks. Each tank received 500 $mL.min^{-1}$ of water, regulated by flowmeter (Manostat Corp., New York). Mariotte bottles (Leduc, 1966) were used to dispense the appropriate concentration of toxicant stock-solution (PMP, as above; NaCN, Fisher Chem.; $CuSO_4.5H_2O$, Fisher Chem.) into each dilution-water stream. Toxicant concentrations were measured daily in each tank, Cu by atomic absorption spectrophotometry (APHA, 1976), HCN by a pyridine-pyrazolone spectrophotometric method (Epstein, 1947) and PMP by ultraviolet absorption at 220 nm. The values reported here are measured levels. Fish were not fed during waterborne exposure.

Experimental techniques

Fish were anaesthetized with MS 222 (100 $mg.L^{-1}$) prior to sampling. Blood was drawn from the caudal artery of each fish with a 10 mL plastic syringe fitted with a 20 gauge needle. Each blood sample was immediately transferred to a glass centrifuge tube and held on ice to allow clot formation. Within 30 to 45 min the blood was centrifuged for 5 min at 8000 rpm to isolate serum. The serum was placed in 1 mL BEEM vials and frozen in liquid nitrogen until analysis.

Blood samples for hematocrits were taken from the caudal artery using 75 uL heparinized micro-hematocrit tubes after severing the caudal peduncle. Wet weight and sex were recorded for each fish. The liver was weighed to determine liver somatic index (LSI = [liver weight/body weight] x 100). In some cases (see design), livers were fixed in buffered formalin and examined for histopathological lesions by Dr. B. Hicks, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

SSDH activity was determined with a kinetic ultraviolet spectrophotometric assay at 25^0C.

The rate of conversion of fructose to sorbitol by SDH was followed by measuring the decrease in absorbance as the co-factor NADH was converted to NAD. Since the optimal assay conditions for rainbow trout are virtually identical to those of the mammalian assay (d'Apollonia and Anderson, 1980), a "kit" was used (No. UV-50, Sigma Chemical Co., St. Louis; Anon., 1974a). Activity was expressed as $mU.mL^{-1}$ serum, where 1 mU (milli International Unit) is the amount of enzyme that will convert 1 nM of substrate per minute. Results were also expressed as $mU.mg^{-1}$ serum protein. Serum protein levels were determined by the Biuret method (Anon., 1974b).

Design

Experiment 1: Precision

To establish the precision of the method, assays of control serum from a 0.9 and a 2.4 kg rainbow trout were repeated 10 times per fish to compute 'within-fish' or analytical error. The 'between fish' error was estimated for the activities of the 10 control fish of each of the experiments described below. Variability between experiments (repeatability) was computed from the mean control activities of each these experiments. Since between fish error was non-homogenous (see 'Data analysis'), all data were transformed to logarithms. This meant that relative standard deviations could not be used as a basis for comparing error. Instead the width of the 95% confidence interval about the geometric mean was expressed as a percentage of the mean.

Experiment 2: Serum storage temperature

Serum was sampled from 20 rainbow trout with a mean weight of 324 g (SD, 32) after three weeks of holding. Hematocrit and plasma protein were also measured for each fish. Each serum sample was randomly subdivided into 4 storage treatments: 20^0C (room temperature); 0^0C (ice bath); - 20^0C (freezer); or - 195^0C (liquid nitrogen). The SSDH activity was determined in an aliquot of each treatment after specified times: 15, 30, 60, 120 and 240 min at 20^0C; 2, 4, 8, 24 and 32 h at 0^0C; 1, 2, 4, 8, and 16 d at - 50^0C; and 2, 4, 8, 16 and 32 days at - 195^0C.

Experiment 3: Effect of fasting

The impact of fasting on SSDH activity was assessed in a 10 wk study. Thirty rainbow trout were acclimated to Waterloo conditions for 4 wk, after which feeding was stopped. Serum was sampled from 10 fish after a further 0, 3 and 6 wk of holding, for a total of 30 fish (10 fish per time x 3 times).

Experiment 4: Effect of sex and weight

Throughout this research a total of 50 control fish were saline-injected and held for 48 h prior to sampling. In addition, 20 fish were injected with CCl_4 at 0.55 of the 96h IP LD50 and held for 48h. Correlation coefficients were calculated for the relationship between sex and SSDH activity for the two treatment groups. A similar comparison of control activity to fish weight included all control fish (N = 173) to test the maximum range of weights (52-217g).

Experiment 5: Carrier effects

To determine the effect of toxicant carrier on SSDH activity, fish were either not injected (control), sham-injected, saline injected (1.0 mL.100 g^{-1}) or corn-oil injected (1.0 mL.100 g^{-1}). A total of 40 fish were treated (4 treatments x 10 fish per treatment) and held for 48 h prior to sampling.

Experiment 6: Sampling interval

Two factorial designs were used to test the effects of toxicant dose and time on SSDH activity. In the first, fish were injected with a PMP dose equal to 0, 0.25, 0.50 or 0.75 of the 96 h IP LD50 of 0.73 mmoles.kg^{-1} (Hodson et al., 1984) and sampled after 12, 24, 48 or 96 h. A total of 160 fish were injected (10 fish per dose x 4 doses x 4 times). Liver samples for histopathology were taken from five fish drawn from each treatment group.

For the second study, fish were dosed with benzene at 0 or 0.50 of the 96h IP LD50 of 25.8 mmoles.kg^{-1} (loc. cit) and sampled ater 24, 48, 60, 72, 80, 96 or 120 h. A total of 120 fish were injected (10 fish per dose x 2 doses x 6 times). Liver samples for histopathology were taken from

five fish drawn from each treatment group.

Experiment 7: Dose-response

The relationships between the IP dose of P, CP, or PPP and SSDH activity were measured by injecting rainbow trout with 0, 0.075, 0.135, 0.24, 0.42 or 0.75 of the respective IP LD50s of 4.3, 0.91 and 0.87 mmoles.kg^{-1} (loc. cit.). For CCl_4, fish were injected with 0, 0.18, 0.26, 0.38, 0.55 or 0.79 of the 96 h IP LD50 of 29.6 mmoles.kg^{-1} (previously determined). A total of 240 fish were injected (4 chemicals x 10 fish per dose x 6 doses). The fish were sampled after 48 h.

Experiment 8: Concentration-response

The effect of exposure to waterborne toxicants was determined by exposing fish to either 28 uM PMP, 0.5 uM HCN or 2.1 uM Cu. Control fish were held under identical conditions in clean water. Ten fish from each group were sampled after 24, 48 and 96 h, for a total of 120 fish (4 treatments x 10 fish per treatment x 3 times). All fish were examined for liver pathology.

Experiment 9: Impaired liver function

The effect of impaired liver function on the SSDH response to toxicants was tested in CCl_4-dosed rainbow trout. Liver impairment was induced by feeding trout with a diet containing 20% digestable carbohydrate (Dixon and Hilton, 1981). A control group was fed a diet that was isocaloric and isonitrogenous but contained 0% digestable carbohydrate. The test conditions (Waterloo) were identical to those outlined above. After 15 weeks on the test diets, the fish were injected IP with CCl_4 at a dose of either 0 or 0.55 of the IP LD50 and sampled after 48 h. A total of 60 fish were injected (15 fish per dose x 2 doses x 2 diets). Five fish per treatment were examined for liver pathology.

Data Analysis

Prior to analysis, the data were tested for normality (D'Agostino's D test, Zar, 1984) and

homogeneity of variance (Bartlett's test, loc. cit.). When fish were exposed to toxic chemicals, both mean SSDH activity ($\bar{x}$) and the standard deviation (s) of activity increased ($s = 1.28 + 0.212\ \bar{x}$; $N = 40$; $r = 0.81$); i.e. variance was a function of the mean and was not homogenous. In these cases, $\log_{10}$ transformations were made before analysis but the antilogs of means and 95% confidence limits are presented here to aid in biological interpretation. Transformed data were subsequently analyzed with standard SAS programs from the library of the Department of Computing Services, University of Waterloo. With the exception of Experiment 4 (see above), results were subjected to factorial analysis of variance followed by Tukey's comparison of means (Steel and Torrie, 1960). In addition, dose-reponse relationships (Experiment 7) were described by regressing $\log_{10}$ SSDH activity against $\log_{10}$ toxicant dose. The statistical significance of the results was assessed at the 0.05 probability level.

Results

Experiment 1: Precision

The geometric mean SSDH activities within the two fish were 11.1 and 11.6 $mU.mL^{-1}$ serum; the relative errors (95% confidence interval/mean x 100) equalled 3.6 and 4.3 percent respectively. Geometric mean activity of control fish from each experiment ranged from 8.0 to 14.6 $mU.mL^{-1}$ serum and the relative error 'between fish' from 24 - 81 percent, with an average of 47 percent (Table 1). On a protein basis, control activities ranged from 0.44 - 0.65 $mU.mg^{-1}$ protein while relative error 'between fish' ranged from 25 - 83 percent of the mean with an average of 45 percent. The overall geometric means of control activities from each experiment were 10.7 $mU.mL^{-1}$ serum or 0.52 $mU.mg^{-1}$ protein. Their confidence intervals, a measure of error between experiments (repeatability), were equal to 17.8 and 13.5 percent of the mean respective means.

Experiment 2: Serum storage temperature

The temperature and duration of serum storage affected measured SSDH activities in all treatments, with the exception of -195^0C (Figure 1). SSDH activities declined by 18% after storage at 20^0C for 4 h, by 26% after storage at 0^0C for 30 h and by 86% after storage at -5^0C for 360 h (16 d). No appreciable change in SSDH activity occurred after 768 h (32 d) of storage at -195^0C. Mean wet weights (SD) of the five fish in each of the four treatment groups ranged from 294.4 (36.9) to 336.6 (41.2) g and were not significantly different. The same was true for mean hematocrit (SD), which ranged from 29.8 (2.9) to 33.3 (3.6) percent, and mean serum protein (SD) which ranged from 19.6 (2.8) to 22.2 (3.4) $mg.mL^{-1}$.

Experiment 3: Fasting

Although a six-week period of fasting did not affect either SSDH activity or serum protein (Table 2), both LSI and hematocrit decreased as starvation progressed.

Experiment 4: Effect of sex and weight

SSDH activity was not correlated to sex for either saline-injected (r=0.09) or CCl_4-dosed (r=0.02) rainbow trout. Similarly, activity did not vary with weight in cotrol fish (r=0.075).

Experiment 5: Carrier effects

Neither sham injection nor saline injection changed SSDH activity relative to controls (Table 3). Corn-oil injection caused a slight (25%) but non-significant increase in SSDH activity. None of the treatments affected serum protein, LSI or hematocrit.

Experiment 6: Sampling interval

The duration of post-injection holding had a definite effect on SSDH activity in both PMP and benzene-dosed rainbow trout (Fig. 2). In fish dosed with PMP (0.75 of the IP LD50), SSDH levels were significantly elevated relative to controls at all four test times, but reached a maximum after 48 h and subsequently declined. Similar trends (not shown) were apparent in fish dosed with 0.25 and 0.50 of the IP LD50. No histological lesions were evident in liver tissue.

Fish dosed with benzene at 0.50 of the IP LD50 showed elevated SSDH levels, relative to controls, 24 and 48 h after injection. Once again maximum activity occured at 48 h. For both chemcials, neither toxicant nor time had a significant impact on serum protein level, hematocrit or LSI. The mean weights (SD) of the 10 fish in each treatment group ranged from 120.3 (27.5) to 135.9 (32.5) g with no significant differences between the 22 groups.

Experiment 7: Dose-response

Significant dose-dependent increases in SSDH activity were apparent 48 h after injection for all four toxicants tested (Table 4; Figure 2). Fish dosed with CP, PPP and CCl_4 showed a dose-dependent decrease in serum protein concentration. As well, fish exposed to CCl_4 showed a dose-dependent reduction in hematocrit. None of the chemicals caused liver histopathology or changes in LSI.

Experiment 8: Concentration-response

Waterborne toxicant exposure had a significant impact on SSDH activity, relative to controls, in fish sampled after 24, 48 and 96 h of exposure (Table 5). After 48 and 96 h of exposure to PMP, respective SSDH levels of treated fish were 103 and 295% higher than controls. LSI was elevated after 96 h of exposure. Mean serum protein levels and hematocrits were unchanged. The livers of four of the ten fish sampled showed some degree of parenchymatous edema, a potentially prenecrotic state.

Exposure to HCN did not cause any change in either SSDH or serum protein. Although LSI decreased after 24 h of exposure, it returned to control levels by 48 h. Hematocrit was similarly elevated at 24 h, but decreased to control levels by 96 h. No histological lesions were evident in liver tissue after 96 h of exposure.

Copper exposure increased SSDH activity (85% relative to controls) after 96 h of exposure. Changes in serum protein, LSI, hematocrit, and liver structure were not apparent.

Experiment 9: Impaired liver function

During the 15 wk pre-experimental growth period, the mean wet weight of fish fed the low-carbohydrate diet increased from 2.5 to 47.5 g. Fish fed the high-carbohydrate diet showed reduced growth, with the mean weight increasing from 2.5 to 37.2 g. Elevated dietary carbohydrate enhanced SSDH activity (Table 6). The SSDH activity of control fish fed a low-carbohydrate diet was 0.58 $mU.mg^{-1}$ serum protein, significantly lower than the mean of 0.98 for controls fed a high-carbohydrate diet. As well, fish fed the high carbohydrate diet had LSIs significantly higher than those of fish fed the low-carbohydrate diet.

For both diets, CCl_4 dosing increased SSDH activity, by 420% in fish reared on the low-carbohydrate diet and by 350% for those on the high-carbohydrate diet. CCl_4 reduced serum protein and hematocrit in both diet groups. Histologically, livers of fish fed the high-carbohydrate

diet had a higher degree of vacuolation than those of fish on the low-carbohydrate diet. The contents of the vacuoles were Periodic Acid Schiff positive, indicating an intracytoplasmic accumulation of glycogen. No other lesions were evident.

Discussion

These studies have shown that SSDH of rainbow trout can be measured reliably and precisely, that serum samples can be stored indefinitely in liquid nitrogen without loss of activity, that activities are unaffected by fasting, sex or fish weight, and that activity clearly reflects exposure of fish to toxic chemicals in a dose-dependant fashion. For routine assays of chemical effects on fish exposed by intraperitoneal injection, several carriers can be safely used, and the optimal time for sampling is 48 hours post-injection. Liver damage induced by diet also elevates SSDH activity but does not interfere with or confound assays of toxic chemicals.

The experiments described in this paper were conducted over a period of 26 months and, for each, separate groups of fish were used. The first experiment showed that analytical error (between subsamples within a fish) and repeatability (error between experiments) were less than error between fish within a sample. Therefore, when testing treatment effects in an experiment, or comparing fish from different natural sampling sites, the average of several samples of fish (replicates) would be a better basis for statistical comparison than a single sample of ten fish. However, since each serum assay involves a kinetic measurement of activity, the increased statistical power of larger sample sizes must be balanced against cost. For laboratory toxicity tests, the precision achieved with 10 fish is adequate to separate treated from control fish, particularly where dose-response relationships permit linear regression analyses (Figure 3). The expression of activity on a serum protein basis ($mU.mg^{-1}$ protein) did not markedly reduce the error of control measurements. Therefore, this is not an argument for expressing activity this way.

The source of error between fish is likely natural variability associated with differences between fish in health, diet (e.g. carbohydrate levels) and sensitivity to hepatotoxicity. The latter was shown by non-homogeneity of variance: as activity increased with chemical exposure (Expts. 7 and 8), the standard deviations of untransformed data increased. Within a sample of 10 fish, some responded dramatically, some a small amount, and some not at all. This differential sensitivity is the source of the non-homogeneity.

Storage in liquid nitrogen (-195^{0}C) is the only adequate method for holding serum prior to determination of SSDH activity. To minimize storage effects, serum should be isolated and placed in liquid nitrogen as quickly as possible after bleeding. Under these conditions SSDH activity should remain stable for at least one month.

Food deprivation was not a significant modifier of SSDH activity, despite liver shrinkage. The observed decrease in LSI is characteristic of starvation, and is probably the result of reduced liver-glycogen reserves (Hochachka and Sinclair, 1962) rather than cell death. The observed decrease in hematocrit during starvation is consistent with previous work (Smirnova, 1965) and is thought to reflect reduced hemopoietic activity (Kawatsu, 1966). Although reduced serum protein levels are characteristic of starvation (Snieszko, 1972), a significant decrease was not noted here. A longer period of fasting would probably have reduced the levels to the point of statistical significance. Limited starvation of fish during laboratory toxicant - exposure or capture in the field would not be expected to prejudice the diagnostic value of SSDH results.

While we found that sex had no impact on SSDH activity, d'Apollonia and Anderson (1980) reported significantly higher levels in male rainbow trout, relative to females. We used sexually immature juvenile fish while d'Apollonia and Anderson (1980) used sexually mature adults. Therefore, sex should be recognized when interpreting results from mature fish, particularly in field surveys where there is less control on the relative numbers of males and females in a sample. The absence of a weight effect on activity should also not be accepted without question due to the limited range of fish sizes tested. Since changes in size may also be associated with sexual maturation and changes in habitat and diet, there may be an effect in feral fish.

Neither saline-ethanol carrier nor sham injection caused a change in SSDH activity relative to uninjected fish. The 25% increase in baseline activity from corn-oil injection is as yet unexplained. We have noticed a similar effect in assays with benzene and p-phenoxyphenol in which the carrier was cod liver oil (Table 1). Control activities per mL of serum for experiments 6 and 7 were 25 percent higher for fish oil-injected compared to saline-injected fish, and the differences were

significant ($p<0.05$). When the data were expressed as activity per mg protein, however, the difference was reduced to 12 percent and was non-significant. This suggests that the oil effect is due to an actual release of enzyme to the serum (i.e. increased protein) rather than to an enhancement or activation of existing serum enzyme activity. The cause of oil-induced liver damage is unknown but oil-borne contaminants (peroxides, pesticide residues) or overloading of hepatic lipid metabolism are two possibilities. Since activity per mg protein is similar between oil-injected and saline-injected fish, there is some advantage to using the protein correction.

For both PMP in saline-ethanol carrier and benzene in cod liver oil carrier, SSDH activity reached a maximum 48 h after IP injection. We had originally speculated that toxicant mobilization from a polar carrier such as saline-ethanol would be more rapid than from a nonpolar carrier such as oil. This was, however, not the case: 48 h postinjection was the optimal sampling time regardless of carrier type.

Significant linear relationships between SSDH activity and toxicant dose were obtained with P, CP, PPP and CCl_4. In all cases the increases were substantial, ranging up to 800% of control levels. Based on control activity of 0.52 $mU.mg^{-1}$ protein (Table 1) and a log standard deviation of 0.13, we calculated that a minimum sample size of 9 fish per treatment would be required to routinely detect a 20% increase in activity with a confidence level of 95% (Steel and Torrie, 1960).

Increased SSDH activity was accompanied by decreased serum protein levels for P, PPP and CCl_4. Changes in serum protein levels under toxicant stress are poorly understood, but can theoretically result from hemodilution, loss of protein to urine following kidney damage, or increased protein utilization without replenishment. The increased hematocrit shown by fish exposed to CCl_4 indicates hemodilution, and is consistent with previous work (Dixon et al., 1985). The absence of altered hematocrit in fish exposed to P and PPP suggests another, as yet unknown, mechanism.

The changes in protein levels introduce a bias to the use of SSDH as an indicator of toxic chemical effects. If protein levels change for a reason other than liver damage, then changes in the

volumetric activity ($mU.mL^{-1}$ serum) can be magnified or diminished when activity is expressed as $mU.mg^{-1}$ protein. Obviously, protein does not provide the stable base that is required for unbiassed results. An alternative might be to use hemoglobin or hematocrit, but chemicals causing hemolysis could generate bias in this case. Clearly, changes in hematocrit, protein, and SSDH must be examined together to understand toxicity and to recognize confounded results.

Liver tissue was chosen for histological evaluation since the level of SDH activity in liver is about an order of magnitude higher than the level in any of the nine other tissues measured to date (unpublished data of the authors). Damage to liver would therefore release relatively more SDH to blood than comparable levels of damage to other tissues. The absence of histopathology after a single IP dose of toxicant indicates that the biochemical lesion (release of SDH to serum) precedes histological damage. This is supported by constant LSIs, which often increase when histological lesions occur, and by the presence of both increased LSIs and histopathological lesions in fish exposed continuously to waterborne PMP. It would appear that while a single toxicant dose caused only changes in SSDH, a more prolonged waterborne exposure was required to cause gross changes in liver morphology.

Elevated SSDH activity is not an artifact of IP dosing, since waterborne exposure to PMP and Cu resulted in significant increases in SSDH activity. With PMP, increased SSDH activity was accompanied by hepatic lesions and elevated LSI. In contrast, Cu exposure caused a slight but significant increase in SSDH activity with no change in LSI or evidence of liver pathology. This implies that while liver damage occurred, it was not of sufficient magnitude to alter LSI and liver pathology. The absence of altered SSDH, LSI or the induction of hepatic lesions by HCN is consistent with its mode of action as a metabolic inhibitor rather than an overt hepatotoxicant.

The increased LSI and liver glycogen vacuolation of fish fed the high-carbohydrate diet, relative to those on the low-carbohydrate diet, is the expected response of rainbow trout to excess dietary carbohydrate (Hilton and Atkinson, 1982). Fish with elevated liver glycogen levels suffer impaired liver function (Dixon and Hilton, 1981; Hilton and Dixon, 1982). This impairment

probably caused the elevated SSDH levels of the control fish fed a high-carbohydrate diet, relative to controls fed a low-carbohydrate diet. IP dosed CCl_4 increased SSDH activity in fish reared on both the low- and high-carbohydrate diets by about the same amount (25.3 and 29.8 $mU.mL^{-1}$ serum respectively). Hence, the elevations caused by excess liver glycogen and CCl_4 appear additive.

Monitoring changes in SSDH activity has potential as a sensitive biochemical indicator of liver damage in salmonids. SSDH responded more quickly, and at lower levels of toxicant exposure, than either LSI or histopathology. Although the response was variable, this was compensated for both by the size of the increases evident, and by the linear nature of the dose response. We have used the response to estimate common endpoints of toxicity for comparison of large numbers of chemicals (Kaiser et al., 1984) and routinely use the response to monitor liver damage during chronic toxicant exposure in the laboratory (Dixon and Hilton, 1985). We are currently undertaking research on the application of SSDH to evaluate toxicant effects in field situations.

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e 1. Serum sorbitol dehydrogenase activities of control rainbow trout. All averages are geometric means unless otherwise indicated.

Experiment	Carrier	Exposure time (h)	SSDH activity ($mU.mL^{-1}$ serum)[1] mean	relative error (%)	SSDH activity ($mU.mg^{-1}$ protein)[1] mean	relative error (%)
2. Storage temperature			9.2	48.9	-	-
6. Sampling Interval - PMP[2]	saline	12	9.3	50.3	0.48	45.8
		24	9.4	80.7	0.48	83.3
		48	8.0	69.9	0.44	80.0
		96	8.9	47.0	0.45	62.2
B[3]	oil	24	11.1	23.9	0.47	23.4
		48	14.1	41.1	0.65	30.8
		60	10.9	45.9	0.52	42.3
		72	13.3	43.6	0.45	26.7
		80	10.6	38.7	0.56	35.7
		96	10.8	34.3	0.58	37.9
		120	11.1	38.7	0.60	33.3
Dose-response						
P[4]	saline	48	10.5	43.0	0.55	44.4
CP[5]	saline	48	10.5	46.2	0.53	52.1
PPP[6]	oil	48	11.0	58.2	0.55	52.7
B[3]	oil	48	14.6	37.5	0.59	24.8
Grand mean			10.7	46.7[7]	0.52	45.0[7]
N			16	16	15	15
95% confidence limits			9.8-11.7		0.49-0.56	
Relative error			17.8%		13.5%	
Mean activity, saline			9.39		0.49	
N			6		6	
95% confidence limits			8.4-10.5		0.44-0.53	
Mean activity, oil			11.86		0.55	
N			9		9	
95% confidence limits			10.7-13.1		0.50-0.60	

Table 1 continued.

$$\text{relative error} = \frac{(95\%\ \text{confidence interval})}{\text{Geometric mean}} \times 100$$

2. PMP = p-methylphenol
3. B = benzene
4. P = phenol
5. CP = chlorophenol
6. PPP = p-phenoxyphenol
7. arithmetic mean

Table 2. The effect of fasting on SSDH activity, serum protein, LSI and hemotocrit of rainbow trout. With the exception of SSDH activity, values are given as means, each with a sample size of 10, and the standard deviation in parentheses. SSDH values are given as geometric means with 95% confidence intervals in parentheses. Values without a superscript letter in common are significantly different.

Period of starvation (wk)	Weight (g)	SSDH activity ($mU.mL^{-1}$ serum)	Serum protein ($mg.mL^{-1}$)	SSDH activity ($mg.mL^{-1}$ serum protein)	LSI	Hematocrit (%)
0	$71.3(18.5)^{a}$	$11.1(9.4-13.1)^{a}$	$19.1(1.4)^{a}$	$0.58(0.50-0.68)^{a}$	$1.01(0.11)^{a}$	$32.5(2.7)^{a}$
3	$66.8(16.2)^{a}$	$11.2(8.2-15.4)^{a}$	$18.2(2.4)^{a}$	$0.62(0.45-0.86)^{a}$	$0.87(0.14)^{ab}$	$29.1(3.5)^{ab}$
6	$59.6(19.1)^{a}$	$9.9(7.6-12.9)^{a}$	$16.1(3.8)^{a}$	$0.63(0.46-0.85)^{a}$	$0.73(0.13)^{b}$	$21.4(3.6)^{b}$

Table 3. Effect of toxicant carrier on control SSDH levels of rainbow trout. With the exception of SSDH activity, values are given as means, each with a sample size of 10 and the standard deviation in parentheses. SSDH values are given as geometric means with 95% confidence intervals in parentheses. Values without a superscript letter in common are significantly different.

Treatment	Weight (g)	SSDH activity ($mU.mL^{-1}$ serum)	Serum protein ($mg.mL^{-1}$)	SSDH activity ($mU.mg^{-1}$ serum protein)	LSI	Hematocrit (%)
Uninjected	157.4 (29.2)[a]	10.5(8.3-13.4)[a]	20.1 (3.1)[a]	0.52(0.39-0.69)[a]	1.06 (0.16)[a]	38.6 (4.8)[a]
Sham injected	130.0 (25.3)[a]	11.0(8.6-14.1)[a]	21.1 (2.8)[a]	0.51(0.39-0.68)[a]	1.04 (0.14)[a]	36.4 (6.1)[a]
Saline injected	147.4 (32.7)[a]	10.5(8.5-13.1)[a]	20.6 (3.4)[a]	0.50(0.40-0.63)[a]	1.01 (0.11)[a]	35.3 (5.3)[a]
corn oil injected	159.8 (34.8)[a]	13.0(10.4-16.3)[a]	19.2 (3.6)[a]	0.67(0.53-0.86)[a]	1.05 (0.12)[a]	37.1 (4.1)[a]

Table 4. The impact of IP doses of phenol (P), p-chlorophenol (CP), p-phenoxyphenol (PPP) or carbon tetrachloride (CCl_4) on SSDH activity, serum protein level, LSI and hematocrit of rainbow trout. Fish were sampled 48 h postinjection. Except for SSDH activity, results are given as means, each with a sample size of 10, and standard deviation in parentheses. SSDH activities are geometric means with 95% confidence limits in parentheses. Within toxicants, values without a superscript letter in common are significantly different.

Toxicant	Dose ($mm.kg^{-1}$)	Weight (g)	SSDH activity ($mU.mL^{-1}$ serum)	Serum protein ($mg.mL^{-1}$)	SSDH activity ($mU.mg^{-1}$ serum protein)	Hematocrit (%)	LSI
P	0.00	118.1(25.6)^{a}	10.5(8.5-13.1)^{a}	19.6(4.7)^{a}	0.55(0.44-0.69)^{a}	43.8(4.9)^{a}	0.82(0.20)^{a}
	0.33	130.9(35.8)^{a}	16.3(12.3-21.8)^{abc}	16.3(2.7)^{a}	1.02(0.78-1.33)^{abc}	39.4(3.8)^{a}	0.76(0.12)^{a}
	0.59	136.5(30.5)^{a}	16.1(12.2-21.4)^{b}	18.7(4.3)^{a}	0.87(0.65-1.16)^{b}	38.8(3.6)^{a}	0.71(0.08)^{a}
	1.04	125.6(33.7)^{a}	18.2(13.1-25.5)^{bcd}	20.5(3.8)^{a}	0.91(0.62-1.34)^{bcd}	41.9(3.0)^{a}	0.73(0.12)^{a}
	1.82	134.3(27.8)^{a}	21.8(15.6-30.7)^{cd}	19.7(2.4)^{a}	1.18(0.86-1.62)^{cd}	39.9(3.4)^{a}	0.72(0.07)^{a}
	3.26	130.3(29.7)^{a}	26.5(22.4-31.4)^{d}	16.9(3.3)^{a}	1.60(1.27-2.01)^{d}	41.1(2.7)^{a}	0.77(0.19)^{a}
CP	0.00	152.6(41.2)^{a}	10.5(8.4-13.2)^{a}	20.3(3.1)^{a}	0.53(0.41-0.69)^{a}	40.2(3.6)^{a}	0.83(0.18)^{a}
	0.07	151.3(39.2)^{a}	15.0(11.8-19.1)^{ab}	19.0(3.2)^{ab}	0.80(0.64-1.00)^{bc}	40.8(4.5)^{a}	0.97(0.14)^{a}
	0.12	122.5(27.5)^{a}	19.2(15.3-24.3)^{bc}	17.7(3.9)^{ab}	1.12(0.81-1.53)^{abc}	44.1(3.7)^{a}	0.90(0.12)^{a}
	0.22	154.1(27.0)^{a}	22.7(18.0-28.8)^{abc}	21.4(4.4)^{a}	1.08(0.85-1.37)^{abc}	38.5(4.3)^{a}	0.95(0.09)^{a}

Table 4 con't.

	0.39	139.4(36.7)^{a}	26.5(20.8-34.0)^{cd}	17.0(1.9)^{ab}	1.57(1.14-2.17)^{cd}	39.1(5.8)^{a}	0.96(0.15)^{a}
	0.69	158.8(36.8)^{a}	32.9(22.0-49.5)^{d}	12.3(8.2)^{b}	3.22(2.31-4.94)^{d}	38.0(6.9)^{a}	0.88(0.13)^{a}
PPP	0.00	161.2(30.5)^{a}	11.0(8.2-14.6)^{a}	20.1(4.0)^{a}	0.55(0.43-0.72)^{a}	34.2(3.6)^{a}	0.88(0.10)^{a}
	0.67	143.7(28.2)^{a}	10.5(8.9-12.5)^{ab}	19.4(5.1)^{a}	0.55(0.45-0.67)^{ab}	38.0(4.5)^{a}	0.94(0.16)^{a}
	1.20	161.8(24.6)^{a}	13.0(11.1-15.2)^{ab}	19.9(2.6)^{a}	0.66(0.51-0.84)^{ab}	39.5(3.8)^{a}	1.02(0.23)^{a}
	2.13	155.2(27.5)^{a}	15.4(12.3-19.4)^{bc}	19.1(2.4)^{a}	0.81(0.67-0.99)^{bc}	38.9(3.1)^{a}	0.91(0.11)^{a}
	3.73	150.6(12.9)^{a}	23.5(19.2-28.8)^{cd}	17.4(3.0)^{a}	1.46(1.13-1.88)^{cd}	39.8(6.6)^{a}	0.89(0.06)^{a}
	6.67	170.2(24.3)^{a}	21.3(16.0-28.3)^{d}	10.9(3.3)^{b}	2.03(1.43-2.88)^{d}	35.2(3.3)^{a}	0.96(0.22)^{a}
CCl_4	0.00	151.1(48.3)^{a}	12.5(10.0-15.8)^{a}	22.3(4.4)^{a}	0.56(0.45-0.71)^{a}	33.4(2.6)^{a}	0.94(0.11)^{a}
	5.33	111.5(46.5)^{a}	17.6(14.5-21.4)^{ab}	20.4(3.6)^{a}	0.87(0.73-1.04)^{ab}	27.9(3.4)^{b}	0.97(0.13)^{a}
	7.70	136.3(62.7)^{a}	23.5(19.9-27.6)^{b}	18.3(4.1)^{ab}	1.24(0.98-1.58)^{b}	27.1(4.1)^{bc}	0.96(0.08)^{a}
	11.25	134.2(31.4)^{a}	37.6(32.7-43.2)^{c}	14.3(5.3)^{bc}	2.62(2.23-3.08)^{c}	24.1(4.3)^{bcd}	1.02(0.07)^{a}
	16.30	142.0(53.5)^{a}	39.3(32.3-47.9)^{cd}	10.1(4.8)^{c}	3.91(3.25-4.70)^{cd}	22.1(4.2)^{cd}	1.04(0.12)^{a}
	23.38	142.3(28.6)^{a}	52.9(44.9-62.4)^{c}	10.2(4.3)^{c}	5.19(4.40-6.12)^{d}	21.3(4.4)^{d}	0.99(0.12)^{a}

Table 5. The effect of exposure to waterborne PMP, HCN or copper on SSDH activity, serum protein, LSI and hematocrit of rainbow trout. SSDH values are given as geometric means with 95% confidence intervals in parentheses. All other results are given as means, each with a sample size of 10, and the standard deviation in parentheses. Within toxicants, values without a superscript letter in common are significantly different.

Toxicant	Concentration (uM)	Exposure time (h)	Weight (g)	SSDH activity (mU.mL^{-1} serum)	Serum protein (mg.mL^{-1})	SSDH activity (mU.mg^{-1} serum protein)	LSI	Hematocrit (%)
Control	0	24	87.3(21.8)^{a}	14.0(11.7-16.9)^{a}	22.3(4.1)^{a}	0.64(0.56-0.74)^{a}	0.98(0.09)^{a}	33.3(3.8)^{a}
		48	103.2(26.2)^{a}	15.3(13.4-17.5)^{a}	23.1(3.8)^{a}	0.68(0.62-0.75)^{a}	0.98(0.11)^{a}	30.8(4.1)^{a}
		96	90.4(31.4)^{a}	13.1(11.3-15.3)^{a}	20.2(4.0)^{a}	0.60(0.53-0.68)^{a}	1.03(0.13)^{a}	29.1(2.7)^{a}
PMP	28	24	93.6(28.4)^{a}	15.6(12.9-19.0)^{a}	20.0(3.6)^{a}	0.81(0.73-0.89)^{a}	1.01(0.08)^{a}	33.4(4.8)^{a}
		48	113.4(20.9)^{a}	23.3(19.8-27.5)^{b}	17.3(3.3)^{a}	1.37(1.23-1.52)^{b}	1.18(0.11)^{a}	30.0(3.6)^{a}
		96	71.3(19.5)^{a}	35.7(30.0-42.4)^{c}	15.2(3.0)^{a}	2.39(2.18-2.63)^{c}	1.42(0.19)^{b}	28.3(3.7)^{a}
HCN	0.5	24	88.5(20.7)^{a}	13.0(10.8-15.6)^{a}	20.3(3.6)^{a}	0.64(0.57-0.72)^{a}	0.68(0.13)^{a}	41.4(6.2)^{a}
		48	121.3(28.8)^{a}	12.0(9.8-14.7)^{a}	21.2(2.1)^{a}	0.57(0.47-0.69)^{a}	0.95(0.11)^{b}	33.5(4.1)^{ab}
		96	88.3(33.1)^{a}	13.5(10.9-16.8)^{a}	19.8(4.1)^{a}	0.67(0.53-0.85)^{a}	0.99(0.13)^{b}	30.6(3.9)^{b}

Table 5 con't.

Cu	0.0021	24	79.3(38.5)[a]	13.8(11.1-17.0)[a]	20.3(3.8)[a]	0.70(0.61-0.79)[a]	0.97(0.12)[a]	30.8(4.3)[a]
		48	91.4(27.5)[a]	21.1(18.4-24.1)[b]	21.2(4.2)[a]	1.00(0.89-1.12)[ab]	0.96(0.11)[a]	31.2(4.1)[a]
		96	111.3(22.7)[a]	22.5(18.7-27.0)[b]	20.4(3.4)[a]	1.12(0.99-1.26)[b]	0.94(0.13)[a]	28.7(5.1)[a]

Table 6. Effect of increased available dietary carbohydrate on SSDH activity, serum protein, LSI and hematocrit of control and CCl_4-dosed rainbow trout. The fish were reared on the diets for 16 wk. With the exception of SSDH activity, values are given as means, each with a sample size of 15, and the standard deviation in parentheses. SSDH values are given as geometric means with 95% confidence units in parentheses. Values without a superscript letter in common are significantly different.

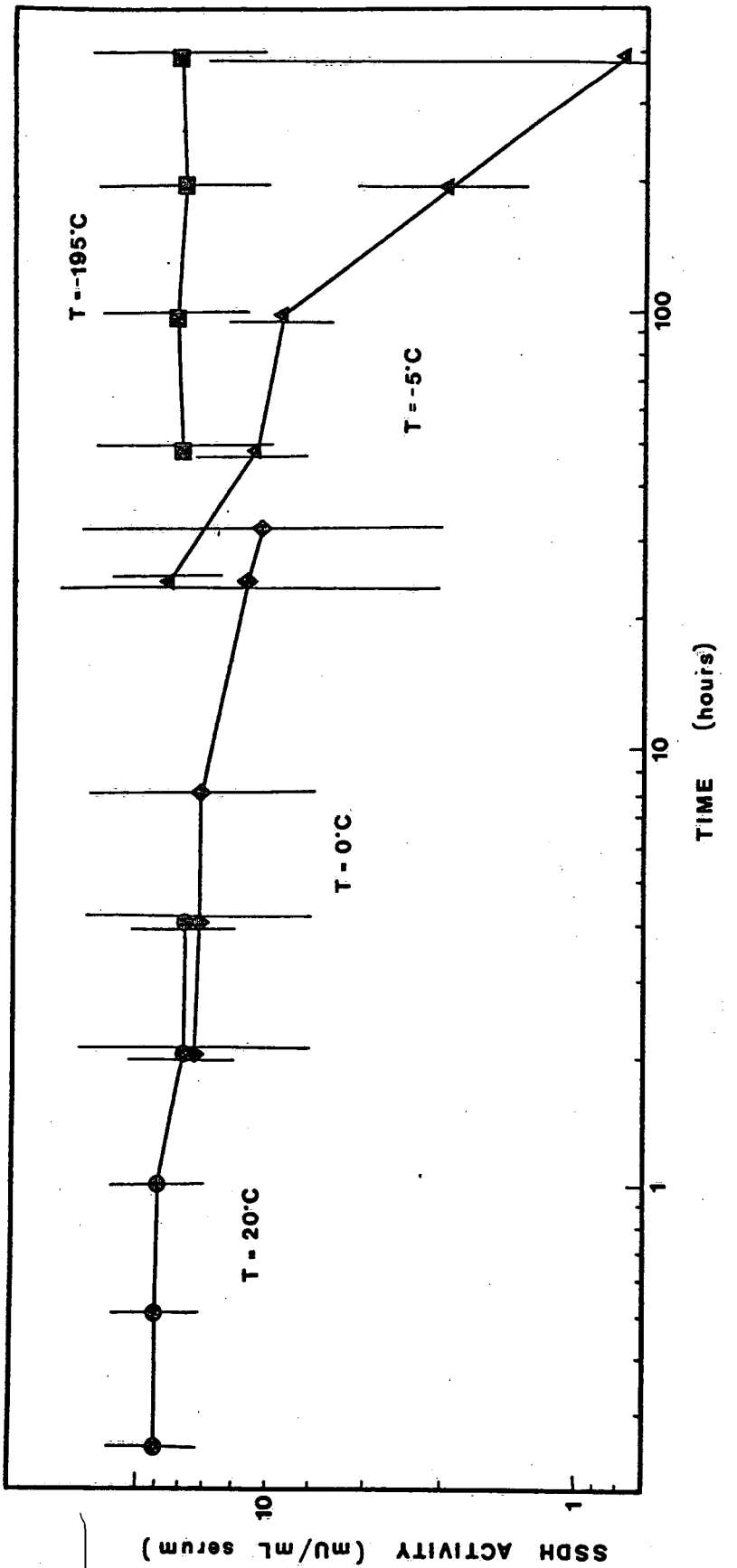
Diet	CCl_4 dose ($mm.kg^{-1}$)	Weight (g)	SSDH activity ($mU.mL^{-1}$ serum)	Serum protein ($mg.mL^{-1}$)	SSDH activity ($mU.mg^{-1}$ serum protein)	LSI	Hematocrit (%)
Low carbohydrate	0.0	46.0(2.1)[a]	12.7(10.8-14.8)[a]	21.8(5.1)[a]	0.56(0.47-0.66)[a]	0.94(0.11)[a]	38.4(4.6)[a]
	16.3	47.1(2.4)[a]	37.5(33.0-42.6)[b]	12.7(4.8)[b]	2.75(2.71-3.58)[b]	1.01(0.13)[a]	26.5(2.6)[b]
High carbohydrate	0.0	40.0(2.7)[b]	21.1(19.1-23.3)[c]	23.4(4.3)[a]	0.96(0.86-1.08)[c]	1.38(0.18)[b]	37.2(3.8)[a]
	16.3	38.6(3.1)[b]	50.0(44.2-56.6)[d]	11.3(5.1)[b]	4.14(3.36-5.10)[d]	1.48(0.26)[b]	24.4(3.1)[b]

Fig. 1. The relationship between serum storage duration and SSDH activity at four temperatures: 20, 0, -5 and -195^{0}C. Results are given as geometric means with 95% confidence intervals.

Fig. 2. Effects of postinjection holding period on SSDH activity in p-methylphenol (0.75 of the 96 h LD50) and benzene (0.50 of the 96 h LD50) dosed rainbow trout. Results are given as geometric means with 95% confidence intervals.

Fig. 3. The relationship between SSDH activity and toxicant dose for p-chlorophenol ($\log_{10}Y = 0.545 \log_{10}X + 0.497$; $r = 0.93$), phenol ($\log_{10}Y = 0.211 \log_{10}X + 0.033$; $r = 0.77$), p-phenoxyphenol ($\log_{10}Y = 0.595 \log_{10}X - 0.206$; $r = 0.94$) and carbon tetrachloride ($\log_{10}Y = 1.307 \log_{10}X - 1.014$; $r = 0.99$). The results are given as geometric means with 95% confidence intervals; the regression statistics are based on geometric means. The shaded bands are the 95% confidence intervals for control fish.

Fig. 1


T = -195°C
T = -5°C
T = 0°C
T = 20°C
100
10
1
TIME (hours)
10
1
SSDH ACTIVITY (mU/mL serum)

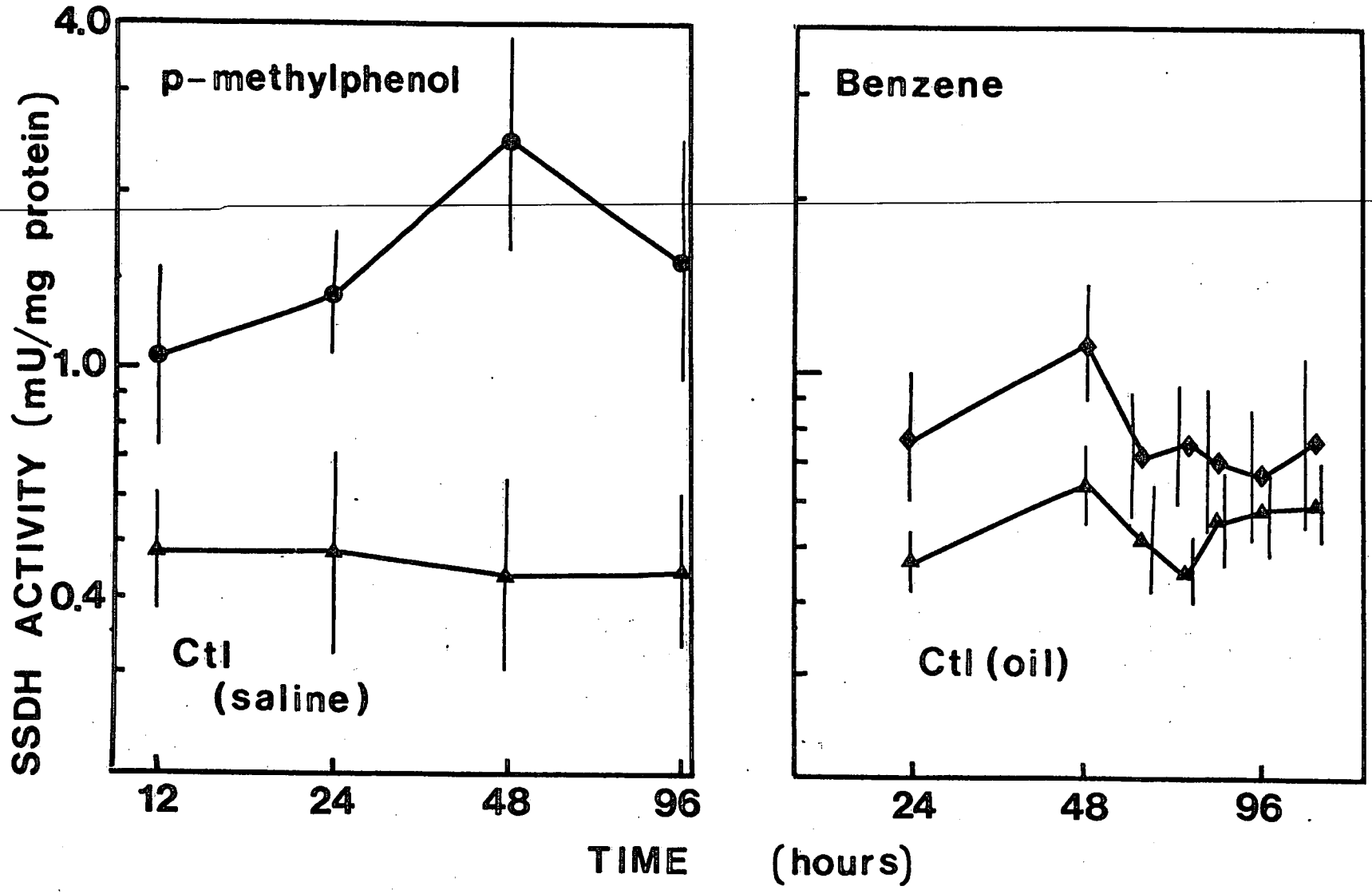

4.0
1.0
0.4
SSDH ACTIVITY (mU/mg protein)
p-methylphenol
Ctl
(saline)
12
24
48
96
TIME
Benzene
Ctl (oil)
24
48
96
(hours)

Fig. 2

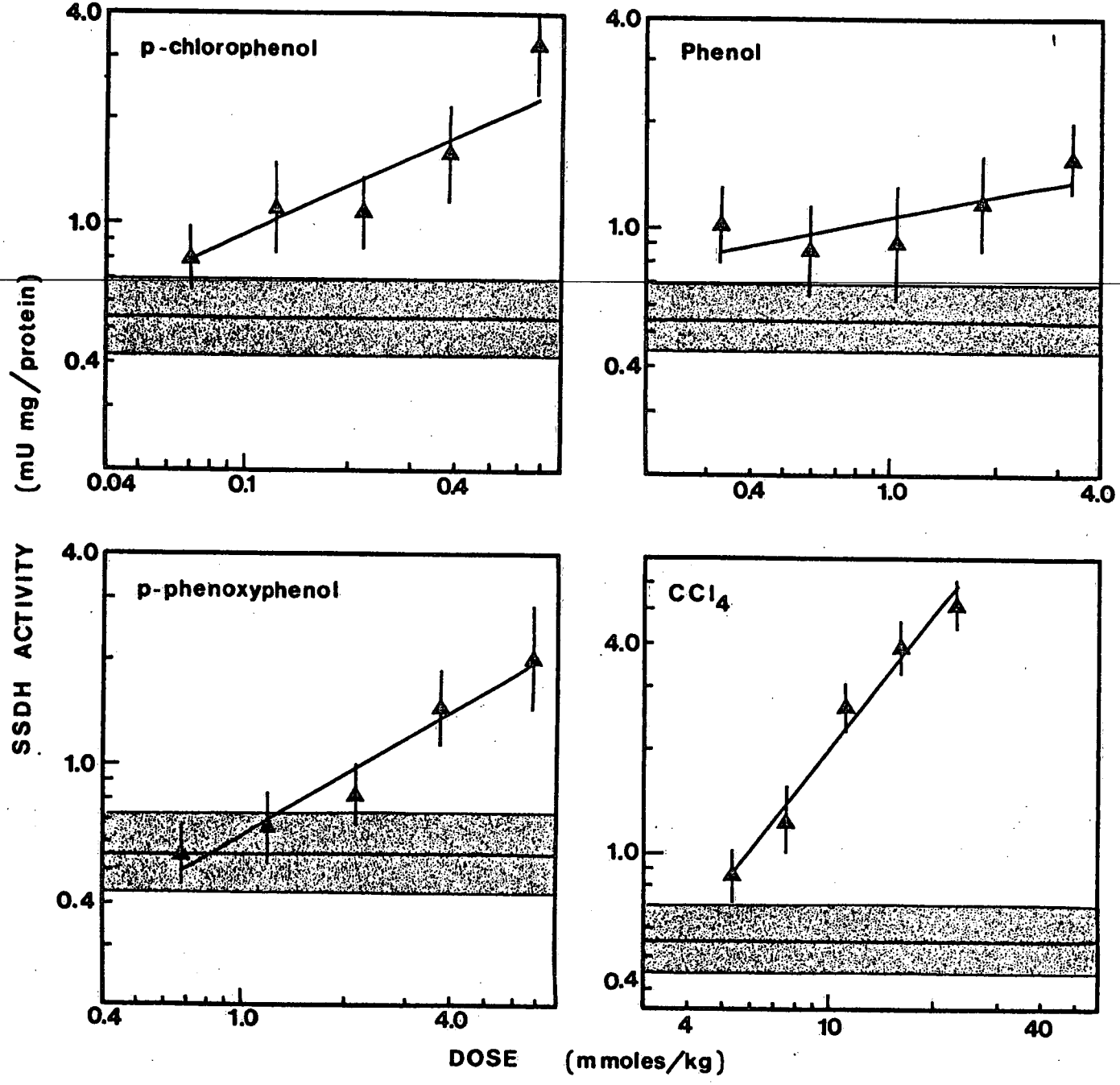
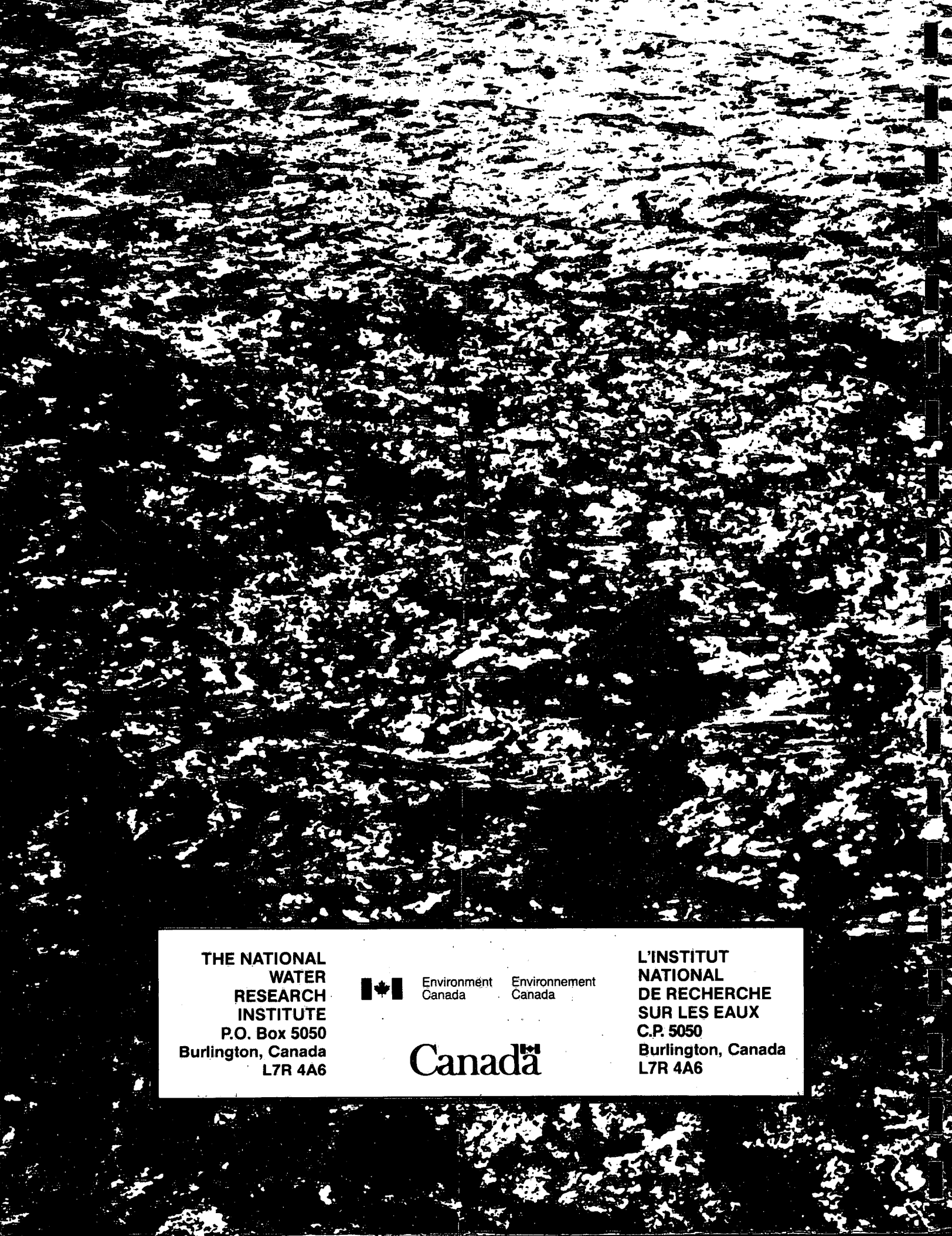

p-chlorophenol
Phenol
p-phenoxyphenol
CCl_4
4.0
1.0
0.4
0.04
0.1
0.4
1.0
4.0
10
40
SSDH ACTIVITY (mU mg/protein)
DOSE (mmoles/kg)

Fig. 3



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