

Avoidance-Preference Reactions of Rainbow Trout after Prolonged Exposure to Chromium (VI)

I. Anestis

**A Manuscript Report Prepared for the
Canadian Environmental Assessment
Research Council
1986**

Avoidance - Preference reactions of Rainbow Trout (Salmo gairdneri) after prolonged exposure to Chromium (VI)

I. Anestis and R.J. Neufeld

Faculty of Engineering
McGill University
3480 University St.
Montreal, Quebec
Canada
H3A 2A7

Keywords: avoidance - preference, Salmo gairdneri, chromium (VI)
toxicity, toxic pre-exposure

ABSTRACT

Avoidance-preference reactions were studied in rainbow trout (Salmo gairdneri) pre-exposed for 7-20 weeks to potassium dichromate solutions ($K_2Cr_2O_7$) ranging from 0.01 to 3.0 mg/l as Cr(VI). Experiments were performed in a hydraulic channel 9.15 m long by 30 cm wide, partly divided along its length and at a flow depth of 30 cm, combining steep and shallow gradient characteristics. An avoidance threshold value of 0.028 mg/l was determined for a population which had not been previously exposed to chromium while avoidance thresholds for pre-exposed fish increase linearly with the level of pre-exposure. Pre-exposed fish exhibited lower avoidance reactions compared to the nonexposed population. Avoidance reactions decreased with increasing level of pre-exposure. Fish exposed to test concentrations matching their pre-exposure

level clearly preferred this same concentration over any adjacent lower or higher test concentration. After 7 days of acclimation in clear water fish previously exposed at 0.01-0.8 mg/l Cr(VI), behaved similarly to the nonexposed population, indicating functional recovery of chemoreceptive capacity, while fish pre-exposed beyond the 0.8 mg/l Cr level did not recover fully within the same 7 day acclimation period. The 0.8 mg/l Cr(VI) level is proposed as a critical pre-exposure level for short term recovery of normal chemoreceptive capacity. Times of pre-exposure within the range used for the study had no influence on avoidance reactions.

INTRODUCTION

Lethal bioassays are routinely used to assess the toxicity of a particular pollutant. Sublethal effects, although more difficult to evaluate, provide much more meaningful information on the environmental impact of the toxicant, since sublethal levels are those commonly encountered in natural waters. The objective of laboratory sublethal toxicity testing is to establish effluent threshold levels, below which fish would not be exposed to hazards. This aim may be achieved by conducting dose-response studies for several concentrations and pre-exposure levels. Further, by comparing data on sub-lethal thresholds with expected effluent levels in receiving waters, the long term health of the fishery resource may be assessed. However, the influence of long term exposure to such levels on changes in behavioural reactions has

received little investigation. Resulting changes in behaviour could have significant environmental implications and might be of considerable importance in research carried out for the purpose of setting standards for water quality. Researchers testing the effects of sublethal levels of toxicants invariably use fish maintained in clean water, neglecting the effects of pre-exposure and adaptation of fish to low-levels of pollution.

The effect of pre-exposure to chromium (VI) was investigated by assessing the avoidance behaviour of rainbow trout following exposure to $K_2Cr_2O_7$ solutions for extended periods to different sublethal levels. Chromium was examined since it is a common pollutant, yet its toxicity is not well understood. The LC50s in the literature are widely scattered, ranging from 11.2-100 mg/l of Cr (Bills, 1977; Benoit, 1976; USEPA, 1976). Proposed 60-day no-effect values range from 51-105 mg/l (Sauter et al., 1976). Chromium is widely used in different industries and occurs in natural waters in Quebec, far from any pollutant source, at levels up to 0.04 mg/l (Goulet et al., 1982), while in the vicinity of industrial discharges, levels as high as 20 mg/l have been reported (EPS, 1983).

Sublethal effects were evaluated using avoidance-preference techniques. Many investigators have dealt with avoidance responses using a variety of apparatus, procedures, species and chemicals (Sprague 1970, 1971, 1976, Larrick et al., 1978, Cherry and Cairns, 1982; Hara et al., 1983; Beitinger and Freeman, 1983; Giattina and Garton, 1983). The last-named authors and Mello (1975) have pointed out that whole-organism behavioural responses

should be included in considerations for water quality criteria and environmental impact, since such reactions cannot be predicted from physiological and histological studies.

MATERIALS AND METHODS

Yearling trout were obtained from a commercial hatchery and maintained under flow-through conditions. Average test weight was 52 (range 44-64) g and total length 15 (range 13-17) cm. Density of fish in holding tanks never exceeded 0.75 g per litre of water per day. Holding tanks had a capacity of 1200 l and activated carbon filtered water was provided in the experimental channel at a flow rate of 114 l/min (175 l/gr fish/day). Sufficient aeration was provided to maintain the O₂ concentration above 90% saturation. The water temperature was maintained at 14.5 ± 1.0°C (McCauley et al., 1977) and water characteristics averaged 100 mg/l total hardness as CaCO₃, 50 mg/l alkalinity as HCO₃⁻, and pH 7.2.

Trout were pre-exposed to each level of potassium dichromate in 300 l static solutions containing 0.01, 0.1, 0.3, 0.8, 1.0 and 3.0 mg/l Cr(VI). Pre-exposure concentrations were nominal. The rate of chromium loss from the solution was determined by atomic absorption to be an average of 9% (range 4.5-13.5) after 1 day exposure. Pre-exposure solutions were changed daily.

All chromium in solution was assumed to be in the hexavalent form, due to the very slow reduction of chromium (VI) to chromium

(III), with the half life varying between 40 and 60 hours depending on the conditions (Kemp 1972, Benson, 1968). Three ionic species are present in the channel, namely $\text{Cr}_2\text{O}_7^{2-}$, HCrO_4^- and CrO_4^{2-} . $\text{Cr}_2\text{O}_7^{2-}$ is the predominant species under the experimental conditions while the reactive form of **Cr(VI)** in oxidative mechanisms is the HCrO_4^- monomeric species (Kemp 1972).

Avoidance-preference testing began 2 weeks after **stabilization** of pre-exposed populations i.e. when no apparent differences in fish mortality existed between non-exposed and pre-exposed populations. Fish were pre-exposed for a minimum of 7 weeks and the pre-exposure period in some cases was extended for a total of 20 weeks. The length of pre-exposure beyond the initial 7 week period was determined to have no significant effect on the response of the fish, (ANOVA, Probabilities varying from 0.063 - 0.64 > 0.05) indicating that fish were at a behavioural steady state and that small differences in size (± 2.0 cm) did not influence their avoidance reaction.

The 9.15 m test channel as first reported by Spraggs et al. (1982) was extensively modified. The channel as is illustrated in Figure 1 was structurally divided into five sections of equal length. The sides of the channel were made of glass and shielded from visual disturbances along the sides of the channel. The flow was split into two streams in the first upstream section of the channel. Concentrated **toxicant** was introduced into one side of the test channel by means of a pump through a planar discharge diffuser. Uniform vertical dispersion was achieved by gentle

mixing, with a vertically mounted multistage impeller. Uniform **toxicant** concentration in the **toxicant** zone could be maintained with the diffuser-impeller arrangement. Identical conditions were maintained by clear water injection in the clear water zone. The channel flow was maintained constant at 57 l/min in each separated zone yielding a velocity of 2.1 cm/sec throughout the length of the channel. The test concentration varied by changing the concentration of **toxicant** injected into the channel. A valve allowed for alternate use of the separated sides of the channel as the clear or **toxicant** zone and alternating side of injection yielded no statistically different results (ANOVA, Prob > F = 0.54 > 0.05). A honeycomb barrier prevented access by the fish to this upstream section. The fish were restricted to the second to fourth channel sections. The final downstream section was also inaccessible to the fish by means of a perforated end gate providing a uniform discharge region for the channel to ensure that its laminar flow characteristics were not disturbed. The actual testing zone was 5.49 m in length, with a lengthwise barrier separating the clear water zone from the **toxicant** discharge zone in the second and third sections of the channel (3.66 m). The fourth section (1.83 m) was not divided and served as a decision and gradual concentration gradient area.

The flow characteristics and concentration distribution in the channel were established by methylene blue injection. Samples were collected to yield lateral, longitudinal and depth profiles throughout the channel for channel flow and **toxicant** discharge

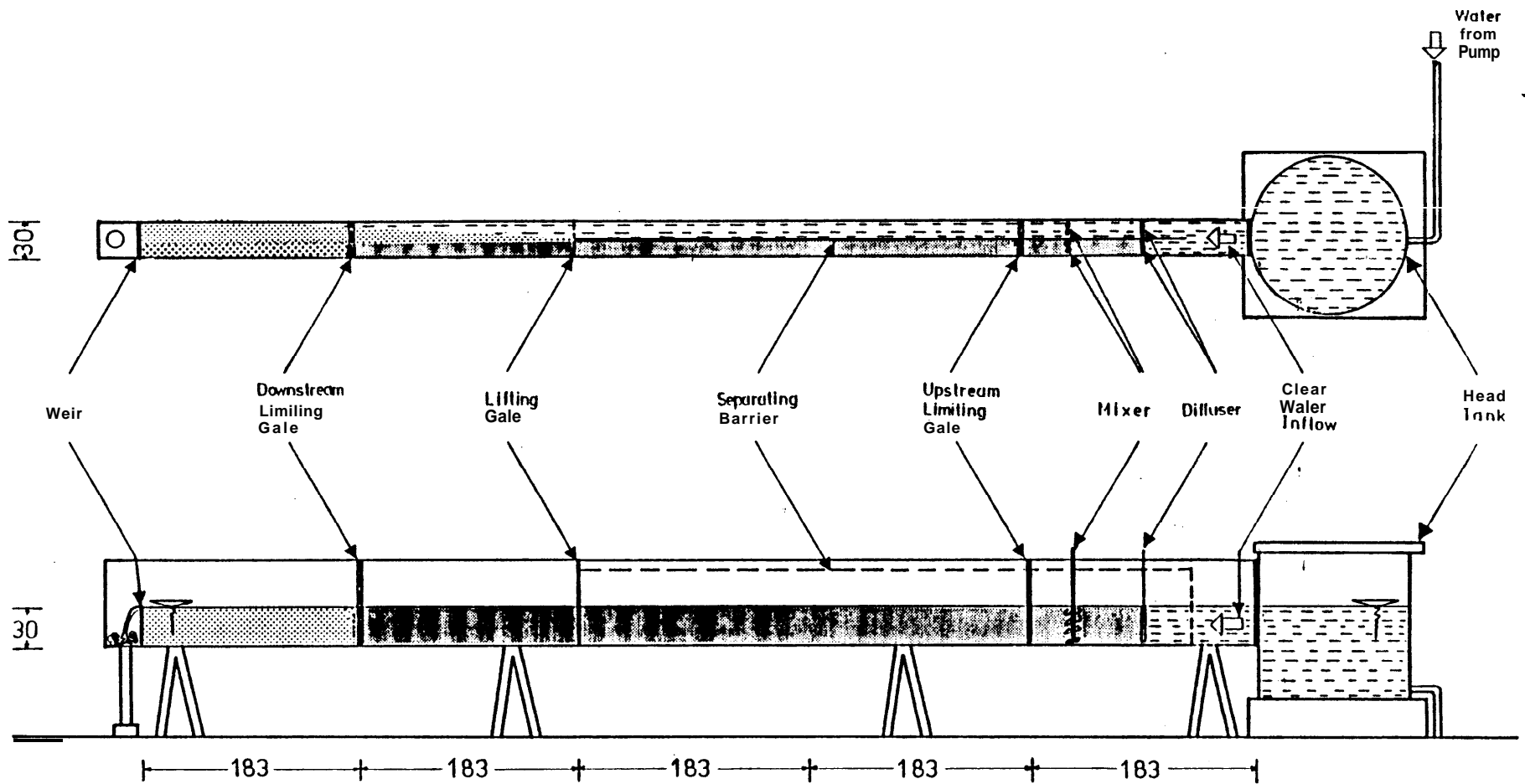


Figure 1. Experimental channel (all dimensions in cm)

Scale 1:40

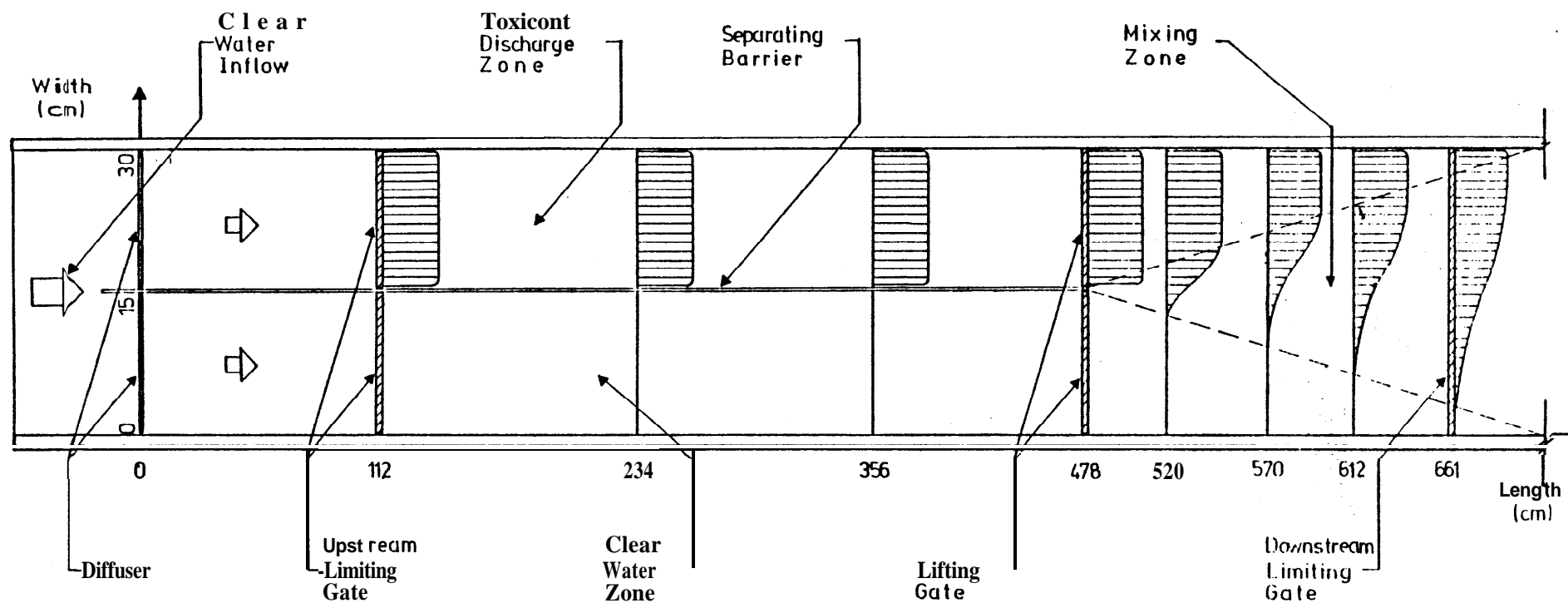


Figure 2. Lateral concentration distribution along the experimental channel for the selected test conditions as determined by dye injections (Dilution ratio in the toxicant discharge zone is measured to be $S = 0.0116$), where $S = \text{toxicant discharge/channel discharge}$).

conditions which represent those under actual testing (Figure 2). Concentration of dye was determined spectrophotometrically. Steady state was achieved in the channel, 15 minutes after commencement of tracer injection, at which time a uniform dye concentration was established throughout the divided section of the channel.

The mixing zone can be **characterized** by three regions (Figure 2). All mixing took place inside the cone region downstream from the edge of the dividing barrier, while on both sides of the cone, both clear and **toxicant** stream concentrations are maintained at the separated stream levels. Concentrations of methylene blue dyes measured in the channel were those expected from channel flow characteristics. Occasional sampling and analysis for chromium was performed during the actual experiments to establish any deviations, between expected and actual levels in the **toxicant** zone. Deviations ranged from -7 to **+13%** of the theoretical values.

Fish were acclimated for two days in the channel with clear flowing water prior to testing. The pre-test distribution was recorded and used as the reference level.

Two experimental techniques were employed in measuring the avoidance response to the **dichromate**. The first consisted of exposing the fish to a single concentration per experimental test and the second procedure involved exposing the fish to increasing concentrations in a step-wise fashion.

The bulk of the avoidance testing was performed using a **grad-**

ually increasing dose method for its time and economic efficiency, based on the results from preliminary tests that suggested no difference between this method vs. one single concentration per experiment. The sequence of doses in the channel was 0.08, 0.3, 0.8, 3.0 and 8.0 or 0.001, 0.01, 0.1, 1.0, 10, 33 mg/l Cr (VI).

Data obtained from experiments using single concentrations per experiment yielded results not significantly different from results obtained using the sequential increase method, (ANOVA, Prob > F = 0.32-0.69 >0.05) indicating that for practical purposes avoidance reactions are independent of the dose sequence.

Experiments were performed to establish the optimum number of fish to be used in a preference avoidance test based on the size of the fish used and the size of the apparatus so as to minimize the effect of territoriality and yield reproducible results. This was established at 18 fish per experimental run.

Prior to initial toxicant discharge, the fish were forced into the non-separated channel section for 15 minutes during which period steady state conditions with respect to toxicant concentration was established in the channel. An additional 5 minute period, 20 min from commencement of injection, was provided for fish to reach a steady-state distribution which was then recorded for 15 minutes on video tapes (20-35 minutes from initiation of a pollutant level). The next concentration level was then introduced and the procedure repeated without forcing the fish in the non-separated section again.

Additional experiments were performed on the pre-exposed

trout after 7 days acclimation in clear water following the pre-exposure period instead of the standard 2 days.

Three replicate experiments were routinely performed for each set of experimental conditions. All data obtained under the same experimental conditions were combined to produce a single avoidance curve.

Data analyses

An experiment which was recorded on video tapes was analyzed visually, by counting the number of fish in clear water regions every 1 minute over a 15 minute period. The percentage of fish avoiding the toxicant was determined using the formula:

$$\% \text{ Fish in clear} = \left[0.5 + \frac{\text{NFC} - \text{PTC}}{\text{TNF}} \right] \times 100$$

where: NFC = Number of fish during experiment in
clear water regions

PTC = Pre-testing number of fish in clear water
regions. (average of 15 readings)

TNF = Total number of fish in experimental channel

Compliance of all data with assumptions for normality and homogeneity of variability was established prior to analysis. Significant differences between means obtained from different populations under the same experimental conditions were assessed

by one way **ANOVA** followed by Duncan's tests (Morrison, 1976). Significant differences of means within the same experiment (sequential exposure of same population) were determined by growth curves analysis (**Capizzi** and Burton, 1978). The statistical significance of all results was assessed at 0.05 probability level. All mean values reported are statistically different from the pre-testing fish distribution denoted as **50%**, unless otherwise stated.

RESULTS

The avoidance behaviour of trout which had not been previously exposed to $K_2Cr_2O_7$ is illustrated in Figure 3. The results are presented as the per cent of fish on the clear water region of the test channel, plotted against concentration of **Cr(VI)** in the **toxicant** discharge stream of the channel. Values higher than 50% indicate avoidance, whereas lower values would suggest preference for the toxicant. No significant avoidance was observed at the lowest test concentrations 0.001 and 0.01 mg/l Cr, while at 0.08 mg/l, 54% of the fish population preferred the clear water zone (**ANOVA**, Prob $> F = 0.0011 < 0.05$). Avoidance was more pronounced with increasing concentration in the channel. A linear relationship on a log-normal scale as plotted in Figure 3 can be represented by equation (1).

$$\% \text{ of fish in clear} = 67.53 + 11.25 \log [Cr(VI)] \quad (1)$$

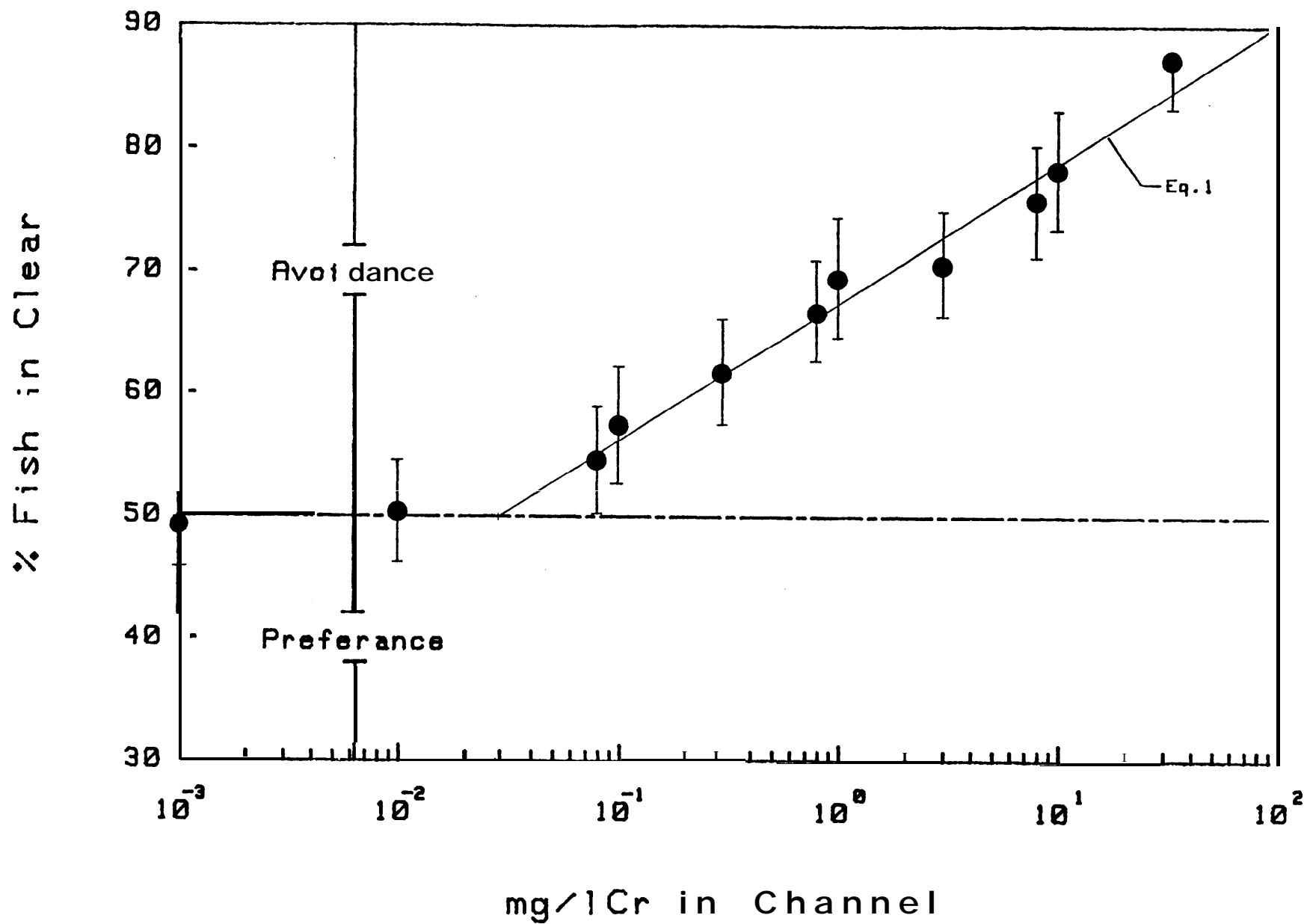


Figure 3. Avoidance reaction of non-exposed rainbow trout (Salmo gairdneri) to potassium dichromate.

where $[Cr(VI)]$ is the concentration of Cr in mg/l, with a coefficient $R^2=0.98$. This expression indicates an avoidance threshold level of 0.028 mg/l, and an avoidance response of 90% at the 96 hour LC50 value of 100mg/l proposed by USEPA (1976).

The avoidance behaviour of rainbow trout pre-exposed at 0.01 mg/l Cr is presented in Figure 4. At concentrations within the range of 0.001-0.1 mg/l, there was no statistically significant reaction of the trout. The actual mean of % fish in clear at 0.01 mg/l Cr in the channel was 51.7% (ST.D. = 5.6) compared to 52.2 (ST.D. = 4.1) and 53.5 (ST.D. = 5.5) for 0.001 and 0.1 mg/l Cr respectively, indicating a minimum avoidance reaction at concentrations equal to the pre-exposure level. For channel test concentrations higher than the pre-exposure level, the fish avoidance behaviour increased with increasing levels of chromium. This behaviour can be represented by equation (2) with a coefficient $R^2 = 0.92$ for concentrations above 0.01 mg/l Cr (VI).

$$\% \text{ fish in clear} = 59.87 + 8.59 \log [Cr(VI)] \quad (2)$$

The slope of equation (2) is less than that of the non-exposed fish population, however the calculated avoidance threshold value 0.071 mg/l Cr is higher than that derived for the control population. Results presented in Figure 4 also indicate that the avoidance response of fish acclimated for 7 days in clear water following the pre-exposure period was similar to that of the non-exposed population.

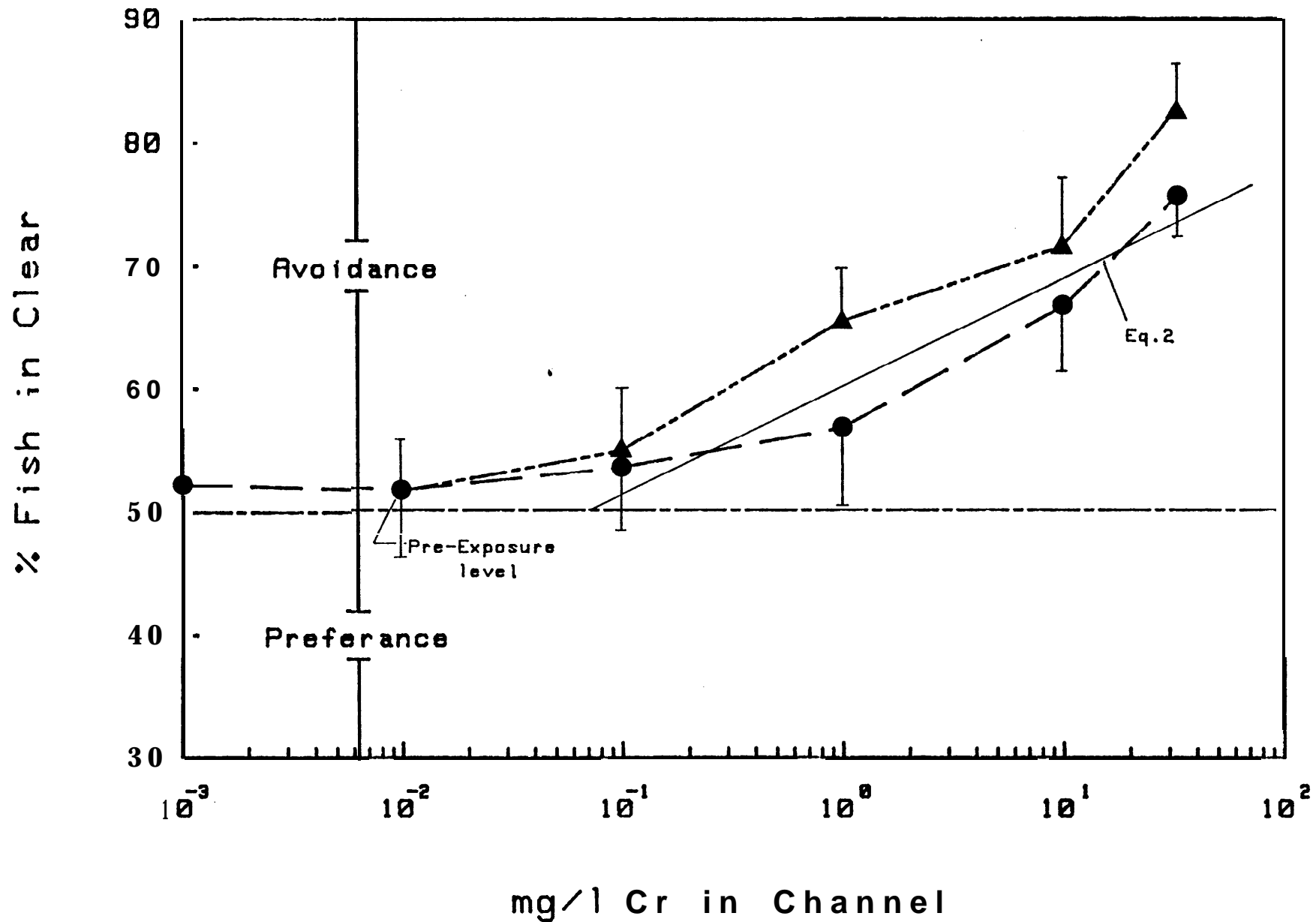


Figure 4. Avoidance reaction of rainbow trout pre-exposed to 0.01 mg/l Cr(VI)(●) and after 7 days of acclimation in clear water (▲).

The avoidance behaviour of rainbow trout pre-exposed at 0.1 mg/l Cr (VI) is presented in Figure 5. At concentrations well below the pre-exposure level (0.001 mg/l) a slight preference 45.4% (ST.D. = 5.2) was observed. At the next higher test concentration, fish exhibited a slight avoidance 54.2% (ST.D. = 6.2). When the toxicant level in the channel reached the pre-exposure level, an inflection point was observed on the avoidance curve, with 46.5% (ST.D. = 4.0) of fish in clear water. As concentrations of Cr in the channel increased above the pre-exposure level so did the avoidance reaction of the fish. Equation (3) represents the avoidance behaviour of rainbow trout for concentrations equal to or greater than the 0.1 mg/l pre-exposure level.

$$\% \text{ fish in clear} = 57.96 + 12.32 \log [\text{Cr(VI)}] \quad (3)$$

with coefficient $R^2 = 0.99$.

The slope of equation (3) was steeper than that of the non-exposed population, while the actual threshold value of 0.22 mg/l was 8 times higher than in the case of the non-exposed population. Results presented in Figure 5 indicate that the avoidance response of fish pre-exposed at 0.1 mg/l and acclimated for seven days in clean water was similar to that of the non-exposed population.

The avoidance behaviour of trout pre-exposed to 0.3 mg/l Cr(VI) is presented in Figure 6. At the lowest concentration tested (0.08 mg/l), the avoidance response was not statistically different (ANOVA, Prob > F = 0.43 > 0.05) than that of the non-

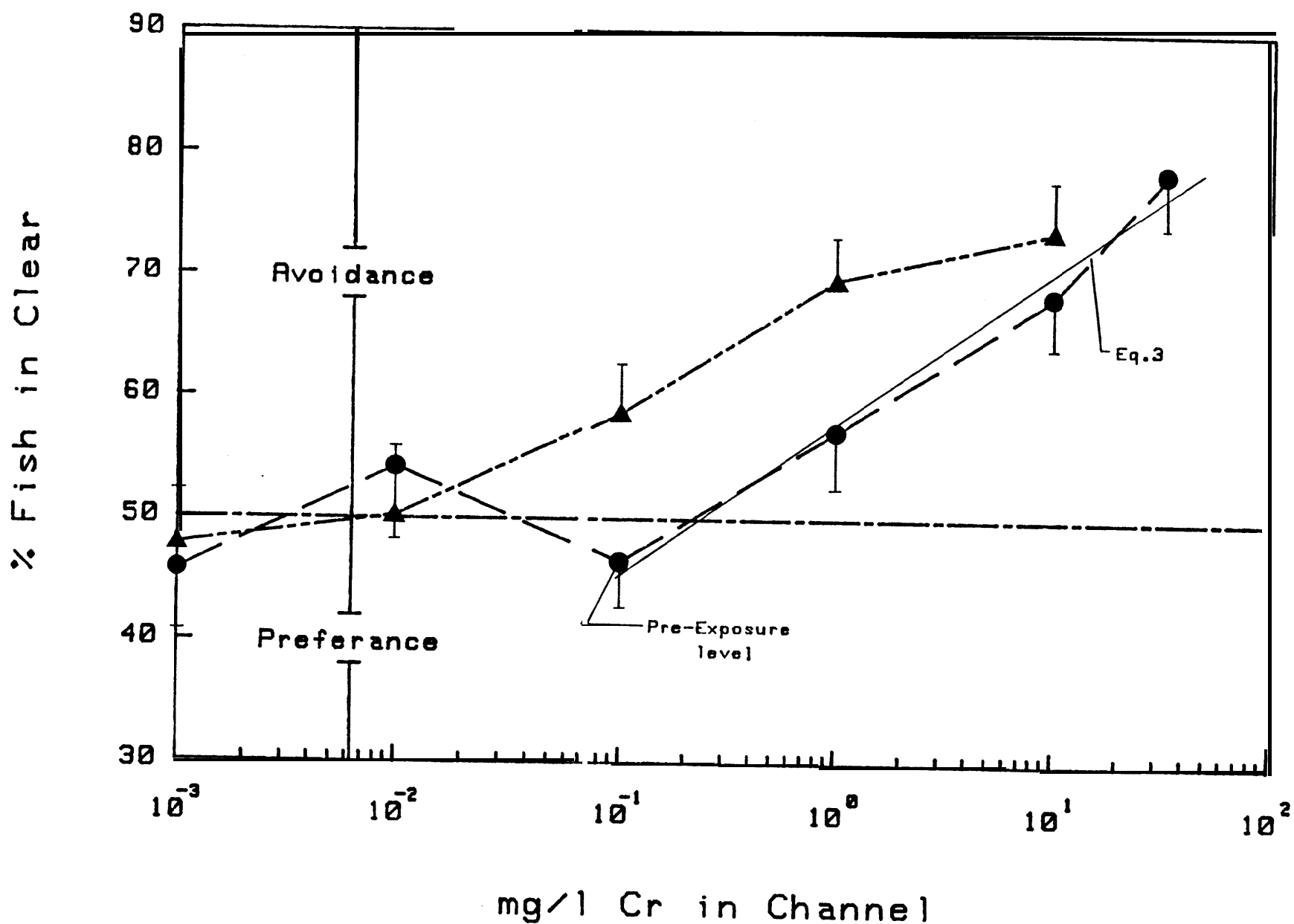


Figure 5. Avoidance reaction of rainbow trout pre-exposed to 0.1 mg/l Cr(VI) (●) and after 7 days of acclimation in clear water (▲).

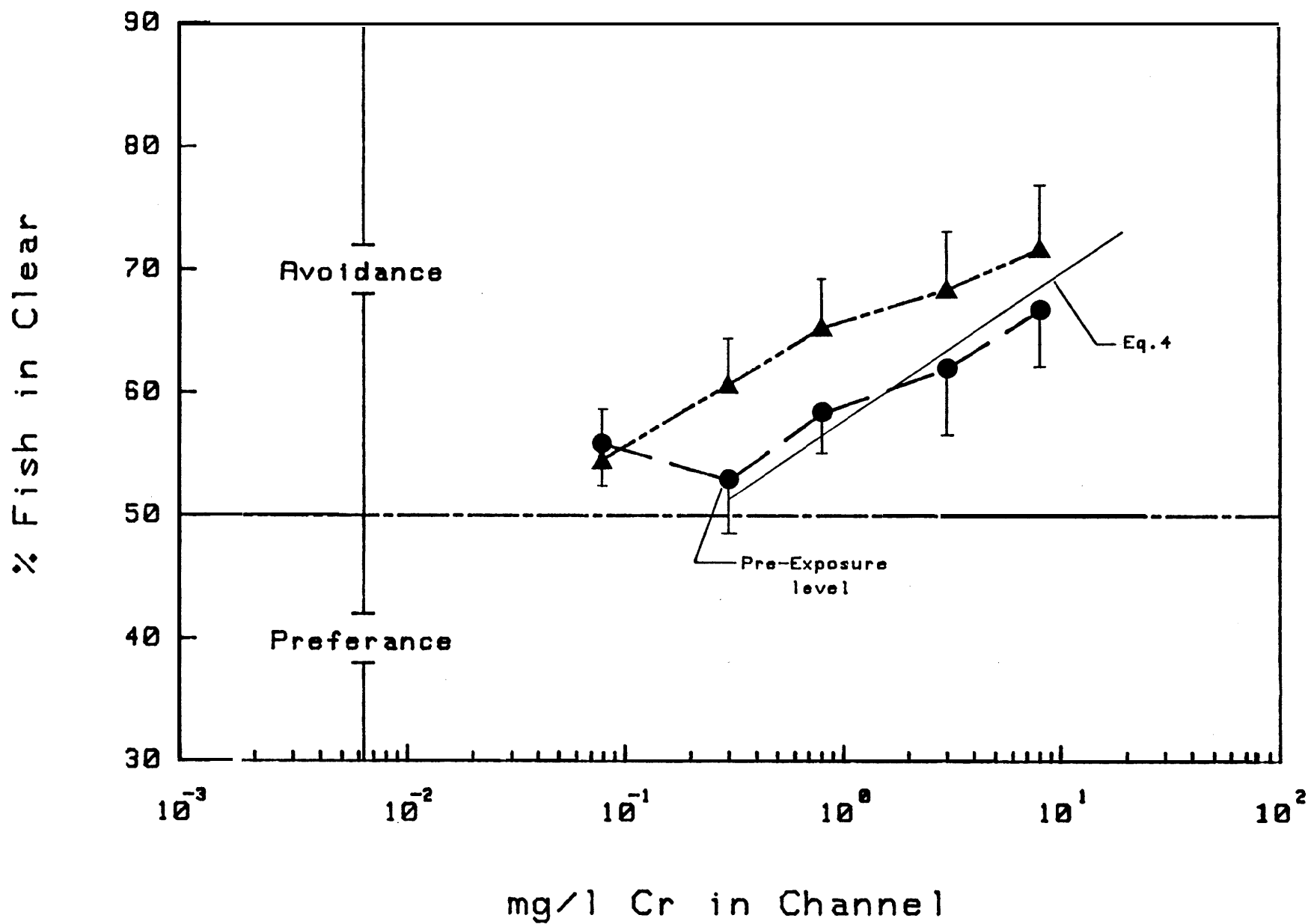


Figure 6. Avoidance reaction of rainbow trout pre-exposed to 0.3 mg/l Cr(VI) (●) and after 7 days of acclimation in clear water (▲).

exposed population. However, at channel test concentrations equal to or greater than the pre-exposure level, the response was significantly different from that of the non-exposed population.

(ANOVA, Prob > F = 0.0000-0.03 < 0.05). Again a behavioural change was observed at the point where the level of Cr(VI) in the channel was similar to the pre-exposure concentration. An inflection point in the avoidance curve was observed at this concentration which suggests that the degree of avoidance is a minimum at concentrations equal to the pre-exposure level, with increasing avoidance at concentrations less than or greater than pre-exposure concentration levels. At channel test concentrations higher than the pre-exposure level, the fish avoidance behaviour can be represented by equation (4).

$$\% \text{ fish in clear} = 57.64 + 9.96 \log [\text{Cr(VI)}] \quad (4)$$

with a coefficient $R^2 = 0.89$.

The slope of equation (4) is less than the one which characterizes the avoidance response for non-exposed fish. This expression yields an avoidance threshold level of 0.17 mg/l Cr., which is 6 times higher than the one for non-exposed. Results presented in Figure 6 indicate that the avoidance response behaviour of fish acclimated for 7 days in clear water following the 0.3 mg/l pre-exposure period was similar to that of the non-exposed population.

The avoidance behaviour pattern of trout pre-exposed to 0.8

mg/l Cr(VI) is presented in Figure 7. At the lowest channel test concentration (0.08 mg/l), no significant difference in response was observed from a pre-test fish distribution. At higher channel test concentrations, increasing preference for the **toxicant** was observed, reaching an inflection point on the curve representing maximum preference at the pre-exposure concentration.. This preference behaviour was in sharp contrast to the avoidance response of a non-exposed population or populations pre-exposed to lower concentrations.

Equation (5) describes fish avoidance behaviour for concentrations higher than 0.8 mg/l Cr (VI), the pre-exposure level.

$$\% \text{ fish in clear} = 36.78 + 16.76 \log [\text{Cr(VI)}] \quad (5)$$

with a coefficient $R^2 = 0.97$. The slope of equation (5) is steeper than that of the non-exposed population and the experimentally determined avoidance threshold value is 5.8 mg/l Cr(VI), twenty times higher than the control value. After 7 days acclimation in clear water following the pre-exposure period at 0.8 mg/l, the avoidance response of the fish (Figure 7) was similar to that of a non-exposed population.

The avoidance behaviour of rainbow trout pre-exposed at 1 mg/l Cr(VI) is presented in Figure 8. At concentrations well below the pre-exposure level, a slight preference was exhibited 44.4% (ST.D. = 4.7) and 43.6% (ST.D. = 3.3) at 0.001 and 0.01 mg/l respectively. At a concentration 10-fold lower than the pre-

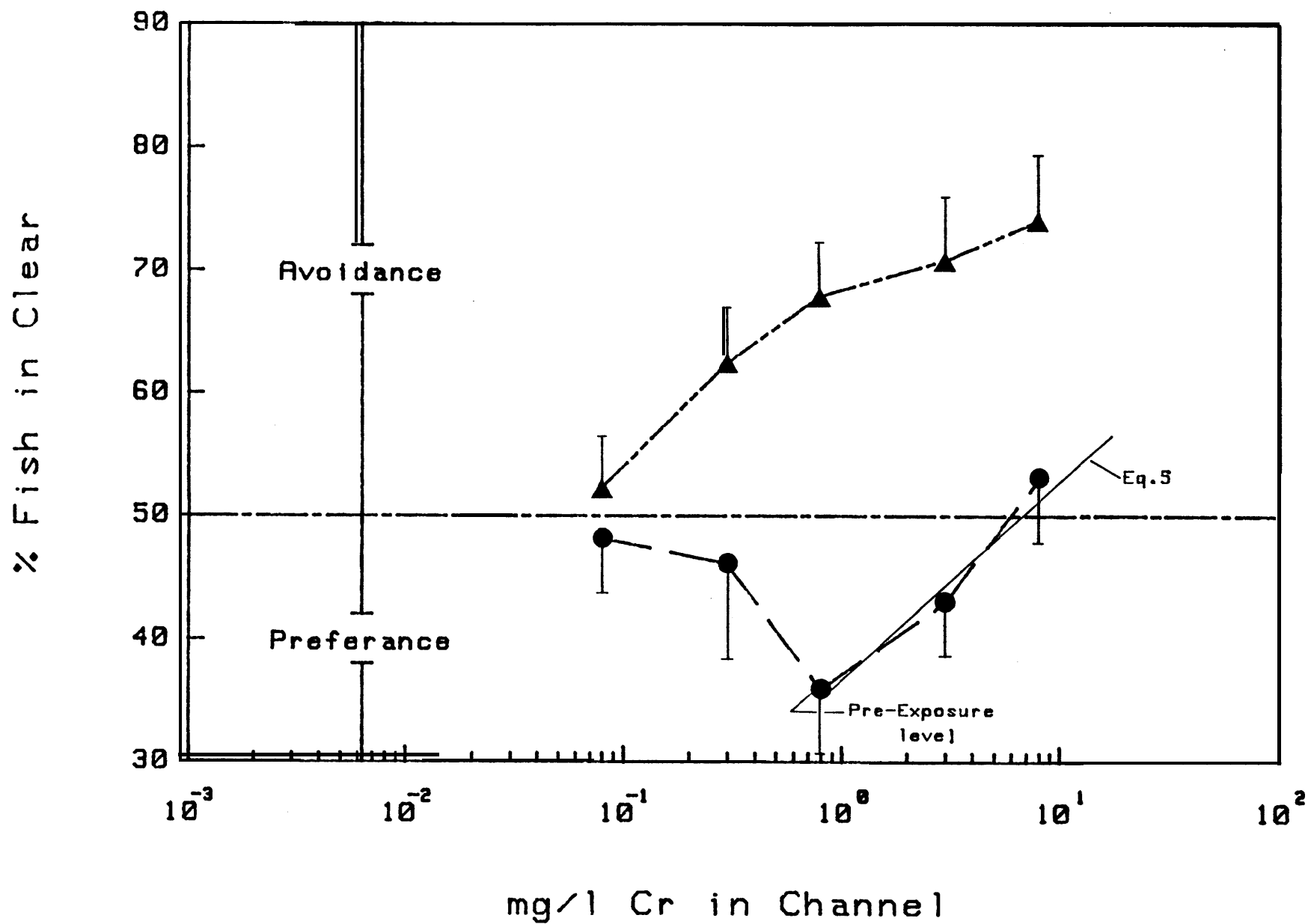


Figure 7. Avoidance reaction of rainbow trout pre-exposed to 0.8 mg/l Cr(VI) (●) and after 7 days of acclimation in clear water (▲)

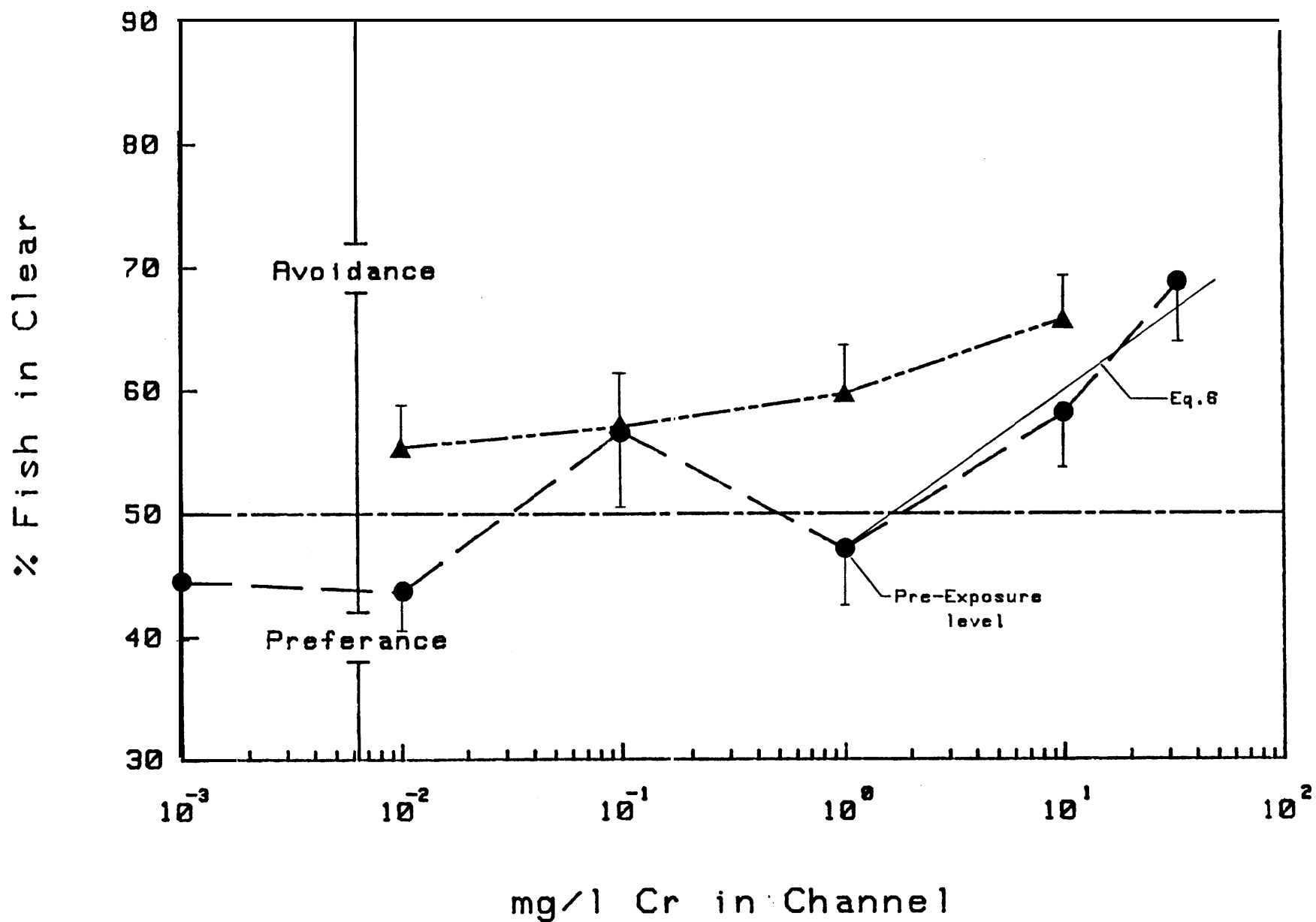


Figure 8. Avoidance reaction of rainbow trout pre-exposed to 1.0 mg/l Cr(VI) (●) and after 7 days of acclimation in clear water (▲).

exposure level, a net population shift was noticed towards the clear water which was again reversed as the concentration approached the level of pre-exposure. From then on a clearly increasing avoidance with concentration levels was exhibited. Equation (6) describes the avoidance behaviour of rainbow trout pre-exposed at 1 mg/l for concentrations higher than the pre-exposure level.'

$$\% \text{ fish in clear} = 46.35 + 13.80 \log [\text{Cr(VI)}] \quad (6)$$

with coefficient $R^2 = 0.98$. The actual threshold value was 1.8 mg/l Cr(VI) while the slope was steeper than the control.

Results obtained after 7 days acclimation of fish in clear water indicate that fish behaviour was not similar to that of the non-exposed population. Such behaviour suggested that fish had not fully recovered their chemoreceptive capacity.

The effect of pre-exposure at 3.0 mg/l Cr(VI) on the avoidance response behaviour of the fish is presented in Figure 9. An increase in the degree of avoidance was observed with increasing channel test concentrations reaching a maximum avoidance at a test concentration of 0.8 mg/l. An inflection point on the avoidance curve was again observed at a channel test concentration equal to the pre-exposure level.

Avoidance threshold values from all pre-exposure levels are presented in Figure 10. There was a linear increase in threshold values with increasing levels of pre-exposure with slight devia-

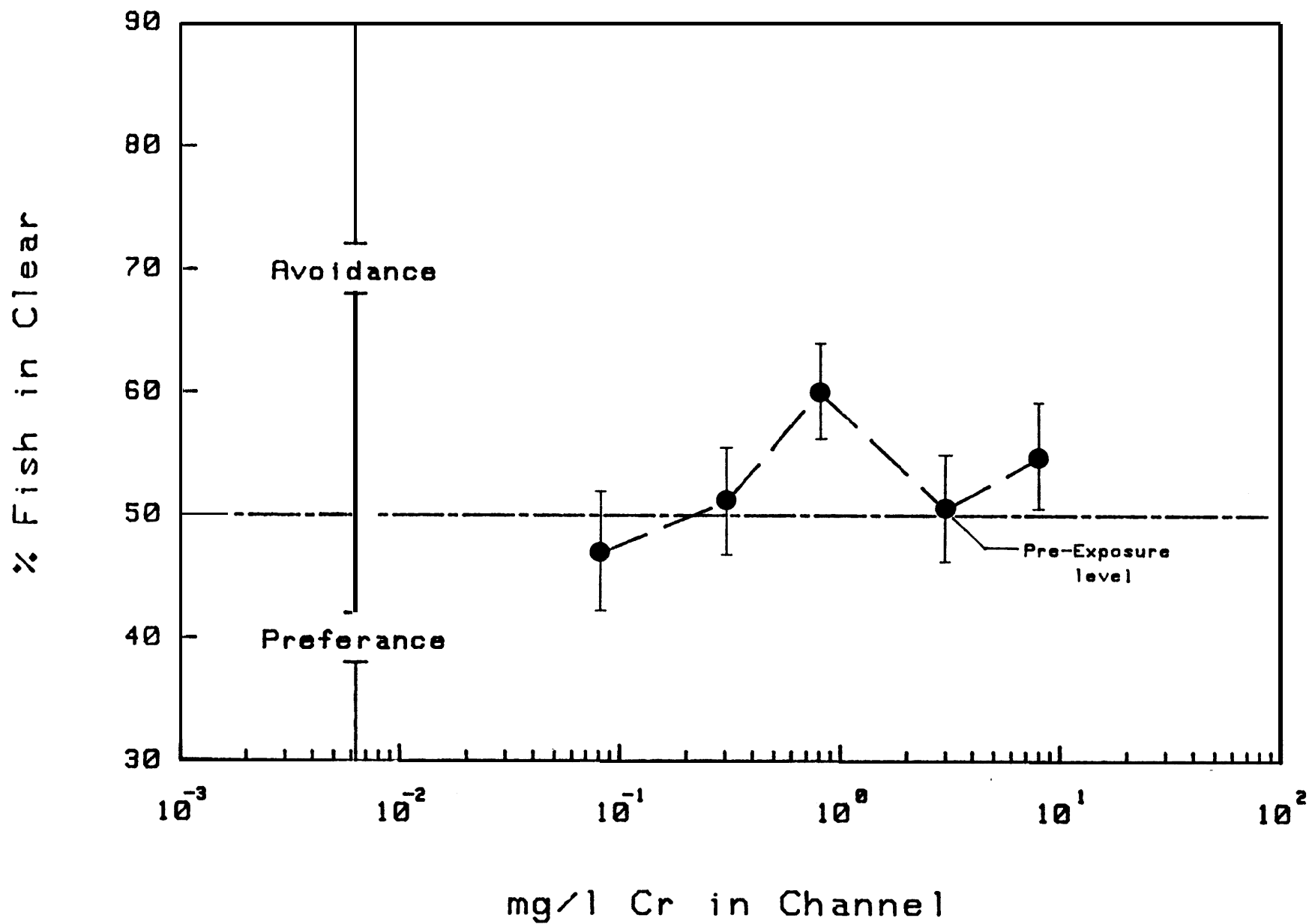


Figure 9. Avoidance reaction of rainbow trout pre-exposed to 3.0 mg/l Cr(VI).

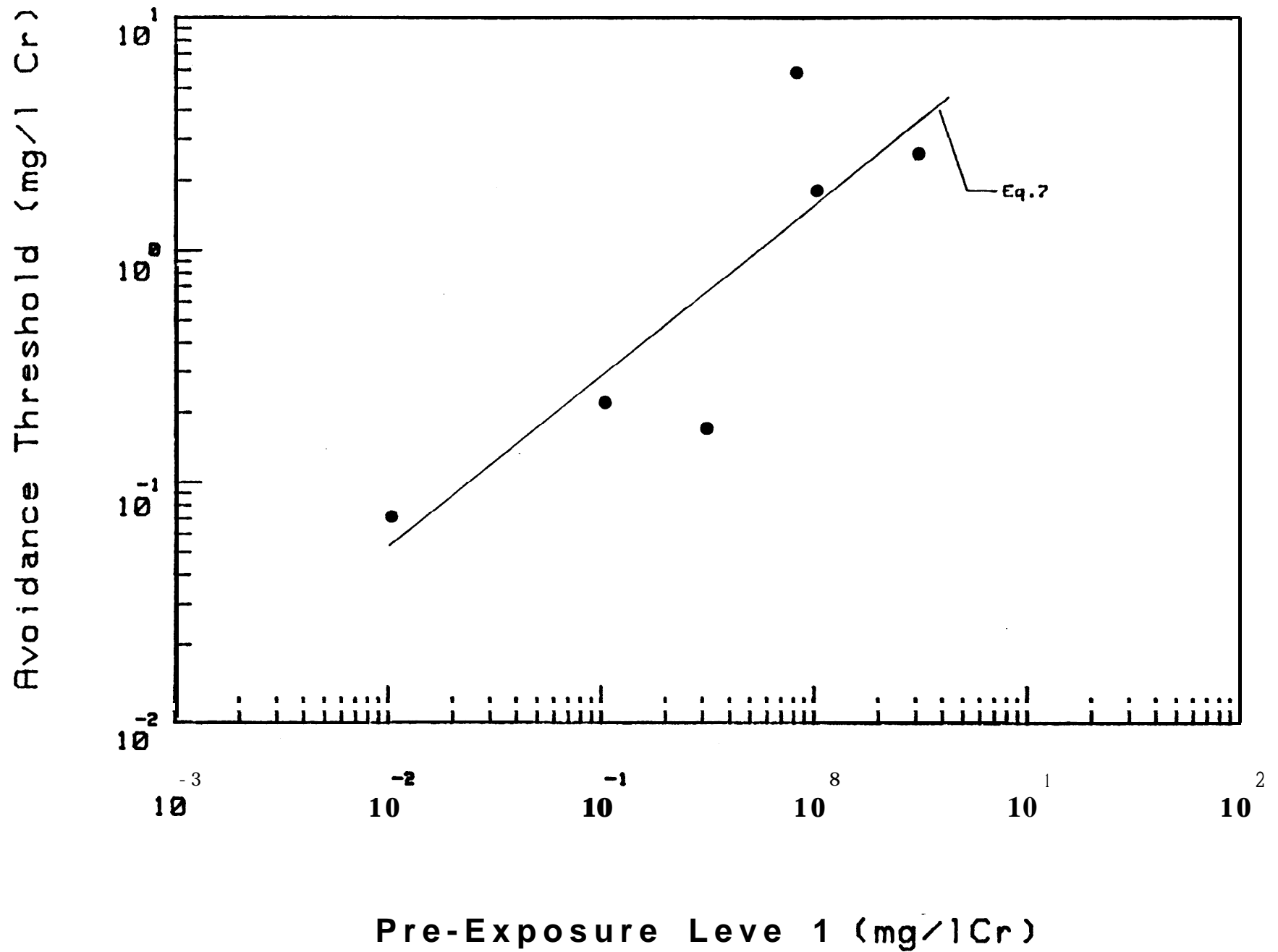


Figure 10. Rainbow trout avoidance threshold variation with increasing level of pre-exposure.

tions at 0.3 and 0.8 mg/l. Equation 7 describes the change in avoidance threshold values vs. the level of pre-exposure.

$$\log [\text{Avoidance threshold}] = 0.201 + 0.746 \log [P-E] \quad (7)$$

with $R^2 = 0.73$, where $[P-E]$ is the pre-exposure concentration.

Units on both sides of equation (7) are in mg/l Cr (VI).

DISCUSSION

The hydraulic channel used for avoidance response testing in this experimental work provided a means of establishing a uniform concentration toxicant zone, totally separated from a clear water zone. A well defined concentration and flow regime downstream from the barrier combined both steep and shallow concentration gradient characteristics. In addition, the non-separated section concentration distribution simulates a more natural concentration gradient ranging from clear water to the level of the toxicant zone.

Experiments performed on rainbow trout, which had not been previously exposed to Cr(VI), yielded an avoidance response curve described by equation (1). The predicted avoidance threshold value was 0.028 mg/l of Cr(VI), below which no significant response was detected. This avoidance threshold level is below the suggested value of 0.05 mg/l Cr(VI), proposed as an acceptable limit for water quality criteria (Clarke, 1974). The ability of

trout to detect and avoid concentrations below the acceptable limit would suggest that existing standards be re-examined.

Figure 11 is a three dimensional representation of the avoidance behaviour of rainbow trout pre-exposed at different concentration levels and reacting to a series of doses in the experimental channel. The surface produced may be used as a predictive tool of the actual avoidance-preference reaction of rainbow trout given any combination of pre-exposure and dose levels.

An increase in avoidance reaction for all dose concentrations higher than the pre-exposure levels may be observed in Figure 11. Avoidance reactions generally decrease with pre-exposure. A minimum in the avoidance reaction occurred at the 0.8 mg/l level of pre-exposure, represented by the valley region on Figure 11, in which case fish exhibited clear preference for the toxicant for all concentrations below their pre-exposure level. Populations pre-exposed to chromium concentrations higher than 0.8 mg/l, exhibited increasing avoidance reactions with increasing levels of pre-exposure. This change of trend in behaviour could be attributed to significant biological alterations to fish populations pre-exposed beyond 0.8 mg/l Cr(VI). Van der Putte (1982) reported that trout pre-exposed at 2.0 mg/l Cr(VI) were biologically different than a non-exposed population. In the present study, a higher mortality occurred in populations pre-exposed at 1.0 and 3.0 mg/l Cr(VI) compared to non-exposed fish populations indicating biological differences. In addition fish avoidance behaviour after 7 days acclimation in clear water following the pre-exposure

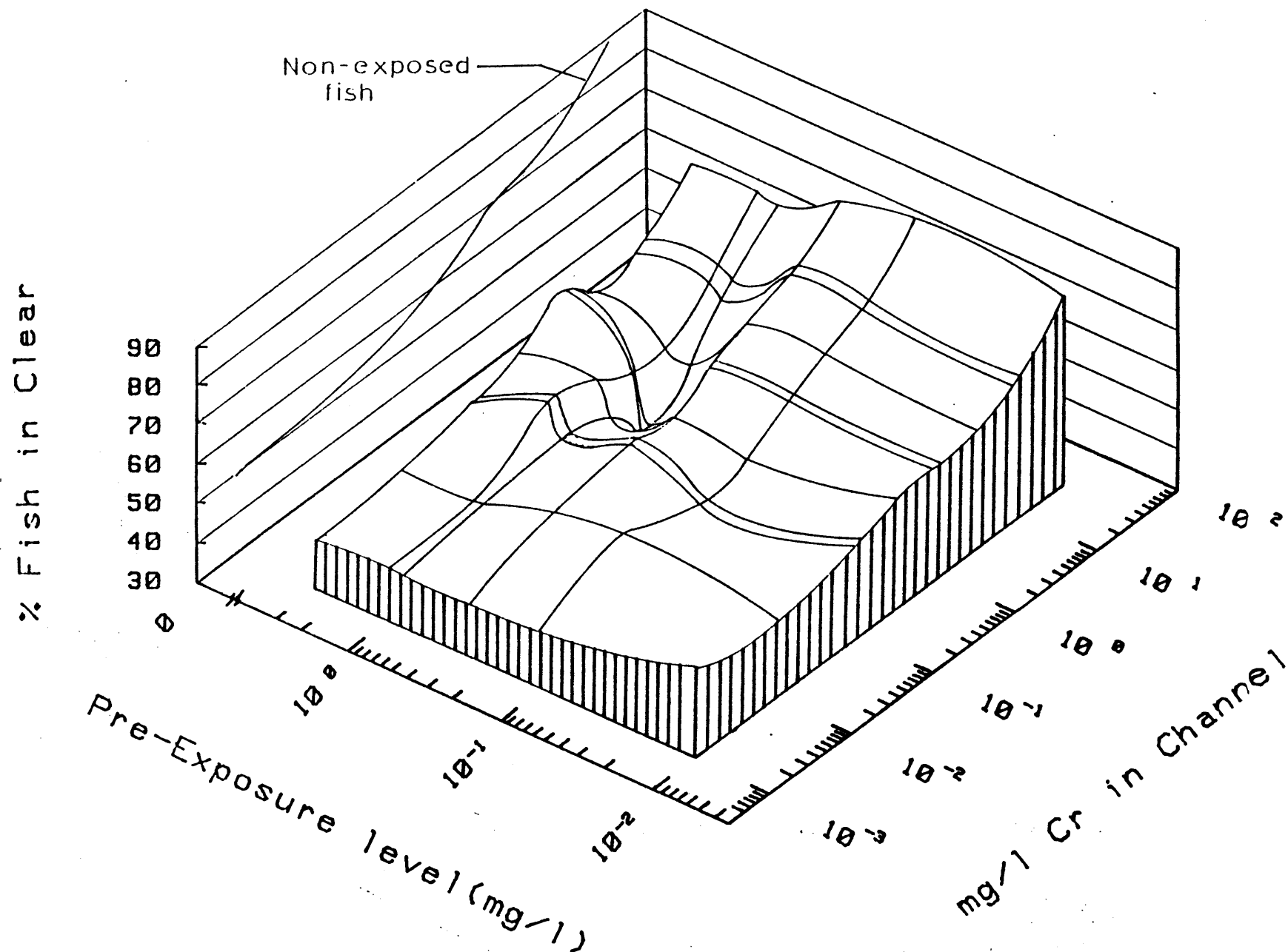


Figure 11. Avoidance reaction of rainbow trout to chromium (VI) after pre-exposure to varying levels of the toxicant.

period (Figure 8) was significantly different than the non-exposed population response indicating a longer recovery period at this higher level of pre-exposure.

All pre-exposed populations of trout demonstrated an attraction towards a familiar environment for concentrations that approached their pre-exposure level. Such preference is demonstrated graphically by the inflection points on all avoidance curves (Figures 5-9), and can be also depicted as a saddle on the surface of Figure 11 along the diagonal of X-Y plane. Despite the fact that fish were acclimated in clear water for 2 days prior to testing, they still demonstrated a distinctive attraction towards their familiar environment. This behaviour has never been reported in the literature. If this behaviour was applicable in natural waters, regulatory standards should be reconsidered in light of this physiological trap and of the additional consideration that avoidance of a pre-exposed population is lowered with increasing pre-exposure levels.

Equations (2) to (6), were derived to describe fish avoidance behaviour for concentrations higher than their pre-exposure level. These equations provided a tool to predict avoidance reactions given the pre-exposure concentration and can be used to determine avoidance threshold values for pre-exposed populations. Data for avoidance threshold values from pre-exposed populations yielded equation (7), which can be used to estimate the avoidance threshold value for any given level of chromium pre-exposure. Threshold values increase linearly with level of pre-exposure.

This behaviour is similar to that reported by Dixon and Sprague (1981), where lethal tolerance of rainbow trout to copper increased with levels of pre-exposure.

Fish populations pre-exposed up to the critical level of 0.8 mg/l Cr(VI) followed by acclimation for 7 days in clear water demonstrated a functional recovery of their chemoreceptive capacity within that period of time, since their respective avoidance curves were similar to the non-exposed populations (Figures 4-7). Singh and Ferns (1978) and Hara et al. (1983) suggested a 12 week rehabilitation period for biologically affected fish after pre-exposure to a pollutant. The present study suggested a much shorter period for functional recovery from chromium exposure to behavioural levels comparable with those of non-exposed populations.

Conclusions

- 1) Rainbow trout demonstrate an avoidance reaction to $K_2Cr_2O_7$ at levels greater than 0.028 mg/l as Cr(VI), the avoidance threshold level.
- 2) Populations of rainbow trout pre-exposed to $K_2Cr_2O_7$ exhibited a reduced avoidance reaction that reached a minimum at 0.8 mg/l as Cr(VI) pre-exposure. Pre-exposure levels higher than this critical level resulted in increasing levels of avoidance.
- 3) Trout pre-exposed to chromium demonstrated a pronounced preference towards their familiar environment, that is for a concentration similar to the pre-exposure level. Increased avoidance was observed at concentrations higher and lower than this pre-exposure level.
- 4) Threshold avoidance values increase linearly with increasing levels of pre-exposure.
- 5) Fish pre-exposed to chromium at levels below the critical level (0.8 mg/l), followed by 7 days acclimation in clear water exhibited similar avoidance reaction levels to non-exposed populations indicating a short functional recovery period. A slower recovery period was observed at pre-exposure levels higher than the critical level.

Acknowledgement

The authors wish to thank the Canadian Environmental Assessment Research Council for financial support to I. Anestis.

References

Beitinger T.L. and Freeman L. (1983) Behavioural avoidance and selection of fishes to chemicals. Residue Reviews 90, 35-55.

Benoit D.A. (1976) Toxic effects of hexavalent chromium on brook trout (Salvelinus fontinalis) and rainbow trout (Salmo gairdneri). Water Res. 10, 497-500.

Benson T.J. (1968) Mechanisms of Inorganic Reactions in Solution. An Introduction. McGraw-Hill, London.

Bills T.D. (1977) Effects of residues of polychlorinated biphenol Anedor 1254 on sensitivity of rainbow trout to selected environmental contaminants. Progressive Fish Culturist 39, 150-158.

Cairns J. Jr. (1981) Biological monitoring: Part VI - Future needs. Water Res. 15, 941-952.

Capizzi T. and Burton O.T. (1978). A Statistical technique for analyzing time response curves. In Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses (Edited by K.L. Dickson, Cairns J. Jr. and R.J. Livingston) ASTM 652, 137-149.

CEARC

Canadian
Environmental Assessment
Research Council

CCREE

Conseil Canadien de la
Recherche sur les
Évaluations environnementales

PROGRESS REPORT
to October 1985

INFORMATION

Cherry D.S. and Cairns J. Jr. (1982) Biological Monitoring Part V. Preference & Avoidance Studies. Water Res. 16, 263-301.

Clarke R. McV. (1974) A Summary of toxicity information for major effluent components from inorganic chemical industries. Dept. of Env. Fish and Marine Services, Tech. Rep. Ser. No. CEN/T-74-9, Aquatic Toxic Studies Division - Res. Manag. Branch, Winnipeg.

Dixon D.G. and Sprague J.B. (1981) Acclimation to Copper by Rainbow Trout (Salmo gairdneri) - A modifying factor in toxicity. Can. J. Fish Aquat. Sci. 38, 880-888.

Environmental Protection Services, Environment Canada (1983) (unpublished data) Cr pollution from various industries in Quebec.

Giattina J.D. and Garton R.R. (1983) A review of the preference-avoidance response of fishes to aquatic contaminants. Residue Reviews 87, 44-90.

Goulet M., Potvin P. and Primeau S. (1982) Toxiques inorganiques dans l'eau des rivières et des lacs du Québec Meridional. Envirodoc #3981. Ministère de l'environnement du Québec.

Hara T.J., Brown S.B. and Evans R.E. (1983) Pollutants and Chemoreception in aquatic organisms. In Aquatic Toxicology, Advances in Environmental Science and Technology. (Edited by Nriagu J.O.), V. 13, pp. 249-306, Wiley, N.Y.

Kemp T.J. (1972) Oxidation - Reduction Reactions between Covalent Compounds and Metal Ions. In Comprehensive Chemical Kinetics (Edited by C.H. Bamford and C.F.H. Tipper). Vol. 7, pp. 275-329. Elsevier, Amsterdam.

Larrick S.R., Dickson K.L., Cherry D.S. and Cairns J. Jr. (1978) Determining Fish Avoidance of Polluted Water. Hydrobiologia 61, 3, 257-265.

McCauley R.W., Elliott J.R. and Read L.L.A. (1977) Influence of Acclimation Temperature on Preferred Temperatures in the Rainbow Trout (Salmo gairdneri). Trans. Am. Fish. Soc. 106, 362-365.

Mello N.K. (1975) Behavioral toxicology: A developing discipline. Fed. Prod. 34, 1832-1841.

Morrison D.F. (1976) Multivariate Statistical Methods. McGraw-Hill, New York.

Sauter S., Buxton K.S., Macek K.J. and Petrocelli S.R. (1976) Effects of exposure to heavy metals on selected freshwater fish -

Toxicity - of copper, cadmium, chromium and lead to eggs and fry of seven species. U.S. Environmental Protection Agency Report, EPA-600/3-76-105, Cincinnati, OH.

Singh S.M. and Ferns P.N. (1978) Accumulation of Heavy Metals in Rainbow Trout Maintained on a Diet Containing Activated Sludge. The Fish Society of British Isles 13, 277-286.

Spraggs L.D., Gehr, R. and Hadjinicolaou J. (1981) Polyelectrolyte toxicity tests by fish avoidance studies. Wat. Sci. and Technol. 14, 1564-1567.

Sprague J.B. (1970) Measurement of Pollutant Toxicity to Fish-II. Utilizing and Applying Bioassay Results. Water Res. 4, 3-32.

Sprague J.B. (1971) Review Paper, Measurement of Pollutant Toxicity to Fish-III. Sub-Lethal Effects and Safe Concentrations. Water Res. 5, 245-266.

Sprague J.B. (1976) Current Status of Sub-Lethal Tests of Pollutants on Aquatic Organisms J. Fish. Res. Bd. Can. 31, 1988-1992.

USEPA (1976) Quality criteria for water-Red Book. U.S. Env. Prot. Ag., Office of Water and Hazardous Materials, Washington D.C.

Van der Putte I., Van der Galien W. and Strik J.J.T.W.A. (1982)

Effects of **Cr(VI)** in Rainbow Trout (Salmo Gairdneri) after Prolonged Exposure at 2 **pH** Levels. Ecotoxicology & Environmental Safety **6**, 246-257.