Proceedings of the Sixth Annual Aquatic Toxicity Workshop

November 6 & 7, 1979 Winnipeg, Manitoba

J.F.Klaverkamp, S.L.Leonhard, and K.E.Marshall

Western Region
Department of Fisheries and Oceans
Winnipeg, Manitoba R3T 2N6

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PROCEEDINGS OF THE SIXTH ANNUAL AQUATIC TOXICITY WORKSHOP

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Edited by

J.F. Klaverkamp, S.L. Leonhard and K.E. Marshall

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Department of Fisheries and Oceans

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6 & 7 NOVEMBER, 1979

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SPONSORS:

The Toxicology Section
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba
Canada, R3T 2N6

Telephone: (204)269-7379

MEETING HEADQUARTERS:

Marlborough Inn Hotel
331 Smith Street
Winnipeg, Manitoba

Telephone: (204)942-6411

SESSIONAL CHAIRMEN:

P.V. Hodson
J.F. Klaverkamp
G. Leduc
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W.L. Lockhart
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D. McLeay
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PREFACE

This volume is the proceedings of the sixth in a series of annual Canadian Aquatic Toxicity Workshops. The Workshop has provided, since its beginning at the Freshwater Institute in 1974, a forum to present and discuss approaches, concepts, methodologies, results and applications of toxicity testing for the protection of aquatic organisms and their habitat. Since the Workshop has maintained an informal atmosphere it also serves as a place to discuss related issues relevant to aquatic toxicology in Canada, to contact colleagues from universities, industries and governmental agencies, and to review "the state of the art".

In 1979 the Organizing Committee called for papers and posters that related to the following general themes:

1. Novel laboratory responses and techniques, including the use of different organisms, life history stages, biological levels of organization, and sequences or networks of multiple tests.

2. Approaches to assessing adverse effects in the field and to relating laboratory studies to the field, including the use of specialized exposure chambers, vessels or corrals and of field sampling methods.

3. Approaches in evaluating and controlling the adverse effects of potential or intentional discharges of chemicals applied to the environment for agricultural or forestry purposes.

After receipt of papers and posters the Workshop was organized into four plenary sessions and six workshops. The plenary sessions considered the following subjects: Field Approaches for Evaluating Pathways and Effects of Chemical Contaminants in Freshwater Systems; Approaches to Developing Bioassays with a Proposal for a Standard Toxicity Test with *Artemia* nauplii, and Aquatic Toxicology and Risk Assessment - Where to Now? The workshops were entitled Novel Approaches for Studying Uptake, Distribution and Effects, Early Life History Stages of Fish, Toxicology of Metals and Other Industrial Wastes, Factors Affecting Pesticide Toxicity, Diet and Fish Behavioural Responses, and Significance of Bioaccumulation Studies.

Each workshop consisted of the presentation of four to six papers or posters followed by a thirty minute open discussion. This discussion assisted the chairman in preparing a five minute summary of the presentations and recommendations for research priorities to the general meeting.

In the Closing Comments section of the Workshop, a lively discussion ensued on the advantages and disadvantages of formalizing the annual meeting possibly through the formation of a professional society. While the workshop is not sponsored by a formal society and is organized by a different volunteer group each year, interest has been continually expressed in the formation of a society of environmental or aquatic toxicology. To provide a basis for further discussion, Dr. Peter V. Hodson of the Great Lakes Biolimnology Laboratory in Burlington, Ontario was asked to investigate the pros and cons of forming a society. His investigation will be presented at the 7th Annual Workshop in Montreal, Quebec. For additional details on the Seventh Annual

Aquatic Toxicity Workshop, please contact:

N. Bermingham — Co-Chairmen — G. Leduc
EPS Canada — Dept. of Biological Sciences
1001 Pierre Dupuy — Concordia University
Longueuil, Quebec — Maisonneuve Blvd.
Montreal, Quebec

Telephone: (514)651-6860 — (514)879-5989

The Proceedings of past meetings may still be available from the following.

Craig, G.R. 1976. Proceedings of the Second Annual Aquatic Toxicity Workshop, November 4-5, 1975, Rexdale, Ontario. Ontario Ministry of the Environment, Box 213, Rexdale, Ontario M9W 5L1

Parker, W.R., E. Pessah, P.G. Wells, G.F. Westlake. 1977. Proceedings of the Third Annual Aquatic Toxicity Workshop, November 2-3, Halifax, Nova Scotia, EPS Technical Report EPS-5-AR-77-1. Fisheries and Environment Canada, Environmental Protection Service, 5151 George Street, Halifax, Nova Scotia B3J 1M5.

Davis, J.C., G.L. Greer and I.K. Birtwell. 1978. Proceedings of the Fourth Annual Aquatic Toxicity Workshop, November 8-10, 1977, Vancouver, British Columbia. F&MS Technical Report 818. Fisheries and Environment Canada, Pacific Environment Institute, 4160 Marine Drive, West Vancouver, British Columbia V7V 1N6.

Wong, P.T.S., P.V. Hodson, A.J. Niimi, V. Cairns and U. Borgmann. 1979. Proceedings of the Fifth Annual Aquatic Toxicity Workshop, November 7-9, 1978, Hamilton, Ontario. F&MS Technical Report 862. Fisheries and Environment Canada, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, 867 Lakeshore Road, Burlington, Ontario L7R 4A6.

EDITORS' COMMENTS

The proceedings of the Sixth Annual Aquatic Toxicity Workshop contain 23 full papers and 12 abstracts as presented during the sessions. Minimal editorial control was exercised by the review committee. There were a few papers presented during the workshop for which no transcripts are available in this manuscript. These include the presentations of the four plenary speakers: D.W. Schindler, Freshwater Institute, Winnipeg - Small Oligotrophic Lakes; J.S. Marshall, Argonne National Laboratory, Argonne, Illinois - Great Lakes; H.E. Welch, Freshwater Institute, Winnipeg - Arctic Lakes, and J.W.M. Rudd, Freshwater Institute, Winnipeg - Large Rivers. Also missing is J. Watson's discussion on the acceptability of toxicology papers for presentation in the Canadian Journal of Fisheries and Aquatic Sciences. The papers are listed in the table of contents in the order of presentation at the workshop so that related papers are grouped together. To facilitate reference and abstraction an alphabetical listing of all authors is provided.

The Editors

ABSTRACT

KLAVERKAMP, J.F., S.L. LEONHARD and K.E. MARSHALL (ed.) 1980 Proceedings of the sixth annual aquatic toxicity workshop, November 6 & 7, 1979, Winnipeg, Manitoba. Can. Tech. Rep. Fish. Aquat. Sci. 975: xi + 291 p.

The proceedings of the Sixth Annual Aquatic Toxicity Workshop consist of 23 papers, 12 abstracts, a review of aquatic toxicity research programs across Canada and a list of participants.

Papers were presented in four plenary sessions: field approaches for evaluating pathways and effects of chemical contaminants in freshwater systems, standardization of aquatic bioassays, Journal of the Fisheries Research Board of Canada policy on acceptability of toxicology papers for publication and aquatic toxicology and risk assessment - where to now?; six workshop sessions: novel approaches for studying uptake, distribution and effects, early life history stages of fish, toxicology of metals and other industrial wastes, factors affecting pesticide toxicity, diet and fish behavioral responses, and significance of bioaccumulation studies; and a poster session.

Key words: Aquatic toxicology; bioassays; toxicity; pH; metals; industrial wastes; pesticides

RESUME

KLAVERKAMP, J.F., S.L. LEONHARD and K.E. MARSHALL (ed.) 1980 Proceedings of the sixth annual aquatic toxicity workshop, November 6 & 7, 1979, Winnipeg, Manitoba. Can. Tech. Rep. Fish. Aquat. Sci. 975: xi + 291 p.

Les comptes-rendus du colloque du sixième atelier annuel sur la toxicité aquatique consistent en 23 communications complètes, 12 résumés, un revu du recherche des programmes en toxicité aquatique à travers Canada et un liste des participants.

Les documents sont présenté en quatre séances plénière: les approches d'étude sur le terrain pour evaluer aux sentiers et aux effets des contaminants chimique en les systèmes des eaux douces, les éssais biologique de toxicité aquatique standardisées, police du Journal de l'office des recherches sur les pêcheries du Canada sur les documents de toxicité qui sont acceptable pour la publication, et la toxicologie aquatique et l'évaluation des risques - où allons-nous maintenant?; six séances d'étude: les approches nouveaux pour l'étude du recouvrement de la distribution et des effets, les etats premières du cycle biologique des poissons, la toxicologie des métaux et des autres déchets industriels, les agents affectant la toxicité des pesticides, les réponses du régime alimentaire et les réponses éthologiques des poissons, et l'importance de la bioaccumulation; et une séance des afficheurs.

Mots-clés: Toxicologie, aquatique; essais biologiques; toxicité; pH; métaux; déchets industriels; pesticides

USE OF LIMNOCORRALS IN STUDYING THE EFFECTS OF PESTICIDES IN THE AQUATIC ECOSYSTEM

K.R. Solomon, K. Smith, G. Guest, J.Y. Yoo and N.K. Kaushik

Department of Environmental Biology, University of Guelph, Guelph, Ontario, N1G 2W1

SOLOMON, K.R., K. SMITH, G. GUEST, J.Y. YOO and N.K. KAUSHIK 1980 Use of limnocorrals in studying the effects of pesticides in the aquatic ecosystem. Can. Tech. Rep. Fish. Aquat. Sci. 975: 1-9.

Techniques used in the construction, assembly and moving of limnocorrals are briefly discussed. The development of a system for applying pesticides to limnocorrals is described. This system makes use of a centrally mounted multi-orifice injector coupled to a 108 m^3/h pump to add pesticides to the corral with a minimum disturbance of sediment and a minimal concentration gradient between incoming solution and that already present in the corral. Injection of fluorescine dye gave localised concentrations not more than 25% in excess of final concentration and total mixing was completed in 60 min. The pyrethroid insecticide, permethrin, was applied to corrals. Rate of disappearance was depth dependent, most probably as a result of temperature differences. Concentration dropped from a mean of 625 µg/L to 0.075 µg/L in a period of 140 days.

Key words: Insecticides; permethrin; water mixing; field screening; pesticide application

SOLOMON, K.R., K. SMITH, G. GUEST, J.Y. YOO and N.K. KAUSHIK 1980 Use of limnocorrals in studying the effects of pesticides in the aquatic ecosystem. Can. Tech. Rep. Fish. Aquat. Sci. 975: 1-9.

L'article qui suit examine brièvement les techniques de construction, d'assemblage et de déplacement des limnocorrals. On y décrit la mise au point d'un système d'application des pesticides dans ces structures. Le système fait appeï à un injecteur à plusieurs orifices monté au centre, couplé à une pompe de 108 m^3/h permettant d'introduire les pesticides dans le corral avec dérangement minimal du sédiment et minimum de gradient de concentration entre la solution introduite et celle déjà présente. L'injection d'un colorant, la fluorescine, donne des concentrations ne dépassant pas 25% de la concentration finale, et le mélange se fait en dedans de 60 min. Nous avons introduit dans les corrals l'insecticide pyréthroïde, la permethrine. Le taux de disparition est relié à la profondeur, très probablement à cause des différences de température. Dans une période de 140 jours, la concentration diminua, passant d'une moyenne de 625 µg/L à 0.075 µg/L.

INTRODUCTION

Originally called the Lund tube, the limnocorral is usually defined as a temporary, flexible, container in which a section of a body of water can be isolated. These enclosures, or limnocorrals, have been used to study the effects of mercury on algae and zooplankton (Blinn et al. 1977; Sonntag and Greve 1977; Beers et al. 1977), for observations on zooplankton (Smyley 1978), phytoplankton (Lund 1972; Lack and Lund 1974) and their use has been suggested for evaluating the fate and effect of contaminants in aquatic ecosystems (Hodgson and Millard 1978).

The use of limnocorrals for studying the effects of pesticides in aquatic systems presents a number of useful research advantages. The limnocorral allows the partitioning of a lake into a number of more or less similar compartments which may then be treated with pesticide in replicate studies. Such replication in whole lakes would be difficult and have an unnecessary environmental impact. A group of limnocorrals would be exposed to similar climatic factors and would all, at least in the beginning, contain chemically and biologically similar water. Heat transfer through the walls of the corral would ensure that the corral maintained a similar temperature profile to the rest of the lake. The relatively low costs of the limnocorral compared to laboratory lake column simulators, suggested that these would be useful for the initial field screening of pesticides for environmental impact.

It is generally accepted that the limnocorral is not a complete substitute for a lake since it is much smaller in volume, contains fewer organisms, has less vertical mixing and has a greater surface to volume ratio than the main body of water. While accepting these limitations, it does allow the impact to a pesticide to be quantified under conditions that would be difficult to maintain in a laboratory. They could be used to identify key organisms that could then be used in laboratory bioassays or as indicator organisms in field evaluation studies. Another possible use is in determining the time required for populations to recover from the effects of a pesticide application.

With these concepts and limitations in mind, we have begun investigating the use of limnocorrals for studying the effects of pesticides in the aquatic ecosystem. This paper describes our initial field experiences, reports on some techniques and methodology we have developed and gives initial results that we have obtained with the pyrethroid, permethrin.

METHODS, RESULTS AND DISCUSSION

HANDLING OF LIMNOCORRALS

Assembly

The corrals used in this work were not self-supporting and had to be supported inside a wood frame (See Fig. 1). Assembly in water was difficult and the corral frames were manufactured unit by unit on shore. As each frame unit was completed, the corral was tied in place and the corral and the frame floated onto the surface of the lake. The sides of

the corral were tightly tied to the floatation to reduce drag during moving. Rafts of 3 and 5 corrals each were constructed in this manner with little difficulty.

Moving of the corral rafts was greatly facilitated by reducing the drag as much as possible. Towing of the corral raft was carried out using a single 4.5m rowing boat fitted with 3 kw outboard motor capable of 360° rotation. Towing was carried out in the reverse configuration with motor, turned 180° and the towline over the bows. Reverse towing with reverse gear equipped outboards was most inefficient as was use of stern mounted towline and the motor in forward gear.

Once in position, the corral raft was anchored by means of 15m lengths of 6mm stainless steel cable and 50 kg scrap cast iron cylinder heads as anchors. Positioning of the anchors was facilitated by attaching a rope to the anchor and by raising it from the bottom during repositioning. The rope can be removed after the corrals are positioned or may be left in position if required later. Anchors were placed at each corner and in the center of the long side of the corral raft. After positioning, the sides of the corrals were released and allowed to drop into the mud bottom. Scuba inspection of the corrals was essential to ensure an adequate seal at the bottom of the corral.

In order to flush the contents of the corral or to move the corral to a different section of the lake it is necessary to raise the sides. To facilitate this, 4 ropes per side were attached to the bottom hem of the corral. Drag from mud and water made raising the sides of the corral a major undertaking. This problem was solved by using a winch such as those commonly used on yachts. The winch was mounted in the bows of the boat to reduce instability during lifting. Nylon rubbing blocks were attached to the edge of the deck and clam cleats were attached to hold the ropes. The winch was also used for lifting anchors as well as for moving corral rafts over short distances in adverse weather conditions.

Corrals were sampled from a raft consisting of a 1.8m x 4.8m floating platform. A 3m x 0.6m cantilever support bridge with a 2.4m overhang was mounted on the one end and additional floatation placed under the mountings to compensate for increased mass. In use the boat was tied fast to the one side of the raft and the whole unit moved with the aid of the outboard motor. The bridge gave a stable working platform with easy access to all parts of those corrals with open water on opposite sides. Sampling times were considerably decreased over other methods.

Based on our field experiences, we wish to suggest a possible design for a corral that would be better suited to multiple use such as in the field screening of pesticides. A suggested construction is shown in Fig. 2. Important design considerations are the use of a rigid bottom frame which would hold the corral open during lowering of the sides. It would also serve as an attachment for a disposable liner (should such be required) and the attachment of a net to exclude fish during lowering. A semi-rigid surface support would provide easy access to all sides of the corral and provide a stable platform from which to raise the sides of the corral, for example, with a portable winch. Attachment would also be provided for a disposable liner, for a bird deterrent system and for cleats to hold the

ropes in place when the sides were raised.

Loops in the side walls through which the ropes could be passed would ensure that the sides of the corral folded (and unfolded) evenly to reduce drag during moving and to keep the shape of the corral as uniform as possible. The construction and field testing of such a corral is being planned at present.

APPLICATION OF PESTICIDES

In the few studies that have been reported the addition of chemical compounds to large volumes of water and the subsequent mixing of these with water has been accomplished by passive diffusion in the case of soluble inorganic compounds such as phosphate (D. Lean pers. comm.) or by the use of air bubbles to induce an accelerated vertical mixing (Sonntag and Parsons 1979).

In such treatments, a major source of variability is likely to be the rate of mixing and the magnitude of the concentration gradient that will exist during the mixing process. Reliance on diffusion process in a large volume of water such as a limnocorral may result in variability between treatments as well as a lack of repeatibility. Complete mixing may never occur in compounds that have low water solubility as well as a density different from that of water.

For these reasons a method that would adequately and rapidly mix the contents of a large body of water was developed. Initial studies were conducted in the laboratory and were followed up by field studies in actual limnocorrals. While complete and rapid mixing is unlikely to occur under natural conditions of contamination, results of rapid and complete mixing would at least serve as a point for comparison with other types of pesticide input and for this reason were considered important.

In developing the mixing system, initial experiments were conducted in 40 l glass aquaria using India ink to visualise water flow and mixing. The efficacy of a mixing current created by a rising column of air bubbles produced by an aquarium pump and an air-stone was compared to the injection of the dye in a stream of water pumped out of the tank and down a centrally located tube with holes drilled in the sides.

Using the injection system complete mixing occurred in less time than air bubble mixing and no upwelling of water occurred. Eddy currents tended to move in the horizontal plane and no significant centrifugal bottom current was observed. Also, because the rate of water as well as dye addition could be accurately measured, the concentration of the solution leaving the injection tube and hence the maximum possible concentration gradient could be calculated. The concentration of dye leaving the injector should not exceed the final concentration in the water by a factor of more than 2-3 fold, provided that a minimum volume (half that of the enclosure) is pumped during injection. For these reasons it was decided to use this technique in the field.

Field studies

The injector tube (Fig. 3) was made from rigid ABS sewer pipe of 100mm internal diameter. Holes were drilled at 150mm intervals in 8 rows staggered by 75mm and spaced 45°apart. The size of the holes ranged from 3mm to 9mm diameter from top to bottom. Cross sectional area of the holes was proportioned to the square of the distance from the inlet to allow for pressure drop and to ensure equal mass flow from each hole.

Sodium fluorescine was selected as the dye to measure mixing in the system. It is completely water soluble but preliminary trials showed that the dye degraded in water when exposed to sunlight. This necessitated that all experiments be done between sunset and sunrise. The fluorescine (4 L of a 1.5% solution) was allowed to flow into the pesticide inlet via a drop counter metering device.

Field trials were conducted in a triangular limnocorral of 7.5m sides, 4.25m deep and a nominal volume of 108000 L. The injector was centrally located. Water samples were taken at the center of the corral and at the edge. Sampling was by suction of samples from depths of 0.5 1.5, 2.5 and 3.5m via Tygon^{R} tubing held in a weighted frame.

In the first trial a pump capable of 10000 L/h was used with an injection period of 240 min. Dye concentration was measured in a spectroflurometer using an excitation wavelength of 495nm and a measuring wavelength of 520nm and the results are shown in Figs. 4 and 5. Both during and after pumping, considerable variation existed at all depths at both sampling points. Even after a post pumping period of 430 min., maximum dye concentration was found at a depth of 1.5m. Since the depth of the intake hose was also at 1.5m, this suggested that the temperature stratification of the water was important in determining mixing at that particular pumping rate. The top to bottom temperature gradient at the time of the trial was 18°-8°C. The relative stability of the dye concentration at the various depths after shut down of the pump suggested that adequate horizontal mixing had occurred, however, vertical concentration differences were as great as 2.5 fold and were considered excessive. Pumping of water appeared to have little effect on zooplankton as examination of water from the pump outlet revealed few dead organisms. No noticeable decrease in zooplankton numbers was observed in Schindler samples from corrals after pumping.

In the second field trial a larger pump capable of 45000 L/h was used and the injection period reduced to 150 min. In addition, the pump was run for 45 min. after dye injection to further mix the water. In order to reduce the effect of thermal layering, the intake hose was raised and lowered during the pumping period as indicated in Figs. 6 and 7. Changes in dye concentration are shown in Figs. 6 and 7.

Once again, little difference between dye concentrations at the edge and at the center of corral was evident, suggesting good horizontal mixing. Variations between the sampling depths did occur but were less than in the first trial and at no time did they exceed the final concentration by more than 25%. The effect of raising and lowering the intake hose could not be observed as changes in the rate of dye accumulation at various depths, suggesting that the more powerful pump ensured more uniform mixing. Although

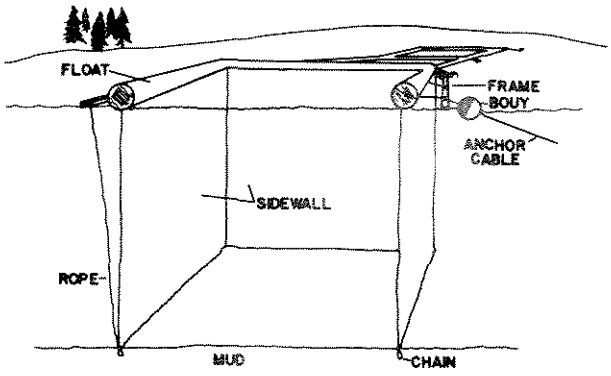

Fig. 1. Constructional details of the limnocorrals.

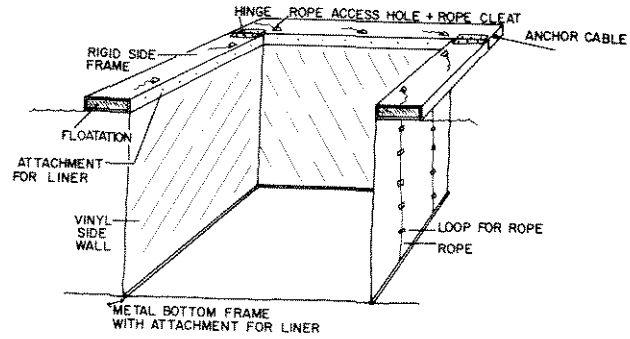

Fig. 2. Proposed constructional details of a corral for use in pesticide screening.

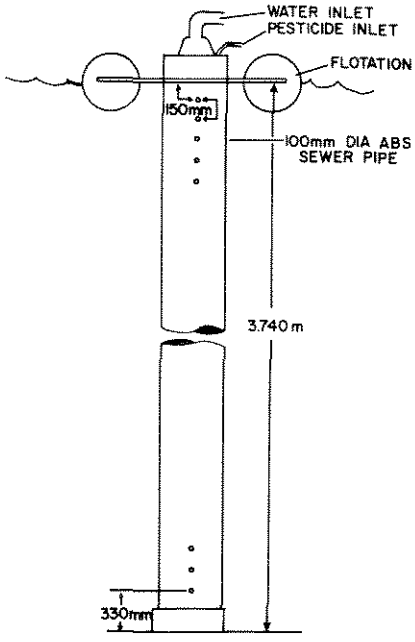

Fig. 3. Constructional details of the pesticide injector.

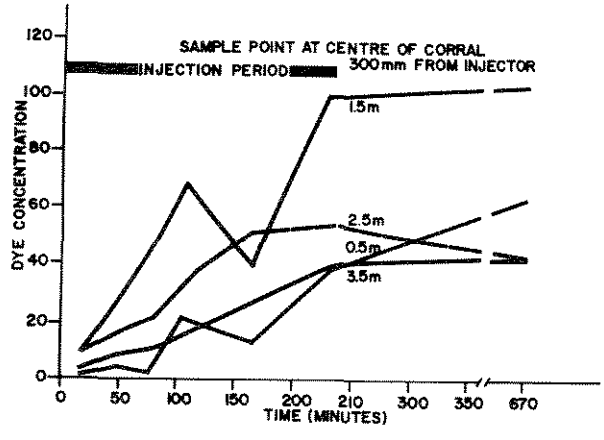

Fig. 4. Variation in fluorescine concentration with depth during injection. Pump rate = 10000 L/h, sampling point =300mm from injector.

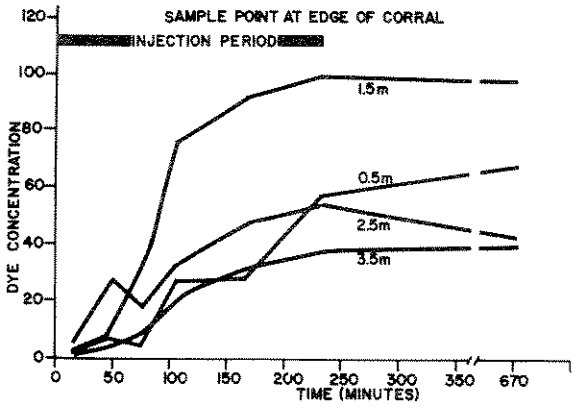

Fig. 5. Variation in fluorescine concentration with depth during injection. Pump rate = 10000 L/h, sampling point = 300mm from wall of corral.

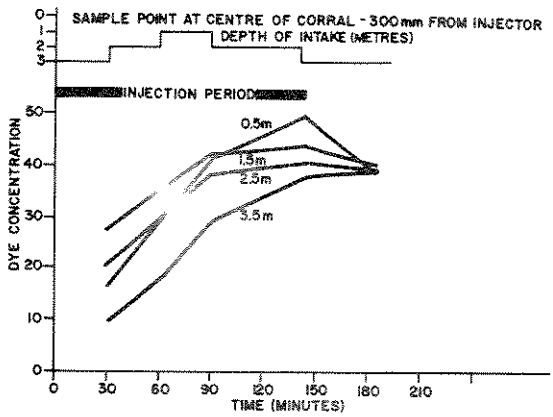

Fig. 6 Variation in fluorescine concentration with depth during injection. Pump rate = 45000 L/h, sampling period = 300mm from injector.

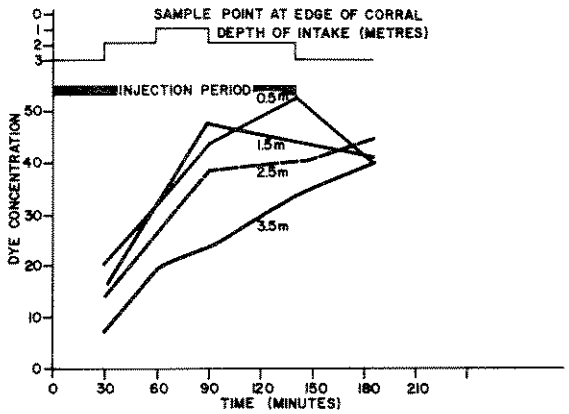

Fig. 7. Variation in fluorescine concentration with depth during injection. Pump rate = 45000 L/h, sampling point = 300 mm from wall of corral.

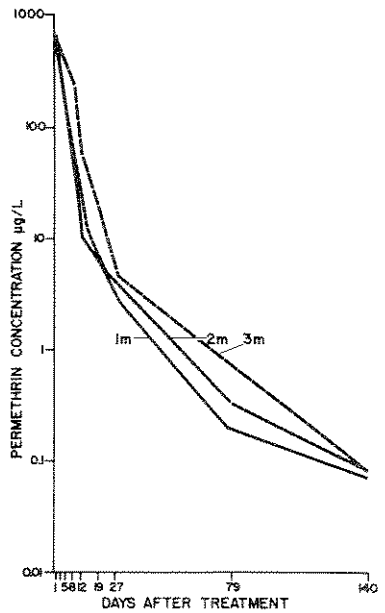

Fig. 8. Permethrin loss from corral #9. Concentrations are the total concentrations in water and suspended matter at the indicated depth.

this trial was conducted at a later date, the surface to bottom temperature gradient was 20° to 12°C. The free running of the pump for a period after injection assured complete mixing in a short time period.

In the third field trial an even larger pump, capable of 108000 L/h was used. Permethrin, as 86.5% pure technical material, was dissolved in 2 L of technical acetone and injected into the pesticide inlet during a 30 min. period to give an approximate final concentration of 1 mg/L in the nominal volume of the corral. This concentration of permethrin is in excess of its reported solubility of ca. 0.25 mg/L and was chosen as a worst case that would present major mixing problems. After injection, the pump was run for a further 30 min. to ensure complete mixing. Samples were taken at depths of 1, 2 and 3m immediately after the pump was shut down, the permethrin extracted in hexane and the concentration determined by gas chromatographic analysis. Results are shown in Table 1.

Table 1. Concentration of permethrin in Corral #9 (1979-07-12).

Depth	Concentration in µg/L
1m	656
2m	624
3m	599

These results indicate that, despite the high concentration at which the pesticide was applied, reasonably good mixing was obtained. The apparent loss of some permethrin in the corral is possibly due to separation of insoluble pesticide from solution or to adsorption on to plastic components of the injector and/or the limnocorral itself. Adsorption to particulate matter followed by precipitation is unlikely because samples were taken immediately after pumping and would have been indicated by higher concentrations at greater depth. The loss of permethrin from the corral is shown in Fig. 8. Disappearance is quite rapid and approximated to first order kinetics in the early phase of the study. The mechanism of loss probably involves both chemical and biological degradation as well as adsorption to mud. A bottom mud sample obtained 4 days after application contained 312 mg/kg (dry mass) permethrin. Loss of permethrin was consistently less rapid at greater depth reflecting the lack of vertical mixing in the corral during the warmer months. Vertical temperature stratification in the corral is the most probable cause of the differences in the rate of loss.

CONCLUSIONS

Preliminary experiments with the technique have suggested that limnocorrals may be of use in determining the effects of pesticides on the aquatic ecosystem. Pesticides can be mixed into the large volumes of water in the limnocorrals with comparative ease and, with some modifications in the design of the corrals, pesticides could be routinely screened for effects on aquatic organisms as well as for the time taken for recovery from the effects of the

pesticide to occur. Other important factors such as the adsorption of pesticides to the walls of the limnocorrals and their subsequent release as well as adsorption to the mud bottom of the lake are currently being studied.

ACKNOWLEDGEMENTS

The authors wish to thank K. Refling and D. Rascher for their technical assistance. The lake on which these studies were carried out is owned by the Metro Toronto Conservation Authority and we are grateful for permission to use the lake in our research. This research was supported by a grant from the National Science and Engineering Research Council of Canada.

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EVALUATION OF IMPACT OF A SYNTHETIC PYRETHROID, PERMETHRIN, ON A LAKE ECOSYSTEM USING LIMNOCORRALS

K. Smith, N.K. Kaushik, K.R. Solomon, and G. Guest

University of Guelph, Guelph, Ontario

SMITH, K., N.K. KAUSHIK, K.R. SOLOMON, and G. GUEST 1980 Evaluation of impact of a synthetic pyrethroid, permethrin, on a lake ecosystem using limnocorrals. Can. Tech. Rep. Fish. Aquat. Sci. 975: 10-11.

To assess effects of permethrin on an entire ecosystem, limnocorrals, approximately 5 x 5 x 4 m deep, were established in a lake that had been previously studied for three years for water chemistry, phyto- and zooplankton. Limnocorrals and three stations in the open lake adjacent to corrals were sampled weekly using a 12 L Schindler sampler for zooplankton distribution at 1 m intervals of depth. Physical and chemical characteristics recorded were temperature and dissolved oxygen at 1 m interval, Secchi disc reading; and pH; conductivity, alkalinity, hardness, dissolved and particulate organic matter, and total N from surface and bottom water samples. Phytoplankton populations were recorded but are not reported here.

To determine concentration of permethrin to be applied to the corrals, static bioassays were conducted using Daphnia spp., Cyclops spp., Ostracods, amphipods (Hyalella and Gammarus), chironomids, crayfish and molluscs as test animals. Sensitivity was found to range from 0.5-10,000 ppb with most animals intolerant of $>$10 ppb. After the corrals had been allowed to stabilize for 2 months, during which period they were sampled, three corrals each received 5 ppb, another three each 50 ppb of permethrin, and three served as controls.

Characteristics of surface water were: temperature - 17-26.5^{o}C; dissolved O_2 6-8 ppm; pH 7.42-8.75; conductivity (micro mho) 122-208, alkalinity (mg/L $CaCO_3$) 71.0-193.8; hardness (mg/L $CaCO_3$) 102.5-208.2; soluble organic matter (mg/L) 4.10-20.1 and particulate organic matter (mg/L) 0.44-10.33 and total N 0.374-1.308 mg/L.

The most sensitive zooplankton populations were Cladocerans (Daphnia spp., Ceriodaphnia, Bosmina and Diaphanosoma) and were completely eliminated at both concentrations. The copepods (Diaptomus spp., Tropocyclops, Diacyclops and Acanthacyclops) and ostracods were slightly more tolerant at 5 ppb, but were eliminated at 50 ppb. Rotifers (Chromogaster, Conochilus Keratella and Polyarthra) appeared to be not affected even at the highest concentrations and so were some species of Protozoa. Changes in planktonic populations possibly because of elimination of certain groups will be discussed.

Key words: Permethrin; ecosystems; phytoplankton; zooplankton; invertebrates; bioassays; water analysis (chemical)

SMITH, K., N.K. KAUSHIK, K.R. SOLOMON, and G. GUEST 1980 Evaluation of impact of a synthetic pyrethroid, permethrin, on a lake ecosystem using limnocorrals. Can. Tech. Rep. Fish. Aquat. Sci. 975: 10-11.

Dans le but de déterminer les effets de la permethrine sur un écosystème entier, on a mis en place des limnocorrals d'environ 5 x 5 x 4 m de profond dans un lac où l'on avait déjà étudié la chimie de l'eau, le phyto- et le zooplancton pendant trois ans. Un échantillonneur de Schindler de 12 L a servi à des échantillonnages hebdomadaires dan les limnocorrals et à trois stations de pleine eau au voisinage de ces derniers, afin de déterminer la distribution du zooplancton à intervalles de 1 m de profondeur. Nous avons enregistré les caractéristiques physiques et chimiques suivantes : température et oxygène dissous à 1 m d'intervalle, profondeur du disque de Secchi; pH; conductivité, alcalinité, dureté de l'eau, matière organique dissoute et particulaire, et azote total dans des échantillons d'eau de surface et de fond. Les populations phytoplanctoniques notées ne sont pas mentionnées ici.

La concentration de permethrine à introduire dans les corrals a été déterminée à l'aide d'essais biologiques statiques avec les organismes suivants : Daphnia spp., Cyclops spp., ostracodes, amphipodes (Hyalella et Gammarus), chironomides, écrevisses et mollusques. Les extrêmes de sensibilité sont 0.5 et 10,000 µg/L, la plupart des animaux étant incapables de tolérer plus de 10 µg/L. Après 2 mois, pour permettre aux corrals de se stabiliser et au cours desquels nous avons prélevé des échantillons, trois corrals reçurent chacun 5 µg/L, trois autres 50 µg/L chacun de permethrine, et trois servirent de témoins.

Les caractéristiques de l'eau de surface étaient les suivantes : température - 17-26.5°C; oxygène dissous 6-8 ppm; pH 7.42-8.75; conductivité (micro mho) 122-208, alcalinité (mg/L de $CaCO_3$) 71.0-193.8; dureté (mg/L de $CaCO_3$) 102.5-208.2); matière organique soluble (mg/L) 4.10-20.1 et matière organique particulaire (mg/L) 0.44-10.33, et azote total 0.374-1.308 mg/L.

Les populations zooplanctoniques les plus sensibles sont les cladocères (Daphnia spp., Ceriodaphnia, Bosmina et Diaphanosoma); elles furent complètement éliminées aux deux concentrations. Les copépodes (Diaptomus spp., Tropocyclops et Acanthacyclops) et les ostracodes sont légèrement plus tolérants à 5 µg/L, mais furent éliminés à 50 µg/L. Les rotifères (Chromogaster, Conochilus, Keratella et Polyarthra) ne semblent pas avoir été affectés, même aux plus fortes concentrations. Il en est de même avec certaines espèces de Protozoa. Nous examinons les changements que subissent les populations planctoniques, possiblement par suite de l'élimination de certains groupes.

EFFECT OF WATER CHEMISTRY ON THE UPTAKE OF ORGANIC POLLUTANTS BY FISH IN RIVER WATER

D.C.G. Muir, D.A. Metner, A.P. Blouw, N.P. Grift and W.L. Lockhart

Department of Fisheries and Oceans, Freshwater Institute
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

MUIR, D.C.G., D.A. METNER, A.P. BLOUW, N.P. GRIFT and W.L. LOCKHART 1980
Effects of water chemistry on the uptake of organic pollutants by fish in river water. Can. Tech. Rep. Fish. Aquat. Sci. 975: 12-26.

Rainbow trout fingerlings were exposed to levels (0.5 to 50 ppb) of nine pesticides (methoxychlor, fenitrothion, permethrin, fluridone, terbutryn, niclosamide, 2,4-D, DDT, ethalfluralin), hexachlorobiphenyl and triphenyl phosphate (all ^{14}C labelled) in river water (Red River, Manitoba) and dilutions of river and lab water (dechlorinated city water). Fish were sampled at regular intervals over 24-32 hr exposures and the samples were oxidized (Packard 306 oxidizer) to determine ^{14}C uptake. The results showed that there were striking differences in the rate and magnitude of uptake of the organics between fish in river and lab water. Concentrations of all compounds were lower in fish in river water at both the initial sampling time (0.5-1 hr after addition of ^{14}C) and after 24-32 hrs. Hexachlorobiphenyl residues showed the greatest differences between the river and lab water treatments while relatively water soluble compounds such as terbutryn showed the least effect. Differences were correlated with physical properties (octanol-water partition coefficient, soil sorption, water solubility) of the compounds.

Key words: pesticides; hexachlorobiphenyl; triphenyl phosphate; bioaccumulation; suspended sediments

MUIR, D.C.G., D.A. METNER, A.P. BLOUW, N.P. GRIFT and W.L. LOCKHART 1980
Effects of water chemistry on the uptake of organic pollutants by fish in river water. Can. Tech. Rep. Fish. Aquat. Sci. 975: 12-26.

Des alevins de truite arc-en-ciel ont été exposés, à des degrés divers (de 0.5 à 50 x 10^{-6}), à neuf pesticides (méthoxychlore, fénitrothion, perméthrine, fluridone, terbutryne, niclosamide, 2,4-D, DDT et éthalfluraline), ainsi qu'à de l'hexachlorobiphényle et à du phosphate de triphényle (marqués au ^{14}C dissous dans de l'eau de rivière (provenant de la Red River, Manitoba) et dans des mélanges d'eau de rivière et de laboratoire (eau d'acqueduc déchlorée). A intervalles réguliers, on prélevait des échantillons de poissons exposés pendant des périodes de 24-32 heures; ces échantillons étaient ensuite oxydés (avec l'oxydant Packard 306) pour déterminer l'absorption de ^{14}C. On a constaté que le taux d'absorption des substances organiques, de même que la quantité de matière absorbée étaient nettement différents, selon que les poissons provenaient de l'eau de rivière ou de la solution contenant de l'eau de laboratoire. Quel que soit le composé, la concentration était plus faible chez les poissons placés dans l'eau de rivière, tant au moment de l'échantillonnage initial (0.5-1.0 h

après l'addition du ^{14}C qu'après 24-32 heures. On a observé les différences les plus marquées avec les résidus d'hexachlorobiphényle; par contre, des composés relativement solubles dans l'eau comme la terbutryne ont eu les effets les moins prononcés. On a établi une corrélation entre les composés et leurs propriétés physiques (coefficient de partage entre l'eau et l'octanol, adsorption par le sol, solubilité dans l'eau).

INTRODUCTION

In studies of pesticide residues in river water it has often been observed that a portion of the residue is associated with the suspended solids. For example, Miles (1976) reported that 64% of p,p'-DDT, 47% of p,p'-DDD and 13% of dieldrin were associated with suspended solids in southern Ontario river water. Methoxychlor has been shown to be bound (40-45%) to suspended sediment in Saskatchewan river water following addition of the insecticide to the river (Fredeen et al. 1975). Runoff studies in which pesticides have been applied to soil in sloped fields and runoff water collecting during rainstorms, have indicated that chlorinated hydrocarbon pesticides are associated with suspended solids while pesticides with water solubility > 100 ppm are found almost entirely in the aqueous phase (Wauchope 1978). Thus the fate of most organic chemicals in natural waters, especially those having water solubilities less than 100 ppm, is highly dependent on their sorptive behavior (Karickhoff et al. 1979). Sorption to sediments or soils is described by the soil sorption coefficient (expressed on an organic carbon basis), K_{OC}, (Kenaga and Goring 1978). The K_{OC} values for a large number of organic chemicals are correlated with their physical properties such as water solubility and octanol-water partition coefficient (K_{OW}) as well as with bioconcentration factors (Kenaga and Goring 1978). A highly sorbed chemical is likely to have a high bioaccumulation potential.

To test the hypothesis that the uptake of organic chemicals by fish from river water should be reduced depending on the physical properties of the chemical, a study was designed to measure uptake of ^{14}C-labelled compounds by rainbow trout fingerlings (*Salmo gairdneri*). Lab water (dechlorinated city water), Red River water as well as 1/3 and 2/3 dilutions of lab water with river water were used in the study. The extent to which the chemicals were volatilized, degraded and sorbed by suspended solids was also investigated.

EXPERIMENTAL

MATERIALS

Water

River water was collected from the Red River, 25 km above Winnipeg near St. Adolphe, Manitoba, and was stored at 5°C in a 40 L polyethylene carboy. The water was used within 10 days of collection. Lab water (dechlorinated Winnipeg city water) was obtained from Dr. M.A. Giles (Freshwater Institute). Water chemistry analyses were carried out on each batch of river water and on lab water-river water dilutions by use of standard methods (Stainton et al. 1977). The range of water chemistry parameters for the river water used in the study is given in Table 1.

Analytical Standards

The organic chemicals used in the study are listed in Table 2. All compounds were ^{14}C- labelled and were greater than 98% radiochemically pure as demonstrated by TLC-autoradiography in our laboratory, or by the manufacturer; in the case of hexachlorobiphenyl and DDT.

Table 1. Characteristics of the Red River water used in the uptake and sorption experiments.

Parameter	Range
pH	8.0-8.2
Total dissolved Nitrogen	1.14-2.90 mg/L
Total dissolved Phosphorus	98-271 µg/L
Dissolved inorganic carbon	1.70-363 mM/L
Dissolved organic carbon	0.30-1.21 mM/L
Suspended carbon	2.31-52.1 mg/L
Total dissolved solids	210-390 mg/L
Chlorophyll - α	5.9-28.2 µg/L
Total suspended solids	60-560 mg/L
Suspended organic carbon[1]	2-4%
% silt in suspended solids[2] (50-2 µm)	8-41%
Clay in suspended solids (< 2 µm)	59-92%

[1] determined by loss on ignition (380°C, 16 hr) divided by 1.724.

[2] determined by the pipet method without pre-treatment of the sample except stirring.

Standards for spiking individual aquaria were prepared in acetone or ethanol at concentrations sufficient to give 50-100,000 DPM/L when a small volume (0.05 to 0.1 mL) of the standard solution was added to each aquarium. In the case of fluridone, 2,4-D, terbutryn, TPP, fenitrothion and niclosamide the standard solution was diluted with non-radiolabelled compound to give higher concentrations of these compounds in the water as indicated in Table 2.

Fish

Rainbow trout varying in size from 0.2 to 1.0 g were obtained from the fish holding facility at the Freshwater Institute. The fish were held at 10°C prior to and during the experiment and were not fed during the study.

METHODS

Design of the Experiment

Fish (18-25/4 L tank) were acclimatized with the four water types overnight (16 hr). The aquaria were then spiked by adding the standard solution with a pipet and mixing with a glass rod or stirring bar. Fish were sampled at intervals following addition of the chemical, generally 0.5, 1, 3, 6, 10 and 24 hrs post-treatment (3-5 fish per replicate each time). The samples were frozen (-20°C) until analysis.

Table 2. Chemical names, concentrations and specific activity of organic chemicals used in uptake experiments.

Common Name	Chemical Name	Conc. (µg/L)	Sp. Act.[1] DPM/µg
Fluridone	(1-methyl-3-phenyl-5-(3-(trifluoro-methyl)phenyl)-4-(1H)-pyridinone)	50	1573
Terbutryn	(2-ethylamino-4-(t-butylamino)-6-methylthio-s-triazine)	50	1099
2,4-D acid	(2,4-dichlorophenoxy acetic acid)	10	11111
Ethalfluralin	(N-ethyl-N-(2-methylallyl)-2-,6-dinitro-4-trifluoromethyl aniline)	33.3	8987
Fenitrothion	(0,0-dimethyl-0-(3-methyl-4-nitro-phenyl) phosphorothioate)	5	10100
TPP	(triphenylphosphate)	50	2000
Niclosamide	(2',5-dichloro-4'-nitrosalicylanilide)	50	741
Hexachlorobiphenyl	(2,4,5,2',4',5'-hexachlorobiphenyl)	0.5	138750
Methoxychlor	(2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane)	25	9665
Permethrin	(3-phenoxy phenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclo-propane carboxylate)	0.5	338701
DDT	(2,2-bis(p-chlorophenyl)-1,1,1-tri-chloroethane)	0.5	185930

[1] Specific activity of standard solutions used for treating each aquaria.

Fish Analyses

The fish were rinsed individually with distilled water, blotted dry and weighed into a tared paper cup. Fish larger than 0.5 g were sliced with a scalpel and transferred quantitatively to the paper cup. The samples were combused on a Packard 306 sample oxidizer after addition of 0.3 mL of Combustaid (Packard) to improve combustion. The $^{14}CO_2$ was trapped in CO_2-M-Met (Amersham) and diluted with PCS (Amersham) in the instrument. Sample vials containing $^{14}CO_2$ were counted on either a Packard 3300 or Beckman 7500 scintillation counter.

Water Analyses

Water samples (1.0 mL) were collected from the aquaria at the same time as the fish samples. The water was mixed with 12 to 15 mL of PCS:xylene (2:1) and counted by scintillation counting. The results enabled losses of radioactivity due to volatilization to be estimated.

Degradation in River Water

In a series of separate experiments the degradation of several of the test chemicals was investigated in river water (niclosamide, TPP, fenitrothion, ethalfluralin) or in sediment-water (1:10 wt/vol) (terbutryn, fluridone) systems. The design of these experiments is described briefly as follows: river water (2 L in duplicate) was stirred (20°C) continuously on a magnetic stirrer and fortified with a small volume of the test chemical at a concentration similar to that used in the fish uptake study. Water samples (100 mL) were taken at 1, 3, 6, 12 and 24 hrs post-treatment. The water was extracted by shaking with dichloromethane (50 mL, three extractions). The dichloromethane extracts were dried by passing through a small column of sodium sulfate and evaporated just to dryness on a rotary evaporator. The sample extracts were dissolved in methanol and analysed by gas chromatography (TPP, fenitrothion, ethalfluralin) or high pressure liquid chromatography (niclosamide) to determine the concentration of the test chemical. In sediment-water studies with fluridone and terbutryn, the sediment was separated from the aqueous phase by filtration and the aqueous phase was analysed for the test chemical as described.

RESULTS AND DISCUSSION

COMPARISON OF UPTAKE RATES IN RIVER AND LAB WATER

The results of the analyses of fish for each ^{14}C-labelled chemical are plotted in Fig. 1 to 11. For sake of clarity only the river and lab water fish results are presented. Uptake in 1/3 and 2/3 dilutions of river water was generally similar to that in river water. The data were analysed by stepwise regression analysis using a combination of backward elimination and forward selection procedures. Independent variables were log weight (g), lot time (hrs), and the dependent variable was log body burden (µg). R^2 values for the regression equation were highly significant ($P < 0.01$). The results indicated that water type (i.e. the presence of river water) significantly reduced the burden of each chemical. In order to compare quantitatively the differences between river and lab water treatments, the initial rate of uptake of each chemical was calculated (Table 3). This was achieved by use of the first two sampling points (generally 0.5, 1 or 2 hrs post-treatment) and by assuming, as a first approximation, that DPM/g = 0 at time = 0 hrs. The use of the initial rate reduced problems of interpretation of the results due to volatilization or degradation of the chemical over time. The difference in initial uptake rates between lab and river water results was then calculated for each compound.

Fluridone, terbutryn and 2,4-D (Fig. 1, 2 and 3, respectively) showed low rates and magnitudes of uptake by fish but the initial rates were significantly lower in river water than in lab water in all cases (Table 3). In comparison with the other compound studied, however, the differences in initial rate of uptake were small ranging from 5 to 16%. At the later sampling times (10-24 hrs) difference in DPM/g levels in the fish in river or lab water were

Table 3. Initial rates of uptake and percent differences in river and lab water exposed fish.

Compound	Water Type	Rate Constant[1] (mL g^{-1} hr^{-1})	% Difference in rate	r^2 Coefficient of Determination
Fluridone	LW	1.17	16.4	0.94
	RW	0.98		
Terbutryn	LW	4.86	5.0	0.77
	RW	4.62		
2,4-D acid	LW	0.99	7.9	0.35
	RW	0.91		
Ethalfluralin	LW	37.04	8.2	0.69
	RW	33.98		
Fenitrothion	LW	24.60	14.1	0.81
	RW	21.10		
TPP	LW	29.61	23.3	0.92
	RW	22.78		
Niclosamide	LW	17.66	54.2	0.93
	RW	8.11		
Hexachlorobi-phenyl	LW	22.20	61.9	0.93
	RW	8.40		
Methoxychlor	LW	73.30	39.1	0.91
	RW	44.67		
Permethrin	LW	7.00	34.8	0.76
	RW	4.60		
DDT	LW	9.67	32.6	-[3]
	RW	6.50		

[1] Rate Constant = $\frac{\text{initial rate (DPM/g hr)}}{\text{concentration at time = 0 (µg/mL)}}$

[2] Coefficient of determination for multiple linear regression model with three independent variables: water type (LW, 1/3, 2/3, RW), log weight, log time and dependent variable (DPM/fish sample). R^2 are all significant at $P < 0.01$.

[3] not analysed.

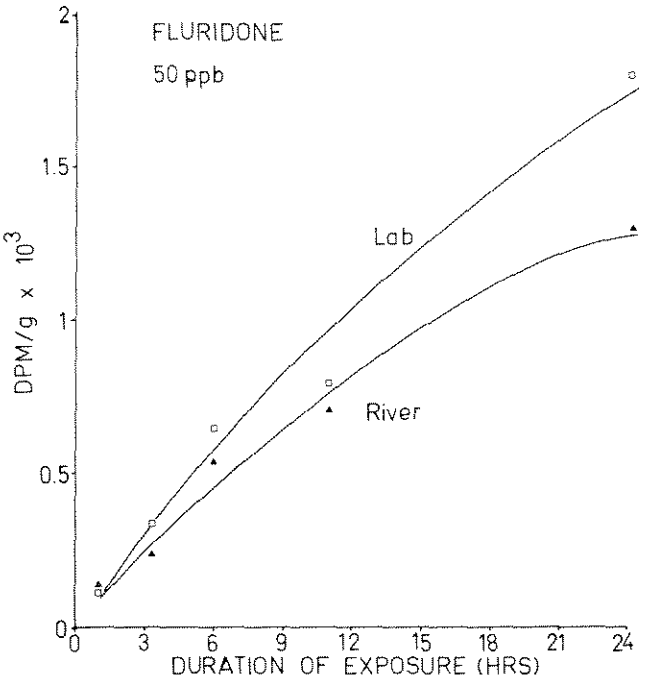
more pronounced (Fig. 1, 2 and 3). These differences were not due to degradation or volatilization of the chemicals since these three compounds are stable in sediment-water (terbutryn, fluridone) or river water (2,4-D) (Watson 1977) and do not degas significantly (Table 4). Increased sorption to suspended solids over time may explain some of the differences in residue levels especially for terbutryn and fluridone which were found to be sorbed to a small extent (5-10%) by suspended solids in river water (Table 5).

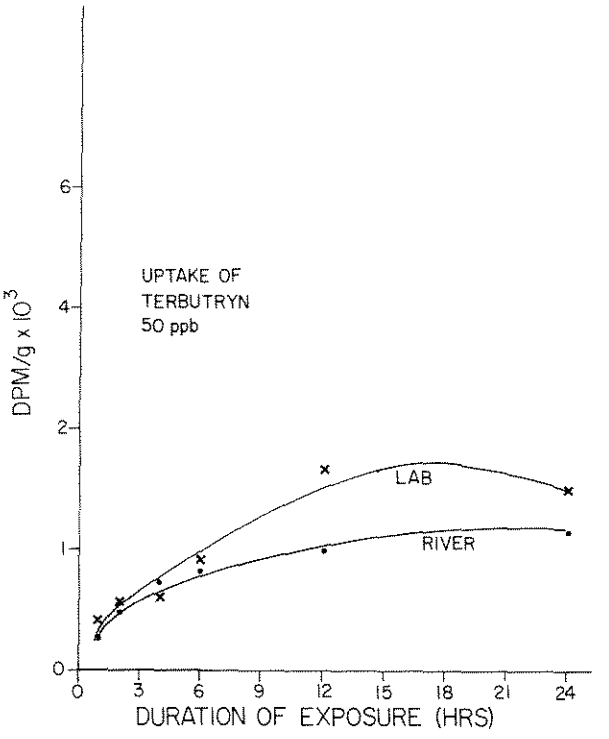
Ethalfluralin, fenitrothion, TPP and niclosamide (Fig. 4, 5, 6 and 7, respectively) showed much greater rates and magnitudes of uptake by fish than the previous three compounds. The initial rates of uptake of these four compounds by fish in river water were all significantly lower than the uptake in lab water (Table 3). The percent differences between the two treatments ranged from 55% for niclosamide to 8% for ethalfluralin. The rates of uptake of all the compounds except niclosamide, during the 10 to 24 hr sampling period in both river and lab water, were much lower than in the earlier sampling times (Fig. 4, 5, 6 and 7). This is explained in part by the lower concentration of radiolabelled chemical due to volatilization (ethalfluralin, fenitrothion) or degradation (niclosamide) (Table 4). Ethalfluralin losses were particularly large (96%) and may account for the small differences in initial rates that were observed. Niclosamide did not volatilize but was almost completely hydrolyzed in river water (Table 4) with a half-life of about 6 hrs (10°C). The unusually large difference in initial uptake rate is due therefore to the uptake of niclosamide in lab water and mainly niclosamide

Table 4. Losses of organic chemicals due to degradation or volatilization during uptake studies.

Compound	Losses after 24 hrs in river water	
	Degradation (%)	Volatilization (%)
Fluridone	< 5	< 5
Terbutryn	< 5	n.d.[1]
2,4-D	n.d.	< 5
Ethalfluralin	n.d.	94
Fenitrothion	< 5	70
TPP	< 5	n.d.
Niclosamide	95	< 5
Hexachlorobiphenyl	n.d.	25
Methoxychlor	n.d.	75
Permethrin	n.d.	30
DDT	n.d.	55

[1] not determined.





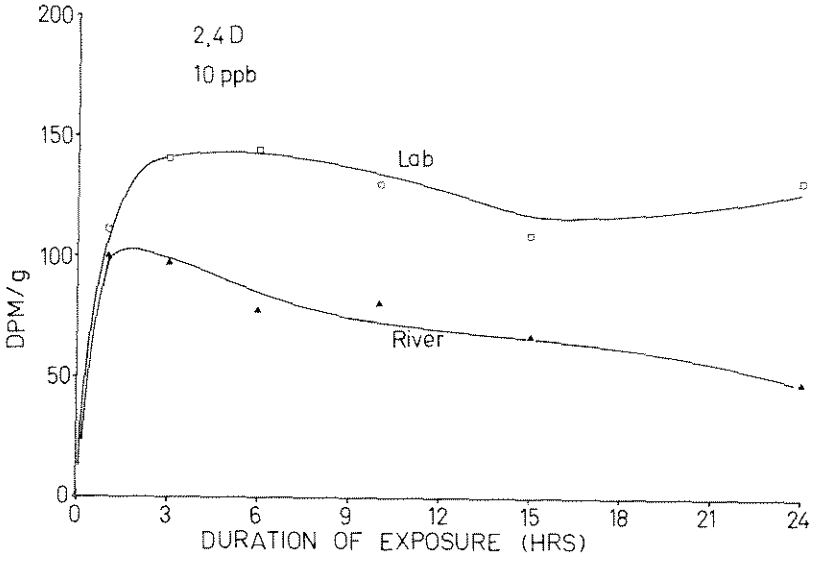
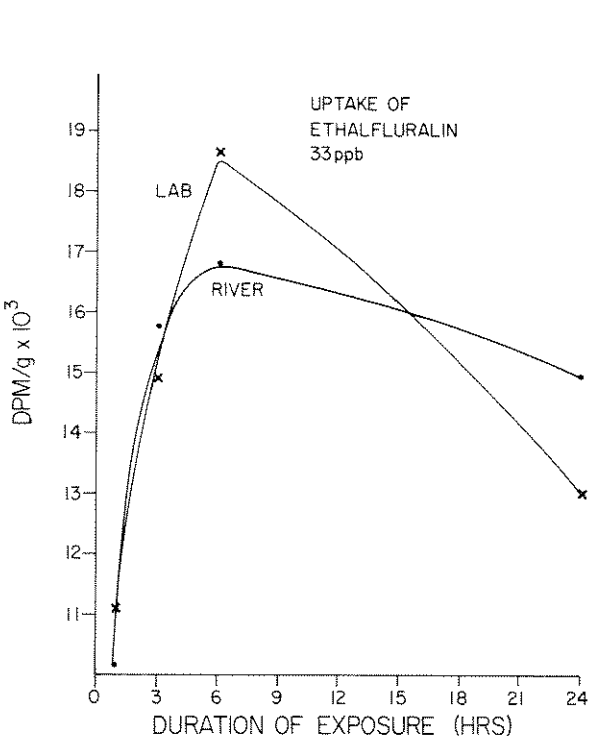
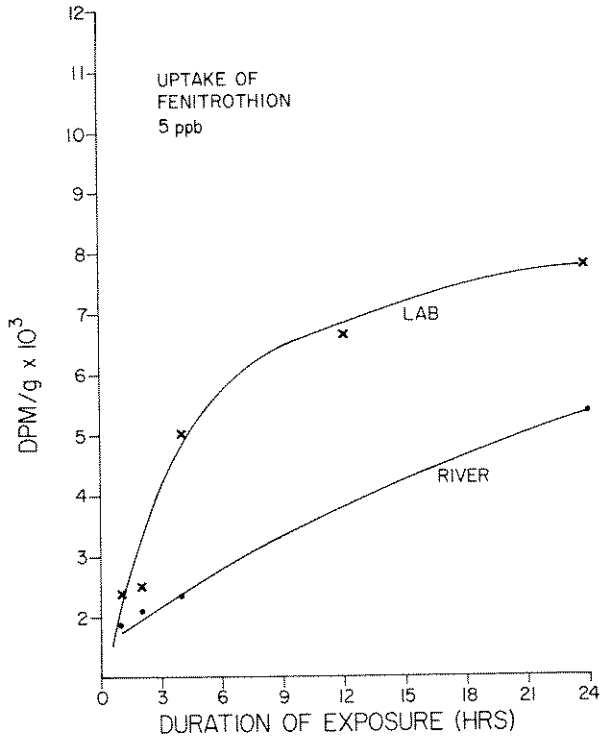
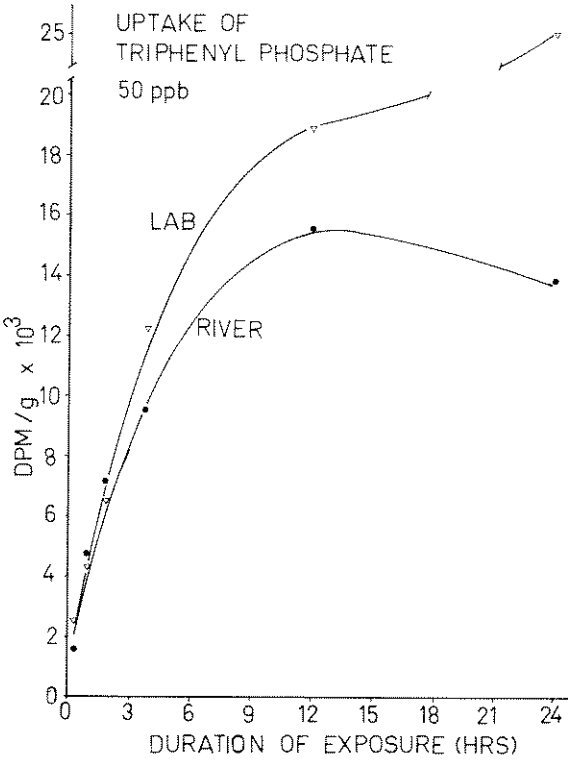


Fig. 1, 2 and 3. Uptake of the herbicides fluridone (1), terbutryn (2) and 2,4-D acid (3) by rainbow trout fry in river water and lab water. DPM/g can be converted to µg/g using specific activities from Table 1.







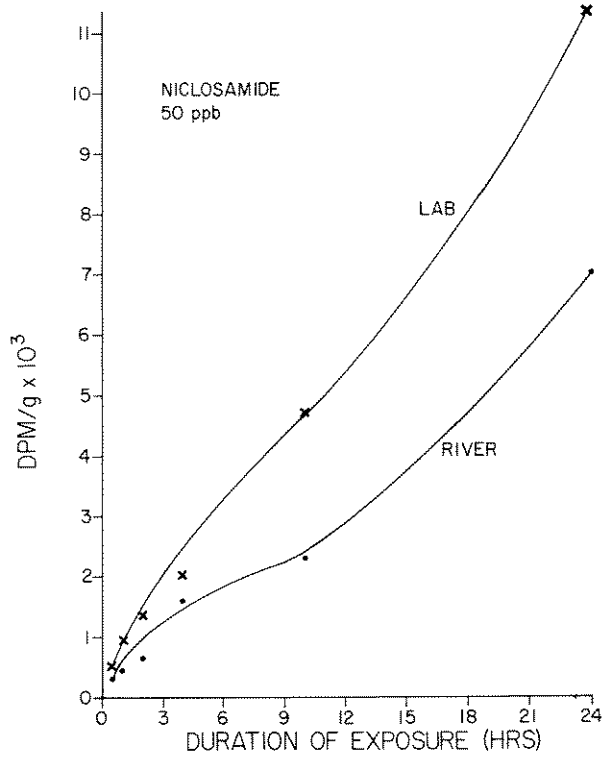


Fig. 4, 5, 6 and 7. Uptake of the compounds, ethalfluralin (4), fenitrothion (5), triphenyl phosphate (6) and niclosamide (7) by rainbow trout fry in river water and lab water. DPM/g can be converted to µg/g using specific activities in Table 1.

degradation products in river water. The niclosamide results are difficult to compare with those of the other compounds because of the much greater rate of degradation.

Hexachlorobiphenyl, methoxychlor, permethrin and DDT (Fig. 8, 9, 10 and 11) had significantly lower initial rates of uptake by fish in river water than in lab water. The differences ranged from 62% for hexachlorobiphenyl to 33% for DDT (Table 3). The relative rate of uptake (DPM/g hr ÷ µg/mL) of methoxychlor was the highest of all the compounds studied (Table 3) but hexachlorobiphenyl, permethrin and DDT had rates of uptake similar to or less than those of ethalfluralin, fenitrothion, TPP and niclosamide. Uptake of hexachlorobiphenyl and DDT (Fig. 10 and 13) was still rapid after 24 hrs with striking differences in the DPM/g levels apparent. The lower rates of methoxychlor and permethrin over the 10 to 24 hr period reflect the metabolism as well as volatilization losses (75% for methoxychlor). Degradation losses for the four compounds in river water were not investigated but would be expected to be small at 10°C based on published reports (Wolfe et al. 1977; Rawn et al. 1980).

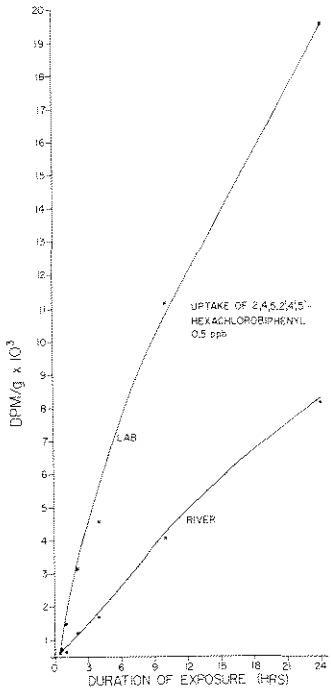
CORRELATIONS WITH PHYSICAL PROPERTIES OF THE CHEMICALS

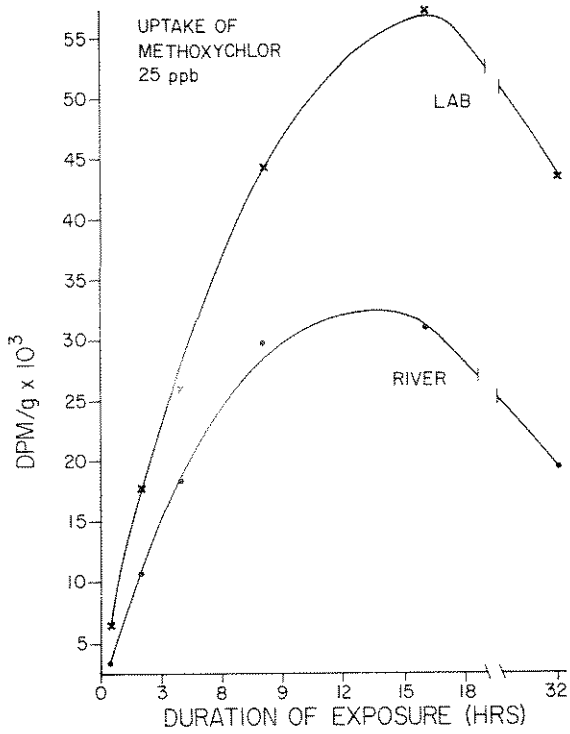
Table 5 lists the K_{OC}, K_{OW} and water solubilities of the test chemicals that were used along with the percent differences in initial uptake rates (Table 3) to calculate correlation coefficients (r) and regression lines of Fig. 12 and 13.

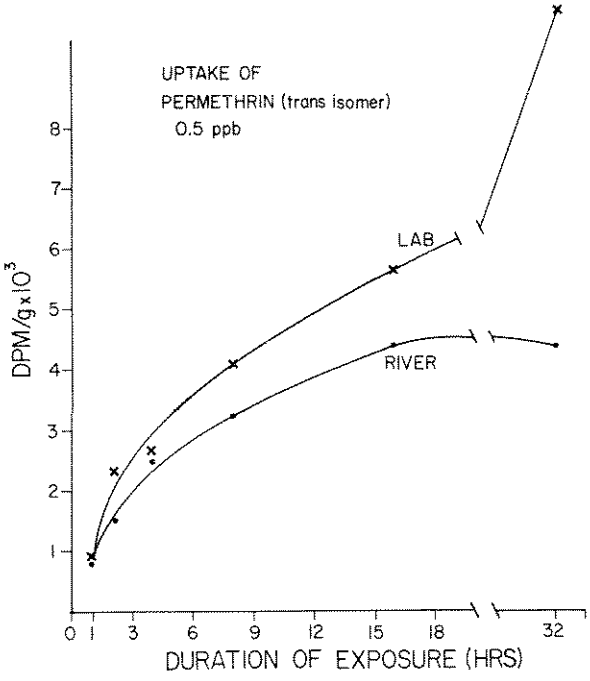
The K_{OC} values are generally higher than those reported in the literature and summarized recently by Kenaga and Goring (1978). The high surface area of the suspended sediment and the low temperature (10°C) at which the adsorption took place may explain these differences (Hamaker and Thompson 1972). The high clay and organic carbon content of the Red River suspended sediment (Table 1) would be expected to yield high K_{OC} values (Karickhoff et al. 1979). A plot of K_{OC} results against percent differences in initial uptake rate between river and lab water treatments (Fig. 12) shows a weak but significant correlation ($r = 0.63$, $P \leq 0.05$ at d.f. = 9). If the niclosamide results are omitted because of the rapid degradation of the compound in river water, a more significant correlation is obtained ($r = 0.80$, $P \leq 0.01$ at d.f. = 8).

Somewhat better agreement was obtained with water solubility versus the percent difference in uptake data (Fig. 13) ($r = 0.66$, d.f. = 9). K_{OW} values for the test chemicals also showed a weak but significant correlation with the percent difference in uptake rate ($r = 0.65$) which is expected since K_{OW} shows an excellent correlation with K_{OC} and water solubility (Karickhoff et al. 1979; Kenaga and Goring 1978). Thus either one of the three physical properties could be used to predict the differences in uptake of organic chemicals between lab studies and those in natural waters.

The results suggest that suspended solids, particularly the organic carbon content of the suspended material, have a great influence on the availability of hydrophobic organic chemicals to fish in river water. While the effects of suspended sediments have always been an important consideration in the fate of pesticides in the aquatic environment (Pionke and Chesters 1973) there are few published reports on the influence of suspended sediment on the uptake of organic chemicals by aquatic organisms. In modelling of fish uptake of organics







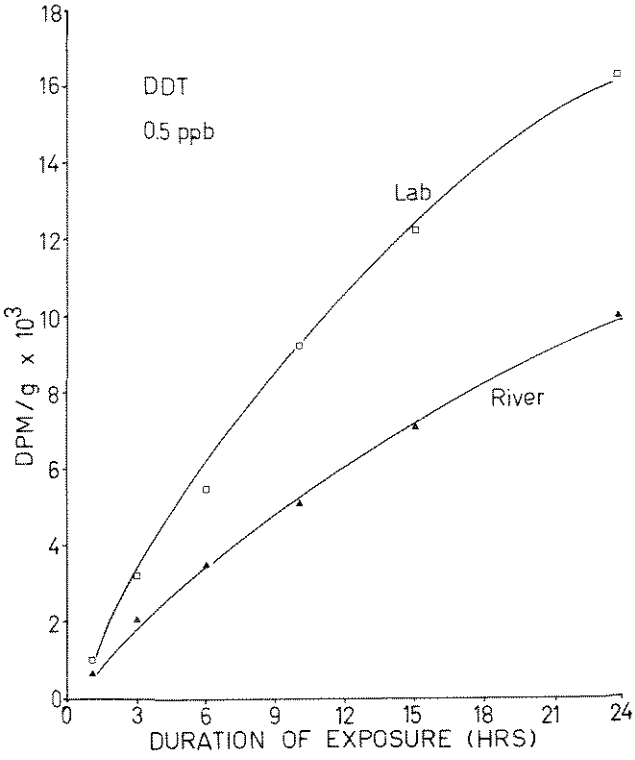
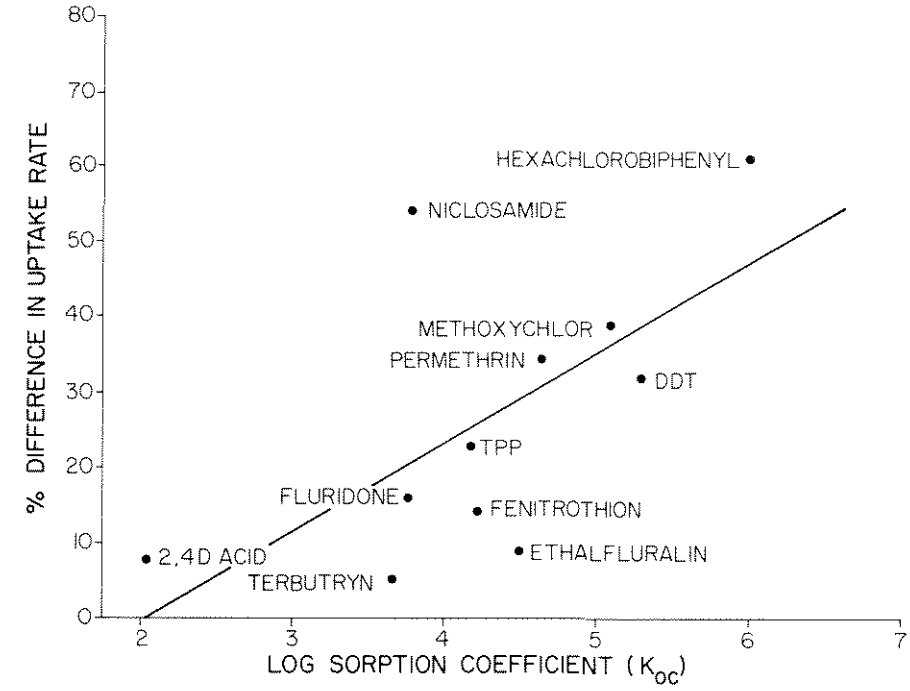


Fig. 8, 9, 10 and 11. Uptake of the compounds hexachlorobiphenyl (8), methoxychlor (9), trans-permethrin (10) and p,p'DDT (11) by rainbow trout fry in river and lab water. DPM/g can be converted to µg/g using specific activities in Table 1.



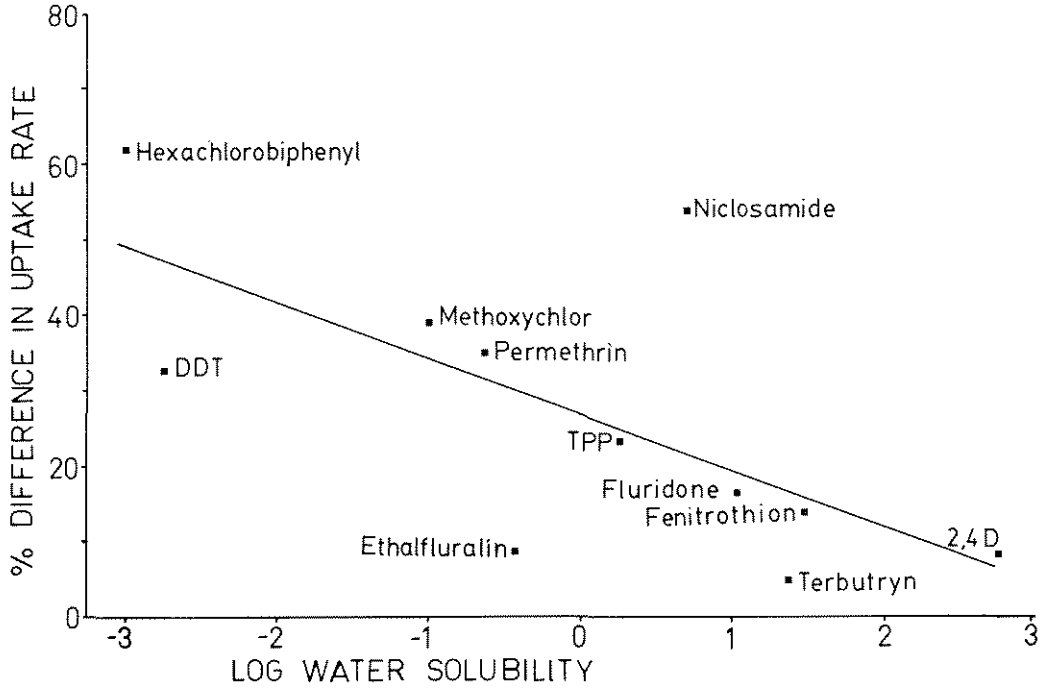


Fig. 12 and 13. Regression lines for correlations of the physical properties K_{OC} and water solubility with percent differences in uptake rate between river and lab water treatments.

Table 5. Physical properties of the organic chemicals used in the study.

Compound	Log K_{oc} [1]	Log K_{ow} [2]	Water Solubility [3] (mg/L)
Fluridone	3.76	2.94	12
Terbutryn	3.68	3.67	25
2,4-D	2.10	1.57	600
Ethalfluralin	4.48	4.59	0.40
Fenitrothion	4.22	3.32	30
TPP	4.17	4.38	1.9
Niclosamide	3.82	3.87	5
Hexachlorobiphenyl	5.99	6.57	0.00095
Methoxychlor	5.12	4.83	0.12
Permethrin	4.63	5.23	0.23
DDT	5.33	5.98	0.0017

[1] measured in Red River water by Muir (1980) unpublished.

[2] measured as described by Metner (1980) unpublished, except for hexachlorobiphenyl, DDT, and 2,4-D which are taken from Kenaga and Goring (1978).

[3] solubility at room temperature (20-25°C) from Kenaga and Goring (1978), except for permethrin (estimated from K_{ow} values) and ethalfluralin (determined in our laboratory).

in aquatic systems (e.g. Neely 1979); it may be necessary to include a term to correct uptake rate constants for the effects of suspended solids or suspended organic carbon. Further studies are needed to assess whether the "protective" effects on the uptake of organic chemicals from water observed in the present work have any long-term significance in terms of lower than expected body burdens of organic pollutants. Work is in progress to evaluate the other water chemistry parameters (Table 1) for their influence on initial uptake rates of the test chemicals.

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UPTAKE OF ETHALFLURALIN AND OTHER PESTICIDES BY FISH

W.L. Lockhart, A.P. Blouw, D.A. Murray, D.A. Metner and D.C.G. Muir

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

LOCKHART, W.L., A.P. BLOUW, D.A. MURRAY, D.A. METNER and D.C.G. MUIR 1980
Uptake of ethalfluralin and other pesticides by fish. Can. Tech. Rep. Fish. Aquat. Sci. 975: 27-37.

Fish were exposed to ethalfluralin, an experimental dinitroaniline herbicide, and several other compounds in order to estimate rates of transfer from water to fish. These rates were expressed relative to calculated oxygen consumption, as suggested by Norstrom et al. (1976). Ethalfluralin was taken up from water by rainbow trout "swim up" fry about as efficiently as oxygen while younger sac fry were approximately 10% as efficient at accumulating ethalfluralin. Uptake and depuration rate constants were determined experimentally for fingerlings, and bioaccumulation ratios of 2200-2500 were calculated indicating that ethalfluralin behaved similarly to trifluralin. Distribution among various body organs and residues associated with death both suggested that a model treating the fish as several compartments will be required in order to use residue measurements as pathological tools in risk assessment.

Key words: Dinitroaniline; herbicides; energy budget; partitioning; bioaccumulation

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L'article qui suit décrit une étude menée en vue d'estimer le taux de transfert de l'eau aux poissons, après avoir exposé ces derniers à l'éthalfluraline, à un herbicide expérimental dinitroaniline et à plusieurs autres composés. Comme le suggèrent Norstrom et al. (1976), nous exprimons ces taux par rapport à la consommation d'oxygène calculée. L'éthalfluraline est captée par les alevins de truite arc-en-ciel au stade de "nage vers le haut" avec autant d'efficacité que l'oxygène. Par ailleurs, les alevins vésiculés, plus jeunes, accumulent ce composé à une efficacité d'environ 10%. Nous avons déterminé expérimentalement les constantes de taux de captation et de dépuration chez des fingerlings, et calculé des rapports de bioaccumulation de 2,200-2,500, signe que l'éthalfluraline se comporte de la même manière que la trifluraline. La distribution des substances dans les divers organes du corps et les résidus associés avec la mort laissent tous deux supposer qu'il faudra un modèle traitant les poissons comme autant de compartiments, si l'on veut utiliser les mesures de résidus comme outil pathologique dans l'évaluation des risques.

Ethalfluralin (Sonalan; α,α,α-trifluoro-2,6-dinitro-N-(2-methyl-2-propenyl)-N-ethyl-p-toluidine) is an experimental herbicide being developed by Elanco Products.

Ethalfluralin is unusually toxic to fish by comparison with most other herbicides. Technical literature supplied by the manufacturer lists LC_{50} values as shown:

Species	LC_{50} (ppb) after 96 hours
Bluegill sunfish (*Lepomis macrochirus*)	32
Rainbow trout (*Salmo gairdneri*)	37
Goldfish (*Carassius auratus*)	260

One component leading to toxicity is uptake of the toxicant by the fish, and we are reporting our preliminary studies of uptake rates of ethalfluralin, as compared with uptake rates for a series of other unrelated pesticides. We report also some experience with the behaviour of ethalfluralin in laboratory situations.

MATERIALS AND METHODS

Ethalfluralin was supplied by the manufacturer, Elanco Products Division of Eli Lilly and Co. as a 99% pure analytical standard, as Sonalan, the formulated emulsifiable concentrate, and as ring-labelled ^{14}C-ethalfluralin.

ANALYTICAL METHODS

One liter of water containing ethalfluralin was extracted with 200 µL n-hexane using an extraction flask described by Murray (1979) and the hexane extract was analysed directly by gas chromatography (GLC). Animal tissues were homogenized in a blender with hexane:ethyl acetate (1:1). Extracts were dried with sodium sulfate and analysed by GLC. GLC was carried out on a Hewlett-Packard 5750 fitted with glass column 1.22 m x 6.35 mm packed with 2% Dexsil on Chrom W and an electron capture detector. Operating temperatures were (°C): detector 250; injection 230; column 200. Peak areas were integrated and quantities of ethalfluralin were estimated by reference to injections of known quantities of ethalfluralin.

For determination of radioactivity in experiments with ^{14}C-ethalfluralin and other compounds, samples to be counted were burned in a Packard oxidizer in preparation for liquid scintillation counting. Prior to use, labelled compounds were purified by preparative thin layer chromatography. After autoradiography to locate spots, labelled materials were recovered by scraping appropriate regions from plates, followed by solvent extraction.

FISH EXPOSURES

Rainbow trout fry (< 1 g) were obtained from the Freshwater Institute's hatchery and were exposed to ethalfluralin and other pesticides in duplicate experiments under static conditions at 10°C. Fish (generally 30) were placed in aerated dechlorinated Winnipeg city tap water in 4-L glass containers for at least 24 hours prior to addition of pesticides. After addition of radio-labelled pesticides, generally in an acetone carrier for water-insoluble compounds, five fish were removed at 1, 2, 4, 8 and 24 hour intervals from each container, rinsed, weighed and stored frozen until oxidized and counted for isotope content. Similar experiments were performed in which trout sac fry were exposed to several concentrations of ethalfluralin.

For estimates of "clearance" rates, fish (5-15 g) were exposed to 10^{-5}M ethalfluralin for 2 hours and then transferred to clean flowing water in a 160-L fiberglass tank. Samples of six fish were taken for GLC analysis at the time of transfer and then at time intervals of 4 hours, 1, 2, 4, 8, 16 and 32 days.

Larger trout were also exposed to ethalfluralin in aquaria in order to obtain individual body organs for analysis.

We have begun investigations of the behaviour of ethalfluralin in water. Sterilized distilled water was fortified with purified ^{14}C-ethalfluralin; flasks were stored in complete darkness at 20°C and samples were removed at times 0, 3, 14, 37, 56, 77 and 136 days for assay by GLC and isotope counting. In addition, proportions of ethalfluralin associated with water and suspended phases were estimated with natural river waters by adding ethalfluralin, mixing for 24 hours, then counting radioactivity before and after centrifugation at 39,000 x g for 15 minutes.

RESULTS AND DISCUSSION

RECOVERIES OF ETHALFLURALIN

Recoveries of ethalfluralin from fish samples were compared using GLC and isotope methods. The radioactivity associated with the extracted tissue residue was determined by combustion and isotope counting. From these results (Table 1) it appears that solvent extraction left approximately 18.2% of total activity associated with insoluble tissue remains. It is not clear whether this amount represents ethalfluralin or other materials, but fortification of dead tissue allowed essentially quantitative recovery of ethalfluralin. Even within the solvent extract, quite variable proportions of the radioactivity were identifiable as ethalfluralin by GLC. Fish C, (Table 1) for example had only about one quarter of its ^{14}C activity present as ethalfluralin. Chromatograms often showed major unidentified peaks. It seems clear that these fish metabolized variable proportions of ethalfluralin to unidentified materials, some of which were not extracted into the hexane:ethyl acetate solvent. The nature of ethalfluralin metabolites in fish is unknown. Kearney et al. (1977) have reported metabolism to unknown compounds following exposures of fish to another dinitroaniline, profluralin. Ability to metabolize profluralin was greater in *Gambusia* than in *Ictalurus*.

STABILITY OF ETHALFLURALIN

Leitis and Crosby (1974) have described a number of products resulting from photodecomposition of trifluralin in water. The transparency of flowing water in much of western Canada is very limited, however, because of high levels of suspended solids. We conducted an experiment to estimate the decomposition of ethalfluralin in sterile lab water kept in the dark at room temperature (about 20°C). The results (Table 2) indicated that ethalfluralin was lost both in terms of GLC assay and by ^{14}C assay. The half-life of intact ethalfluralin under these conditions was about 1 month. Losses of radioactivity were less rapid than losses of ethalfluralin suggesting decomposition to new products.

ADSORPTION OF ETHALFLURALIN

Pesticides with low water solubilities tend to adsorb to suspended material in the water. This may result in reduced availability to organisms accumulating those pesticides by gill absorption. Adsorption of ethalfluralin to suspended solids in Red River water was estimated by adding ^{14}C-ethalfluralin to water and by determining activity present in the water before and after centrifugation. The proportions removed by centrifugation are shown in Table 3. Almost 30% of ethalfluralin was removable from the water column by centrifugation of Red River water, and only 6.7% was removable from distilled water. If the difference between these values represents ethalfluralin adsorbed to river sediments, then about 20% was adsorbed. In her studies of trifluralin contaminating the Wabash River, Spacie (1975) reported 13-26% bound to river solids (river solids were reported as 124-575 ppm).

UPTAKE OF ETHALFLURALIN BY FISH

Rainbow trout fry exposed to ^{14}C-ethalfluralin in water were analysed for ^{14}C-content by sub-sampling exposed groups at 1, 2, 4, 8 and 24 hours after starting exposure. A similar experiment was conducted using unlabelled material and presenting ethalfluralin as the commercial emulsifiable concentrate sonalan. Also, uptake rates by sac fry were compared as a function of ethalfluralin concentration.

A typical uptake curve of fish body burden of ethalfluralin as a function of exposure duration is shown for sac fry exposed to $10^{-6}M$ ethalfluralin (Fig. 1). To evaluate parameters in the expression for uptake from water, as described by Norstrom et al. (1976), the initial slope of this uptake curve was taken since this would minimize such sources of error as metabolism and/or excretion by fish, decomposition and other losses of ethalfluralin from the water. To approximate the slope of the tangent to the initial portion of the curve, the curve was assumed to pass through the origin and the slope to the 1-hr point was calculated. This slope was taken as the value for dp/dt and the value obtained was divided by the initial measured radioactivity in the exposure water (c) to give an expression for the efficiency of uptake of pollutant from water $e_{pw} = (dp/dt)/c$. A calculation for oxygen uptake (e_{ox}) was also carried out using an expression to give the mL of water theoretically "cleared" of oxygen per hour. This value $e_{ox} = Q/q_{ox}/c_{ox}$ (Norstrom

et al. 1976) is calculated from the fish weight ($Q = 0.207\ (\text{weight})^{0.73}$); the "caloric equivalent" of oxygen, q_{ox} = 3.42 kcal/g; and C_{ox} the solubility of atmospheric oxygen at the exposure temperature (calculated as 10.876 mg/L at 10°C). The ratio of values for ethalfluralin uptake and oxygen uptake was calculated to give the efficiency of pesticide uptake relative to oxygen (e_{pw}/e_{ox}). The results shown in Table 4 indicate that ethalfluralin was taken up extraordinarily efficiently from water by fry after resorption of yolk. Stages both younger and older appear to have been rather less efficient than young fry. Prior to resorption of yolk the uptake efficiency was only about one tenth as efficient as it was after resorption. In the test with sac-fry and different concentrations of ethalfluralin the uptake efficiency was similar over the three orders of magnitude in ethalfluralin concentration, as would be expected if uptake rate was indeed controlled by the concentration of ethalfluralin in the water.

It should be stressed that we have attributed differences among efficiency calculations to differences among pesticide uptake rates. It is also possible that parts of these differences may be attributed to differing oxygen consumptions.

In order to compare ethalfluralin uptake efficiencies with those for some other compounds, similar uptake experiments were conducted on other compounds with the results shown in Table 5. Norstrom et al. (1976) listed efficiency values for DDT ranging from 0.073 to 1.90 for fish of varying species and sizes. Values for methyl mercury were more tightly grouped from 0.14 to 0.49 for several species. It appears that ethalfluralin is readily taken up by fish, but that sac fry and fingerlings are substantially less efficient at uptake than fry at the "swim-up" stage. In comparison with other pesticides, ethalfluralin ranked the highest among those tested; an unexplained anomaly in these data is the apparent low uptake efficiency of hexachlorobiphenyl. Several papers have been published showing statistically meaningful relationships between bioaccumulation and physical properties of non-polar compounds. For example, Neely et al. (1974) derived a relationship between bioconcentration factor (BF) and octanol/water partition coefficient (P): $\log BF = 0.542 \log P + 0.124$. Other authors have used water solubility (Chiou et al. 1977) and recently the parachor has been suggested (Tulp and Hutzinger 1978). When uptake values for the compounds in Table 5 were plotted as a function of the octanol/water partition coefficients (using logarithms on both axes), weak relationships were found. When e_{pw}/e_{ox} was plotted, the correlation coefficient was 0.59 (not significant); when $(dp/dt)/c$ was plotted, the value was 0.80 ($p < 0.01$).

We have used the data on ethalfluralin uptake by fingerlings to estimate an uptake rate constant from the expression $dC_f/dt = k_1 C_w$ when C_f and C_w are concentrations of ethalfluralin in fish and water respectively. For this calculation uptake was plotted in terms of concentrations in fish rather than body burden, for both fry and fingerlings, and an estimate of the initial slope dC_f/dt was obtained from the concentrations in fish at the first point, and k_1 was obtained after dividing the slope by the concentration of ethalfluralin in the water. Values for k_1 for the two replicate exposures of fingerlings were 10.23 and 11.32 hr^{-1}.

A clearance experiment was also conducted on fingerlings using chromatographic analyses to measure ethalfluralin depuration. By plotting the natural

logarithm of ethalfluralin remaining against time (Fig. 2) the regression equation [$\log_e$ (ethalfluralin in fish) = -0.00448 (time in hours) + 2.8620] was calculated. The slope in this equation is the clearance rate constant k_2 (Moriarty 1975) in units of hr^{-1}. The bioaccumulation ratio for fingerlings was then estimated from the ratio k_1/k_2, and hence for rainbow trout fingerlings values for bioaccumulation ratios were 2283 and 2527. Using similar calculations with trifluralin, Spacie and Hamelink (1979) reported a bioaccumulation ratio of 3261 with fathead minnows; at least with regard to accumulation in fish it seems that the dynamics of ethalfluralin will be very similar to those of trifluralin.

With compartmental models, one must question whether to treat the fish as one or several compartments. Our study cannot answer the question but data describing distribution of ethalfluralin in various fish organs (Table 6) may be useful in making such a decision. These data suggest rapid transfer from water to fish through gill surfaces, then distribution to internal organs by the blood, with storage in fat deposits.

The meaning of residue measurements, in terms of biological risk to fish, is not clear from data presented in this report, nor indeed in many reports of pesticide residues in fish. Both water concentration and exposure duration seem to determine fish responses, and the residue accumulation by itself may not be meaningful in trying to judge biological risk. For example, fish killed by a 10-minute exposure to 10^{-4}M ethalfluralin (as Sonalan EC) contained body residues of 10-25 µg/g. Others survived 8 days exposure at a theoretical starting concentration of 10^{-6}M, and contained residues as high as 210 µg/g. Obviously a spill of ethalfluralin might result in measurable fish residues, but it would be difficult to associate risk with those measurements. These observations suggest that, for risk assessment, in contrast to the estimation of bioaccumulation, a more complex compartmental model is required.

CONCLUSIONS

Ethalfluralin was rapidly accumulated from water by fish, but not all of the accumulated material was extracted by conventional solvent extraction. It appears that fish metabolized a proportion of ethalfluralin to unidentified products.

In water maintained in the dark under aseptic conditions, ethalfluralin was lost only slowly, with an apparent half-life of about one month; the fate and nature of the lost portion remained unknown. It river water high in suspended solids, a portion of ethalfluralin estimated at about 20% was associated with sediments; presumably this would limit the biological availability of sorbed ethalfluralin in a stream.

The estimated efficiency of ethalfluralin uptake varied with the size of fish, being about 10% that of oxygen for sac-fry, equal to oxygen for "swim-up" fry, and about 50% of oxygen for fingerlings. Ethalfluralin uptake efficiencies were the highest among those encountered for a series of compounds, but ethalfluralin was readily lost from trout fingerlings with a half-life of about 6 days. The proportions of this "loss" due to clearance and to metabolism are not known. Combining uptake and clearance rate data, the potential for bioaccumulation of ethalfluralin was estimated to be about 2400 times the concentration in the water.

Distribution of ethalfluralin among body organs, and residues associated with death both suggested the need for a more sophisticated compartmental model if residue measurements are to be used as pathological tools in assessment of biological risk.

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Dr. Jon Anderson of Elanco Products kindly supplied samples of ethalfluralin.

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Table 1. Quantities of ethalfluralin and radioactivity recovered from rainbow trout exposed to ^{14}C-ethalfluralin for 5 days @ $10^{-7}M$. Hexane:ethyl-acetate extracts were analysed by GLC and by liquid scintillation counting. Insoluble tissue remaining after extraction was oxidized and counted for ^{14}C-content. Results from ^{14}C detection are expressed as ethalfluralin, although they may not have been present as that compound.

Fish	Ethalfluralin (µg) in extract		Percent ^{14}C (as µg ethalfluralin) not extracted by solvent
	Assay by GLC	Assay by ^{14}C	
A	17.2	36.2	15.1
B	29.3	53.3	15.8
C	13.0	54.3	19.6
D	16.7	28.0	23.9
E	20.5	22.8	16.9
			Mean 18.2

Table 2. Percentage of ethalfluralin remaining in sterilized distilled water maintained in darkness at 20°C.

Time after mixing (days)	Percent remaining	
	Assay by GLC	Assay by ^{14}C
0	100	100
3	97.5	100
14	63.6	72.4
37	45.5	56.4
56	16.4	44.4
77	8.4	34.2
136	1.9	46.2

Table 3. Distribution of ^{14}C-ethalfluralin (282 µg/L) between water and suspended solids after 24 hours mixing. The radioactivity in water was determined before and after centrifugation, and the amount removed by centrifuging was taken as the proportion associated with suspended material.

Water Type	Suspended solids (ppm)	% radioactivity with water	% radioactivity with suspended solids
distilled	<1	93.3	6.7
1/3 Red River + 2/3 distilled	20	87.5	12.5
2/3 Red River + 1/3 distilled	42	80.3	19.7
Red River	66	71.8	28.2

Table 4. Parameters for ethalfluralin uptake from water by fish, using conventions of Norstrom et al. (1976). The column designated (dp/dt)/c refers to uptake rate of ethalfluralin from water; that designated $Q/q_{ox}\ C_{ox}$ refers to calculated oxygen consumption and the ratio of these two columns (e_{pw}/e_{ox}) represents the fractional efficiency of ethalfluralin uptake relative to oxygen.

Fish Group	Exposure	(dp/dt)/c (mL/hr)	$Q/q_{ox}\ C_{ox}$ (mL/hr)	e_{pw}/e_{ox}
sac fry	^{14}C-ethalfluralin $10^{-8}M$	.6928	4.8	.144
sac fry	^{14}C-ethalfluralin $10^{-7}M$	.4476	4.8	.093
sac fry	^{14}C-ethalfluralin $10^{-6}M$	.5696	4.8	.119
"swim-up" fry	^{14}C-ethalfluralin $8 \times 10^{-8}M$	8.164	7.424	1.10
fingerlings	sonalan $10^{-6}M$ (assay by GLC)	56.26	121.6	0.463

Table 5. Efficiency of uptake of several pesticides. Rainbow trout fry or fingerlings were exposed to the compounds and uptake rates were calculated as fractions of calculated oxygen consumption.

Compound	$(dp/dt)/c$ (mL/hr)	$Q/q_{ox}\ C_{ox}$ (mL/hr)	e_{pw}/e_{ox}
methoxychlor	5.7581	6.9384	0.83
terbutryn	1.1172	7.2097	0.16
fenitrothion	3.8882	7.2557	0.54
niclosamide	3.1775	7.2307	0.44
fluridone	0.4083	7.1436	0.06
permethrin	3.4110	17.4078	0.20
triphenyl phosphate	8.2594	10.5175	0.79
2,4-D	0.9420	31.9908	0.03
hexachlorobiphenyl	18.0650	28.553	0.63

Table 6. Concentrations of ethalfluralin (µg/g), as determined by GLC in several body organs of rainbow trout.

Organ	After 10 min exposure to 10^{-4}M sonalan	After 10 hours exposure to 10^{-6}M sonalan	After 16 hours exposure to 10^{-6}M sonalan, then 3 days in clean water
Blood	2.86	1.45	0.09
Gill	44.4	0.60	0.11
Brain	1.86	0.77	0.54
Bile	ND	0.34	0.10
Fat	0.44	8.46	24.37
Liver	Trace	0.07	Trace
Kidney	0.30	0.23	0.05
Muscle	0.62	1.35	2.11
Skin	0.30	0.27	0.19

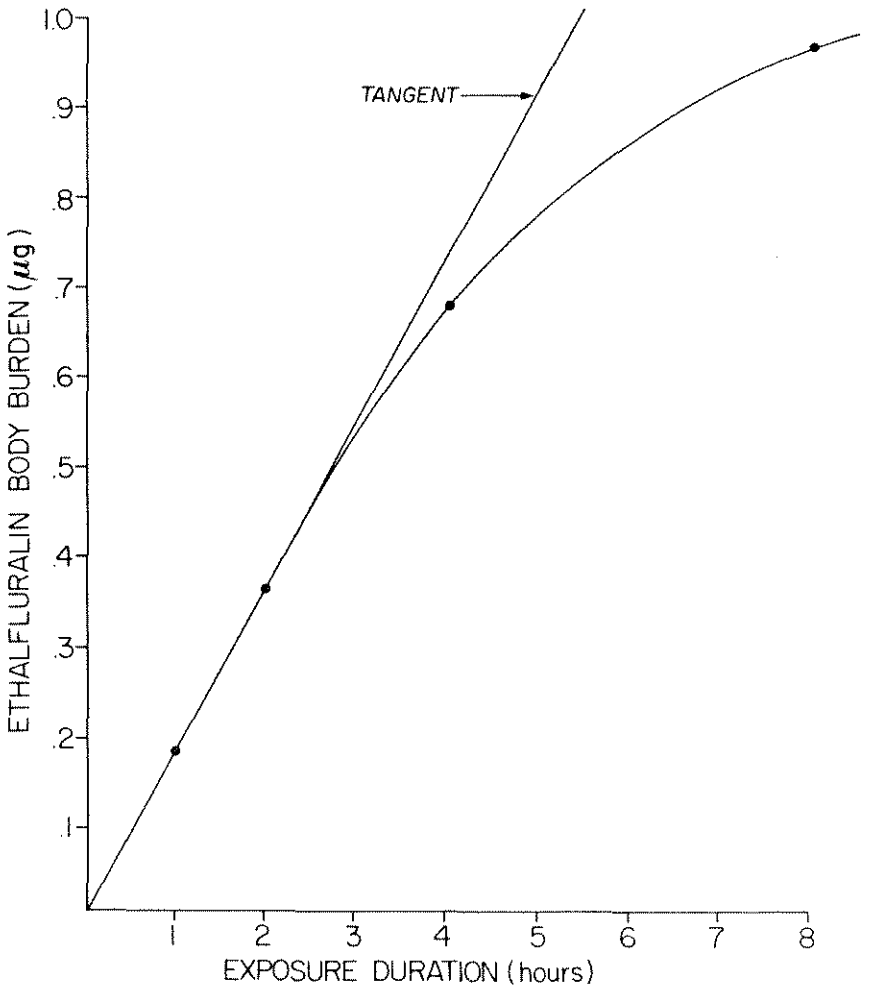

Figure 1. Uptake of ^{14}C-ethalfluralin ($10^{-6}M$) from water by rainbow trout sac fry at 10°C.

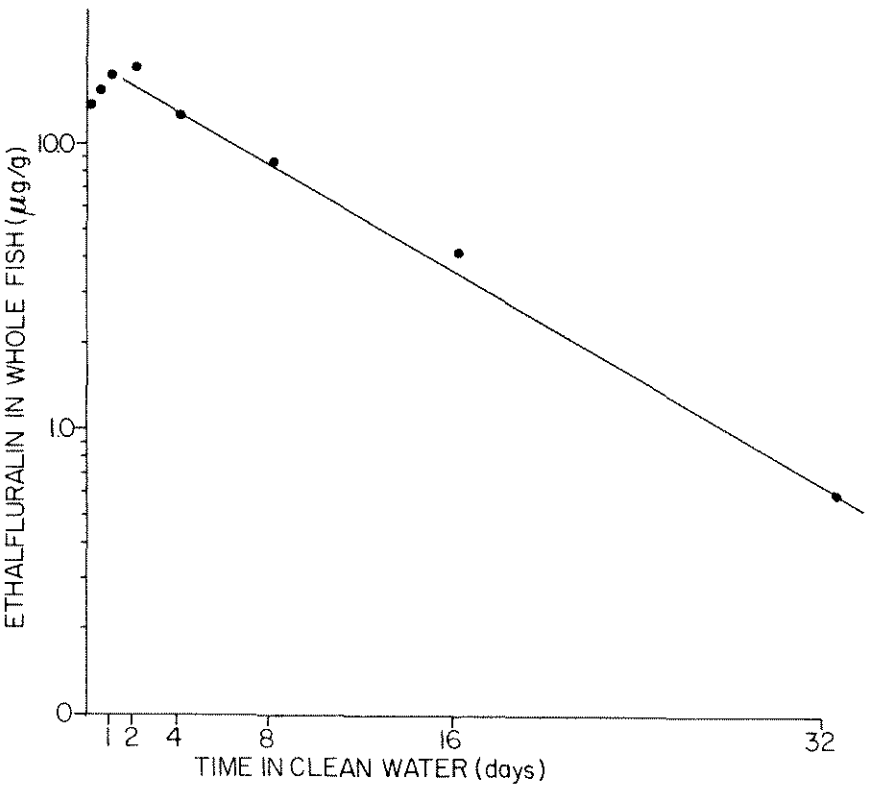

Figure 2. Decline in ethalfluralin concentrations in rainbow trout fingerlings maintained at 10°C in flowing water free from ethalfluralin.

A METHODOLOGY FOR DETERMINING THE DISTRIBUTION AND ELIMINATION OF MERCURIAL COMPOUNDS IN GOLDEYE (Hiodon alosoides)

B.A. Munson

Alberta Oil Sands, Environmental Research Program, Edmonton, Alberta

MUNSON, B.A. 1980 A methodology for determining the distribution and elimination of mercurial compounds in goldeye (Hiodon alosoides). Can. Tech. Rep. Fish. Aquat. Sci. 975: 38.

Goldeye, Hiodon alosoides, sampled in 1973 from the North Saskatchewan in Alberta, contained mercury levels significantly higher than other species of fish examined from the same relatively mercury free environment. Two possible hypotheses exist to explain this data; either goldeye accumulate mercury in some other environment and then migrate into Alberta, or goldeye possess some unusual physiological feature which permits a resident population of goldeye to accumulate and retain mercury from an environment which possesses a relatively low level of contamination.

A methodology to test the latter hypothesis was developed utilizing temperature regulated, restricted-movement chambers. A description of this methodology and preliminary results will be presented.

Key words: Methodology; chemical analysis; mercury (metal); fish

MUNSON, B.A. 1980 A methodology for determining the distribution and elimination of mercurial compounds in goldeye (Hiodon alosoides). Can. Tech. Rep. Fish. Aquat. Sci. 975: 38.

Des laquaiches aux yeux d'or, Hiodon alosoides, récoltées en 1973 dans la North Saskatchewan River (Alberta) contenaient du mercure à des teneurs nettement plus élevées que celles des autres espèces de poissons examinés, provenant du même environnement relativement libre de mercure. On peut expliquer ce phénomène par l'une ou l'autre des hypothèses suivantes : la laquaiche aux yeux d'or accumule le mercure dans un autre milieu avant d'émigrer en Alberta, ou encore elle possède quelque caractéristique physiologique inusitée permettant à une population résidente de laquaiches aux yeux d'or d'accumuler et de retenir le mercure capté d'un environnement à niveau de contamination relativement bas.

L'auteur a mis au point une méthodologie afin de vérifier la seconde hypothèse, à l'aide de chambres à température contrôlée et à mouvements restreints. Suit une description de cette méthodologie et des résultats préliminaires.

THE MICROTOX BACTERIAL LUMINESCENCE ASSAY FOR AQUATIC TOXICITY MONITORING

A.A. Bulich, M.W. Greene and D.I. Isenberg

Beckman Instruments, Inc., Carlsbad, California

BULICH, A.A., M.W. GREENE and D.I. ISENBERG 1980 The microtox bacterial luminescence assay for aquatic toxicity monitoring. Can. Tech. Rep. Fish. Aquat. Sci. 975: 39-40.

Acute toxicity testing of aquatic samples is conventionally accomplished with 24 to 96 hour bioassays using various species of fish or other aquatic biota. Such testing procedures are time consuming, expensive to perform and difficult to standardize. The assay method described here, using luminescent bacteria as the test organisms, correlates well with other bioassays and requires less than thirty minutes to obtain a complete reportable assay. The assay system, designated MICROTOX^{TM}, is an instrumental approach in which the bioassay organisms are handled much like a chemical reagent.

To perform a test, a vial of freeze-dried luminescent bacteria is instantly activated via hydration. Aliquots (.01 mL) of this hydrated cell suspension are diluted into 0.5 mL of testing solution. This suspension (containing about 10^6 individual cells) emits a relatively constant amount of light, which is easily measured and recorded using a specially designed, temperature controlled photometer. After obtaining initial light output values for each of the cuvettes containing test solution, aliquots (0.5 mL) from a second set of cuvettes containing an array of toxicant or effluent concentrations are added.

The array of toxicant concentrations (up to 8) may include one or more controls. After allowing time for the toxicants to effect the organisms (usually 5 minutes) final light readings are obtained for each test cuvette. The decrease in light output corresponding to each toxicant concentration is easily calculated. An EC50 value (that concentration of effluent or toxicant which produces a 50% decrease in light output in time t) may be readily obtained by the usual graphical or computer techniques employed for determining LC50 values (that concentration causing 50% mortality in time t) in conventional bioassays.

Optional data reduction schemes are summarized in a table. Data establishing the reproducibility of the Microtox system for pure toxicants such as phenol and sodium lauryl sulfate are presented. Coefficients of variation of less than 20% are common for pure compounds. The results of over 50 correlation tests on complex effluents using both Microtox and conventional bioassays (such as fish and daphnia) are presented in a summary table. These results demonstrate a high order of correlation for complex effluents. In addition, the Microtox EC50 values obtained for twenty pure compounds are presented in a table together with published values of LC50 for fish and other organisms. Again, the similarity between Microtox and conventional bioassay results is apparent for these compounds which cover an EC50 (5 minute) range of 0.4 ppb (tributyltin oxide) to 3% (ethanol).

Key words: Bioassays; toxicity tests; micro-organisms; bioluminescence

BULICH, A.A., M.W. GREENE and D.I. ISENBERG 1980 The microtox bacterial luminescence assay for aquatic toxicity monitoring. Can. Tech. Rep. Fish. Aquat. Sci. 975: 39-40.

Diverses espèces de poissons ou autres communautés aquatiques peuvent très bien servir à des essais de toxicité aiguë de 24 à 96 h. Mais de tels essais prennent du temps, sont dispendieux et difficiles à normaliser. La méthode décrite dan l'article qui suit, utilisant des bactéries luminescentes comme organismes expérimentaux, s'accorde bien avec les autres essais biologiques et nécessite moins de 30 minutes pour un essai complet. Le système, appelé MICROTOX^{MC}, adopte une approche instrumentale en ceci qu'il implique la manipulation des organismes un peu à la façon d'un réactif chimique.

Dans un essai, des bactéries luminescentes cryodésséchées contenues dans une éprouvette sont activées instantanément par hydratation. On dilue des portions aliquotes (0.01 mL) de cette suspension cellulaire hydratée dans 0.5 mL de solution d'essai. Cette suspension (contenant environ 10^6 cellules individuelles) émet une quantité de lumière relativement constante, facilement mesurable, et qui est enregistrée sur un photomètre spécialement conçu, à température contrôlée. Après avoir trouvé les valeurs initiales de l'émission lumineuse pour chacune des cuvettes contenant la solution d'essai, on ajoute des portions aliquotes (0.5 mL) prélevées à même une deuxième série de cuvettes contenant diverses concentrations du toxique ou de l'effluent.

La série de concentrations de toxiques (jusqu'à 8) peut inclure un ou plusieurs témoins. Après un intervalle suffisamment long pour permettre aux toxiques d'agir sur les organismes (ordinairement 5 minutes), on procède à des lectures finales de lumière pour chacune des cuvettes d'essai. On calcule aisément la diminution de l'émission lumineuse correspondant à chaque concentration de toxique. On trouve une valeur de CE50 (cette concentration de l'effluent ou du toxique qui produit une diminution de 50% de l'émission lumineuse dans le temps t) par les méthodes usuelles, graphiques ou par ordinateur, de détermination de CL50 (la concentration causant 50% de mortalité dans le temps t) dans les essais biologiques conventionnels.

Nous résumons dans un tableau les schèmes optionnels de réduction des données. Nous présentons également les données permettant de reproduire le système Microtox pour des toxiques purs, tels que le phénol et le sulfate lauryl de sodium. Des coefficients de variation inférieurs à 20% se rencontrent communément avec des composés purs. Nous résumons dans un autre tableau les résultats de plus de 50 essais de corrélation sur des effluents complexes, utilisant et le système Microtox et les essais biologiques conventionnels (comme avec poissons et daphnies). On constate un haut degré de corrélation avec des effluents complexes. En outre, les valeurs de CE50 obtenues avec Microtox pour 20 composés purs apparaissent dans un tableau, avec des valeurs publiées de CL50 pour des poissons et autres organismes. Ici encore, la ressemblance entre les résultats par Microtox et par essais biologiques conventionnels sont évidents chez ces composés couvrant une gamme de CE50 (5 minutes) de 0.4 µg/L (oxyde de tributyltine) à 3% (éthanol).

ENVIRONMENTAL ACIDIFICATION IMPACT DETECTION BY EXAMINATION OF MATURE FISH OVARIES

J.H. McCormick, G.N. Stokes and G.J. Portele

U.S. Environmental Protection Agency,
Environmental Research Laboratory-Duluth,
6201 Congdon Boulevard,
Duluth, Minnesota 55804

McCORMICK, J.H., G.N. STOKES and G.J. PORTELE 1980 Environmental acidification impact detection by examination of mature fish ovaries. Can. Tech. Rep. Fish. Aquat. Sci. 975: 41-48.

Fathead minnow egg production and hatching success at pH 5.3 was scant to non-existent. Eggs spawned and incubated until hatching at pH 8.1 and 6.3 produced 39.7 and 5.7% normal larvae, respectively; eggs spawned at pH 6.3 and incubated at 5.3 produced 0.5% normal larvae. Reproductive failure at pH 5.3 may have been predicted by ovarian histology. Ovaries of fathead minnows at pH 5.3 had exceptionally high proportions of pre-ovulatory corpora atretica relative to other oocyte stages present. The fathead minnow data were generated as a basis of extrapolation to estimate reproductive impairment in smallmouth bass inhabiting acid environments by examination of their ovarian condition.

Key words: Acid precipitation; minnow, fathead; bass, smallmouth; gametogenesis; reproduction (biology); pH

McCORMICK, J.H., G.N. STOKES and G.J. PORTELE 1980 Environmental acidification impact detection by examination of mature fish ovaries. Can. Tech. Rep. Fish. Aquat. Sci. 975: 41-48.

La production d'oeufs et le succès d'éclosion de têtes-de-boule au pH de 5.3 varient de faibles à non existants. Des oeufs, pondus et incubés jusqu'à éclosion à des pH de 8.1 et 6.3 produisent 39.7 et 5.7% de larves normales respectivement; les oeufs pondus à un pH de 6.3 et incubés à 5.3 produisent 0.5% de larves normales. L'histologie ovarienne aurait permis de prédire l'insuccès de la reproduction au pH de 5.3. A ce pH, les ovaires de têtes-de-boule contenaient des proportions exceptionnellement élevées de corpora atretica préovulatoires par rapport aux autres stades ovocytaires. Ces données ont été recueillies comme base d'extrapolation pour estimer, par examen de leur condition ovarienne, la dégradation de la reproduction chez des achigans à petite bouche habitant des milieux acides.

Problem Identification

Considerable information is available from both laboratory and field investigations on the levels of pH which cause adverse effects in a number of fishes. Among these are the laboratory studies of Mount (1973) with the fathead minnow and Ruby et al. (1977) with the flagfish; and field studies by Beamish (1976), Beamish and Harvey (1972), and Kennedy (1980), whose paper follows.

Environmental modeling using presently available data will provide information on the trends and expected rates of development of the acidification problem. Past work should indicate at what pH adverse effects can be expected. The work we are involved in is an attempt to provide earlier warning evidence of actual effects, should it be required, to support the need for corrective action.

Approach Rationale

The study area chosen for this work was northeastern Minnesota because of its vulnerability to degradation from acid precipitation. The smallmouth bass was selected as the study species as it has been reported among the most acid sensitive of the area's indigenous fishes (Beamish 1976). Gametogenesis was selected for study because it was reported as probably the most sensitive life stage affected by acid exposure (Beamish 1974; Kennedy 1980, in the following article) and its importance is easily recognized. Field surveys for young-of-the-year (y-o-y) fish were rejected because of the difficulty in obtaining dependable negative data. Ovarian condition was examined by a modified form of the the method developed by Ruby et al. (1977).

Experimental Design

The experimental design called for the acquisition of indices of ovarian conditions (during the spawning and early post-spawning season) of mature fathead minnows reared at known pH levels and relating them to the measured reproductive success within those same populations. The purpose of the fathead minnow work was to serve as a basis of extrapolation from which the ovarian condition of field exposed fishes (smallmouth bass) could be used to predict expected reproductive impairment.

Results and Discussion

Smallmouth bass:

Smallmouth bass ovaries were collected and preserved for histological examination from two of the most acid lakes in northeastern Minnesota where smallmouth bass were reported, and from two non-acid (control) lakes with pH values between 7 and 8. Thumb and McDonald lakes were sampled as the acid lakes; pH values at sampling in June were 6.75 and 6.85, respectively. Spring and Dumbbell lakes were chosen as the non-acid lakes, pH 7.40 and 7.10 respectively, in June. One acid and one control lake were sampled within a day of each other in close geographic proximity (Table 1). (The ovaries from these fish are now undergoing histological examination for the establishment of an index of effect.)

Gonadalsomatic indices[1/] of fish from the two lakes at pH values between 7 and 8 and those of fish from the lakes with pH values below 7 were found to be significantly different (t-test, $p<0.05$) (Table 1). Those indices of the fish from the alkaline lakes (pH above 7) indicated a more advanced stage of maturation. However, analysis of variance did not separate the acid and non-acid lake results into two discrete groups ($p>0.05$), suggesting that the gonadal-somatic index is not a dependable index of subsequent reproductive success.

Fathead minnows:

Adult fathead minnows were sampled from populations exposed to three levels of acidification in artifical stream systems at the U.S. EPA Monticello Ecological Research Station at Monticello, Minnesota. The three exposures were in Mississippi River water: one unaltered, at pH 8.1; and two exposures in H_2SO_4 acidified water, one at pH 6.3 and the other at pH 5.3. Samples were taken four times during the summer of 1979 to determine both their gonadalsomatic indices (Table 2) and the histological condition of their ovaries (Table 3) as related to the progress at the spawning season and the pH of the environment.

Qualitative examination of the ovaries from fish in the artificial streams at the time of first spawning showed that the fish at pH 8.1 were in an advanced stage of developing maturation as much as a week earlier than those at pH 6.3 and pH 5.3 (Hermanutz, pers. comm. 1979). The same stage was, however, eventually reached at pH 6.3 and apparently occasionally at pH 5.3 since spawning, though very limited at pH 5.3, did occur at these lower pH values. Comparisons of the gonadalsomatic indices have not revealed statistically significant differences associated with these three levels of pH ($p>0.05$). Again the gonadalsomatic index does not show promise as a prospective indicator of subsequent reproductive success. Histological indices of gametogenesis are partially completed at this time and the values thus far obtained are presented in Table 3 to be discussed later.

Reproductive impairment as indicated by hatching success of eggs from these same populations has revealed significantly adverse effects (ANOVA, $p<0.05$) when incubated in the artifical streams at pH 6.3 or 5.3 (Fig. 1). Eggs incubated at pH 5.3 were derived from the population at pH 6.3 since no eggs from the fish at pH 5.3 were available. (Eggs from the fish at pH 5.3 were too few to study at any time and none were available at the time of the incubation study.) Incubation studies were performed using 4.6 cm x 4.6 cm stainless steel screen incubation chambers with 2.0 cm deep glass bottoms. The incubating chambers were suspended on styrofoam floats resting in the artifical streams and anchored to the bottom. The chambers were tended daily for removal of dead eggs, and counting dead, deformed and normal larvae. Planaria present at these times were also removed to limit their influence on hatching success of the eggs.

[1/]Gonadalsomatic Index = [ovary weight (g)/total fish weight (g)]x100.

Data from reciprocal transfer of fathead minnow eggs between acid and unacidified stream conditions indicated that parental exposure at pH 6.3 was unimportant in reducing the hatching success of eggs incubated at pH 8.1 (Table 4). Two-way ANOVA found a highly significant effect ($p<0.01$) due to incubation pH and no effect due to parental exposure pH or interaction. Thus it was questionable whether adverse effects of acid environment of pH 6.3, which signficantly reduced hatching success, would be revealed by ovarian examinations. It appears that embryonic development and/or initial larval stages of the fathead minnow are more sensitive parameters of acid impact on year class recruitment than is gametogenesis. To measure this parameter our incubation apparatus or that described by Kennedy (1980; in the following article) seems a good choice, the latter particularly where daily attendance is difficult.

Though the gonadalsomatic indices of the fathead minnows (Table 2) showed no relationship to their pH experience, the histological examination of the ovaries of these same fish indicated that near complete spawning failure at pH 5.3 may have been predicted on the basis of the abundance of pre-ovulatory corpora atretica relative to those of the fish at pH 8.1 (Table 3).

The time of sampling can have an important influence on the results obtained (Table 3). It appears that for the determination of the relative volume of pre-ovulatory corpora atritica sampling the time for the most indicative results would be late in the spawning season to early in the post-spawning period. Pre-ovulatory corpora atretica are a normal phenomenon in the ovaries of mature fish during the reproductive period. Henderson (1963) and others have previously documented this, but the extent and stage of development of the pre-ovulatory corpora atretica found in the fathead minnows at pH 5.3 seem to indicate complete to nearly complete failure of ovulation. Henderson (1963) reports 3 to 5% of the maturing ova became atretic in normal brook trout. Possibly an increase of 15-25% or greater above the number in the species of concern at approximately the same stage of maturation in a similar but unacidified body of water should be cause for concern. The 15-25% was chosen as the critical range since it was this range of values in the fathead minnow that was associated with the poor reproductive success found in the fish at pH 5.3 (Table 3 and Fig. 1), and because scant to no production of juveniles occurred in that artifical stream (Hermanutz, pers. comm. 1979).

Conclusion

It is our opinion that the accrual of large numbers of pre-ovulatory corpora atretica among mature fish exposed to acidified environments shows considerable promise as a means of detecting the adverse impact of environmental acidification on fishes, but that more samples of fish from natural populations where effects are already known will be required before it can be offered as a reliable index of reproductive inhibition.

Acknowledgments

We thank members of the ERL-D staff and others who assisted in collecting ovary samples, some of which required days of canoeing in remote areas. We also wish to thank Messers. J. Arthur and R. Hermanutz for their assistance in the Monticello Research Station portion of our work and their gracious sharing of data and observation related to the aspects of their pH-fathead minnow experiments as it pertained to our particular project.

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Table 1. Gonadalsomatic Indices of Northeastern Minnesota Smallmouth Bass in Lakes with Different pH Values.

	Alkaline Lakes		Acid Lakes	
	Spring 6/5-6/79	Dumbbell 6/23-24/79	Thumb 6/5-6/79	McDonald 6/24-26/79[a]
pH[1]	7.4	7.1	6.75	6.85
$\bar{X}$ G.S.I.[2]	7.24 (4.2-14.7)[b]	4.31 (1.2-8.5)	3.59 (1.0-8.3)	2.85 (0.7-4.2)
Sample size	12	10	9	6
				[3]

[1] The pH values reported are those of the lakes at athe time of fish collections.

[2] G.S.I.= Gonadalsomatic Index.

[3] Lines underscore values not found significantly different, Scheffe's test (p=0.05).

[a] Sampling dates.

[b] (Range).

Table 2. Fathead Minnow Gonadalsomatic Indices from Acid Treated and Untreated Mississippi River Water Artifical Streams.

	pH		
Sampling date	8.1	6.3	5.3
6/19	18.1 (13.2-22.6)	17.4 (11.0-26.0)	15.4 (7.5-28.6)
7/12	12.1 (2.1-17.0)	12.5 (2.1-19.3)	16.6 (6.8-25.8)
8/14 [a]	2.7 (0.5-11.4)	8.6 (1.7-13.2)	5.6 (2.3-8.4)

[a] Fourth sample not yet processed.

Table 3. Mean percent of Fathead Minnow Ovary Oocyte Volume Occupied by Pre-Ovulatory Corpora Atretica When Exposed to Three Levels of pH (fifty oocytes were examined per fish; 5 fish/treatment and date).

Sampling dates	Pre-ovulatory corpora atretica		
	pH 8.0	pH 6.3	pH 5.3
6/19/79	0.0	5.2**	4.0
7/12/79	0.0	7.6	19.6*
8/14-15/79	2.8	22.4	54.0**

*, ** Significantly different (p=0.05, and p=0.01, respectively) Dunnett's one-tailed test using arc sine transformed percent data.

Table 4. Hatching Success, (%) Normal Larvae Produced from Fathead Minnow Eggs When Incubated at Two pH Levels and Influenced by Previous pH Experience (50 eggs/test lot).

Previous pH experience (Gametogenesis-early cleavage)	Egg lots	Replicates	Incubation pH 8.1	pH 6.3
8.1	V	A	56	0
		B	26	64[a]
	VI	A	26	6
		B	36	6
		$\bar{X}$	35	4
6.3	VII	A	48	2
		B	40	4
	VIII	A	30	0
		B	30	4
		$\bar{X}$	37	3

[a] This value is a statistical outlier ($p<0.99$) and not used in the calculated mean reported below. Two-way ANOV using arc sine transfered % data including the outlier for the most conservative test, found a highly signifiant incubation pH effect and no effect due to parental pH exposure or interaction.

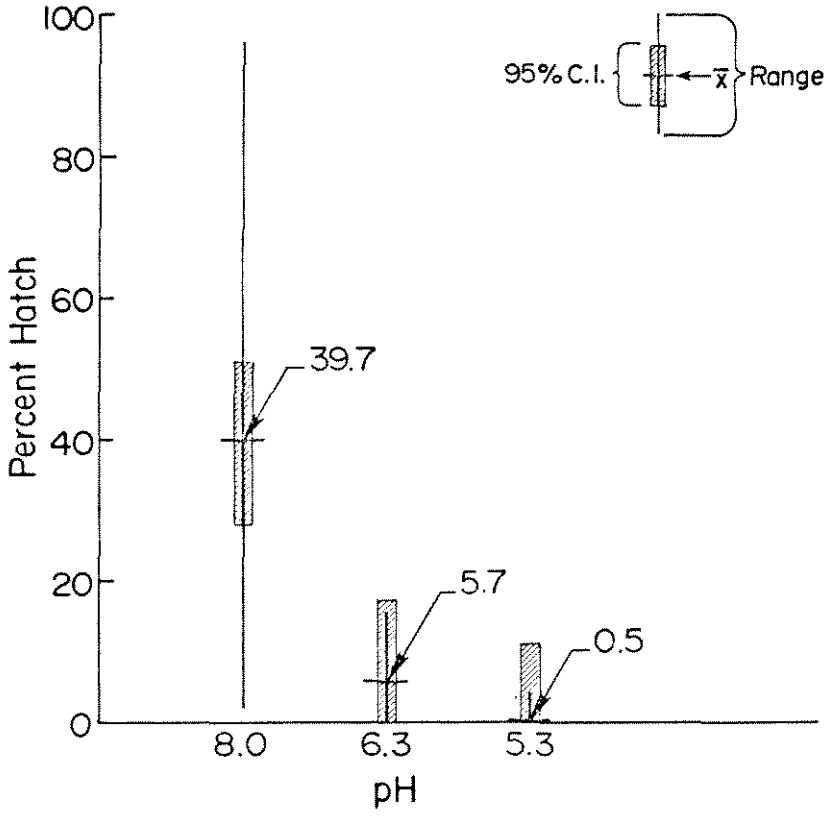

Fig. 1. Percent normal larvae hatched from fathead minnow eggs incubated at different pH's.

THE EFFECTS OF LAKE ACIDIFICATION ON EMBRYONIC DEVELOPMENT OF THE LAKE TROUT SALVELINUS NAMAYCUSH

L.A. Kennedy[1]

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

KENNEDY, L.A. 1980 The effects of lake acidification on embryonic development of the lake trout, Salvelinus namaycush. Can. Tech. Rep. Fish. Aquat. Sci. 975: 49-54.

A high incidence of embryonic mortality and morbidity was seen in an indigenous population of lake trout, Salvelinus namaycush, inhabiting a lake experimentally acidified over a period of three years to a mean summer pH of 5.84. Fertilization appeared to occur normally but after 15 days 59% of blastodiscs had died or failed to gastrulate and 60% of the surviving embryos were morphologically abnormal. Prior to hatching only 6% of the eggs contained formed embryos and all were anomalous. Both the volume and dry weight of eggs of fish inhabiting the acid lake were significantly reduced. Even when incubated in untreated water there was a 50% reduction in egg viability by midgestation when gametogenesis and fertilization occurred in the acidified lake.

Key words: Embryologic failure; gametogenesis; lake acidification

KENNEDY, L.A. 1980 The effects of lake acidification on embryonic development of the lake trout, Salvelinus namaycush. Can. Tech. Rep. Fish. Aquat. Sci. 975: 49-54.

On a observé, dans une population indigène de touladis, Salvelinus namaycush, habitant un lac acidifié expérimentalement pendant une période de trois ans pour atteindre un pH estival moyen de 5.84, une forte incidence de mortalité et de morbidité embryonnaires. La fécondation semble se produire normalement, mais après 15 jours, 50% des blastodisques meurent ou ne subissent pas de gastrulation, et 60% des embryons qui survivent sont difformes. Avant l'éclosion, seulement 6% des oeufs contiennent des embryons formés, et tous sont anormaux. Il y a diminution significative, tant du volume que du poids sec, des oeufs de poissons habitant le lac acide. Même avec incubation dans une eau non traitée, il y a diminution de 50% de la viabilité des oeufs à la mi-gestation, quand la gamétogénèse et la fécondation ont eu lieu dans un lac acidifié.

[1]Present address: Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0.

INTRODUCTION

The acidification of soft water lakes by acid precipitation is known to destroy fish populations (Beamish 1974, 1975). It has recently been estimated that in Ontario alone, 48,000 lakes will be endangered by acid precipitation in the next 20 years (Parrott 1979). Almost half of Canada, as well as much of the northern U.S.A. contains lakes of similar susceptibility to acidification. The dominant sport fish in most of the Canadian lakes is the lake trout, Salvelinus namaycush.

Field studies near Sudbury, Ontario (Beamish 1974) have shown that young fish are the first to disappear from lake trout populations as a lake becomes more acid, which suggests that reproductive failure is the cause. However, it is not known whether this failure is due to impairment of gametogenesis, spawning behavior, fertilization, or to a high mortality among developing embryos or yolk-sac fry.

LAKE 223 ACIDIFICATION EXPERIMENT

Lake 223 is a small lake at the Experimental Lakes Area (ELA) in the Precambrian Shield of Northwestern Ontario. Originally the pH of its surface waters ranged from 6.5 to 7.0 during the course of a year. Over a period of 3 years Lake 223 has been experimentally acidified with sulfuric acid; pH values were lowered by approximately 0.25 units per year. In 1977, the pH of the lake averaged 6.1. In May of 1978, the epilimnion pH was lowered to 5.8 where it was maintained throughout the summer and fall (Schindler et al. 1980). As a part of this whole lake acidification experiment, an indigenous population of lake trout is being studied to determine where in the reproductive cycle chronic and progressive acidification exerts its deleterious effects and at what pH the effects are detectable. Details of the lake 223 acidification experiment are given in Schindler et al. (1980).

EMBRYOLOGICAL FAILURE IN LAKE 223

When lake trout were spawning in early October, 1978, sexually-mature females and males were captured from Lake 223 and from nearby Roddy Lake, which is unpolluted. Roddy Lake is chemically similar to Lake 223 prior to acidification. Both are softwater oligotrophic lakes with little buffering capacity. Eggs from one female and milt from 2 to 3 males were stripped into plastic tubs and mixed with water from their respective lake. This procedure was repeated at least five times for each lake. The day of fertilization was designated day 0 of the experiment. On day 1, 50 fertilized eggs from one spawn were placed in individual chambers in plexiglass incubators and suspended in the appropriate lake. Twelve incubators were placed in each lake. On days 15, 25 and 30, incubators were removed and eggs monitored for mortality and embryonic development. Eggs were also sampled in December immediately prior to hatching (Kennedy 1980).

Eggs from Lake 223 were markedly smaller than those from Roddy Lake. Despite this, the fertilization of eggs from both lakes was highly (95% to 98%) successful. However, the eggs from Lake 223 had a much higher incidence of early embryonic mortality (Table 1). In addition, the surviving embryos from Lake 223 had a marked increase in the incidence of gross morphological

Table 1

	Embryonic mortality, %	Deformities among survivors, %
Roddy Lake (summer pH ≈ 6.8)		
Day 15	2%	0%
Day 30	7%	1%
Day 65 (winter)	7%	0%
Lake 223 (summer pH 5.8)		
Day 15	59%	60%
Day 30	83%	30%
Day 59 (winter)	94%	100%

malformations. Most commonly, there was a unilateral or bilateral sloughing off of caudal segments leaving a shortened stump or a hook-like tail. Immediately prior to hatching only 6% of Lake 223 eggs contained formed embryos and all were either dead or malformed. In contrast, 93% of eggs from Roddy Lake contained normal live embryos. These results suggest that very few viable individuals would be recruited from the 1978 lake trout spawn when gametogenesis and embryogenesis occurred at pH 5.8. That the lake trout did indeed spawn in 1978 was shown by the observation that females recaptured in December were not resorbing their eggs. However, young-of-the-year were captured in the fall of 1979 (Ken Mills, Freshwater Institute, personal communication). It appears, therefore, that at pH 5.84 there are enough acid-resistant individuals in the lake to allow some recruitment into the existing population. Whether or not this recruitment will be adequate to maintain a viable population is as yet unknown.

PARENTAL CONTRIBUTION TO EMBRYOLOGICAL FAILURE

Because of the small egg size and high incidence of early embryonic mortality and morbidity among the fertilized eggs from Lake 223 parents, a second experiment was conducted to investigate the parental contribution to the embryological failure. Fertilized eggs obtained from lake trout in Lake 224 were used as controls. Lake 224 is a slightly more oligotrophic lake of similar size located immediately upstream to Lake 223 in the same drainage system. Lake 224 is being used as a conservative control for fish population studies being conducted on Lake 223. Egg volume and dry weight were significantly lower ($p > .99$) in Lake 223 eggs. No significant variation was found in Ca, Na, K or P content, per gram dry weight.

Although the relationship between the length of the female and fecundity is complex, it has been shown that larger salmonids usually have larger and more numerous eggs than do small fish and that larger eggs yield larger fry which have a higher survival rate (Bagenal 1967, 1969; Pope et al. 1961).

However, for a given age class, the females of Lake 224 are smaller than those in Lake 223 (K. Mills, Freshwater Institute, personal communication). The paradox in Lake 223, of larger fish producing smaller eggs cannot therefore be interpreted as a compensatory increase in fecundity, but may be regarded as an early indicator of acid-related stress. This hypothesis is supported by an analysis of the early embryological development of fertilized Lake 223 eggs when incubated in natural, untreated lake water. Approximately 300 fertilized eggs from each of Lake 223 and Lake 224 were incubated in egg trays set in plastic tubs containing 6 L of untreated Roddy Lake water. They were maintained at 10°C for 31 days after fertilization. Whitened eggs were counted and removed daily, and 50 "apparently viable" eggs were sampled on days 4, 9, 15, 20, 26 and 31 for evaluation of growth and development. Normally, hatching would occur around day 60 at this temperature. By day 31, 45 eggs from the Lake 223 sample had whitened and were removed, in contrast to only 1 among the Lake 224 sample. Among the "apparently viable" eggs sampled for embryological analysis, only 24% of Lake 223 eggs contained eyed embryos compared to 75% among their Lake 224 counterparts. When incubated under these controlled conditions there was no difference in the rate of development between the two groups of eggs, as evidenced in the time of onset of major developmental events. Once gastrulation had been accomplished there was no further embryonic loss in either group as seen at day 31 of gestation. It appears, therefore, that when gametogenesis and fertilization occur at pH 5.8, there results a major embryological failure as seen in the inability of over 50% of fertilized eggs to gastrulate. Further, because there was no difference in the incidence of morphological abnormalities between the two groups of embryos when incubated in untreated water, the 35-fold increase in embryonic malformations observed among the Lake 223 survivors in the previously described *in situ* experiment can be attributed directly to the effect of lake acidification on embryogenesis following gametogenesis in the acid lake.

CONCLUDING REMARKS

These experiments indicate the extent and the nature of the embryological failure observed in lake trout inhabiting a lake which has been experimentally acidified to pH 5.8. The field study suggests that even if the pH of the lake were allowed to go no lower than 5.8, the future population might eventually be seriously affected by the observed embryological failure. Support for these conclusions is obtained by inventory data collected by the Province of Ontario (Martin and Olver 1976) which show that only 9% of the lakes in Ontario which contain lake trout have pH values less than 6.0 and by a survey of 109 lakes in our area (Beamish et al. 1976) which revealed that only 3 lakes with a summer surface pH of less than 6.5 contain lake trout. The above data also show that 43% of lake trout lakes in Ontario have pH values of 6.0 to 6.5, suggesting that very small reductions in natural lake pH, as little as a few tenths of a unit, may impair reproduction of lake trout in a large number of lakes. The laboratory experiments have demonstrated that the parental contribution to the embryological failure is a major cause for the failure of reproduction observed in acidified lakes. Failure of the sexually-maturing female to achieve elevated levels of protein-bound calcium (Lockart and Lutz 1977) and impaired oogenesis (Ruby et al. 1977) are implicated as possible mechanisms underlying the reduction of early egg viability. It is clear from the above evidence and discussion that the continued high emission of SO_2 from smelting and fossil fuel burning in North

America will soon have severe effects on lacustrine fish populations of Eastern Canada and the U.S. and thus on tourism and the sport fisheries in these areas.

ACKNOWLEDGEMENTS

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EFFECTS OF SUBLETHAL PULSE CYANIDE EXPOSURE ON SEVERAL LIFE STAGES OF AMERICAN FLAGFISH Jordanella floridae

S.K. Cheng and S.M. Ruby

Concordia University, Montreal, Quebec

CHENG, S.K. and S.M. RUBY 1980 Effects of sublethal pulse cyanide exposure on several life stages of American flagfish Jordanella floridae. Can. Tech. Rep. Fish. Aquat. Sci. 975: 55-56.

American Flagfish Jordanella floridae received 5-day pulse exposures to sublethal cyanide during several life stages. The relative sensitivities of these stages to cyanide were examined using developmental morphological, histological, and reproductive parameters.

Developmental experiments involved exposure of eggs to 0.065, 0.075, 0.087 and 0.15 mg/L HCN from time of fertilization through hatching in continuously renewed water at 25± 0.5°C. Results indicate increased hatching times ranging from 140 hours at 0.065 to 216 hours at 0.15 mg/L HCN as compared with 114 hours in controls. Hatching success was decreased from 85.6% at 0.065 to 3% at 0.15 mg/L HCN as compared with a control value of 89%. Similarly, fry survival decreased with increasing concentration, with values of 96.96, 91.72, 83.92 and 0% at 0.065, 0.075, 0.087 and 0.15 mg/L HCN respectively, as compared to 97.8% in controls.

Cyanide-induced anomalies included a high incidence of microphthalmia (alteration of optical layers of the eye) and monophthalmia (complete disintegration of the eye), along with body flexures. Measurements of larval pituitary glands indicated significantly reduced sizes at all concentrations tested.

Exposure of eggs from fertilization to hatching significantly lowered fecundity levels of females at sexual maturity and may be related to the reduced pituitary size observed during the pre-hatching period.

Exposure to HCN prior to hatching and again for 5 days during juvenile development further reduced fecundity levels of mature females and also lowered hatching success of offspring which were successfully spawned. It is suggested that the additional reduction may be the result of oocyte damage following exposure during juvenile development. Females exposed to HCN from fertilization to hatching and again for 5 days during sexual maturity showed no significant differences from those which received a single pre-hatching exposure. Sexual maturity was delayed by 14±2 days in all cyanide treated groups. Shortened estrous cycles (from 12 to 8 days) accompanied by reductions in the maximum number of eggs laid at the peak of the estrous cycle were also evident among the cyanide treated fish.

The results indicate that short pulse exposures to low cyanide levels is critical during both the embryonic and juvenile stages of development, with effects being expressed in fecundity levels of the sexually mature adults and also among embryos of the following generation.

Key words: Embryology; sublethal; bioassays; developmental stages; cyanides

CHENG, S.K. and S.M. RUBY 1980 Effects of sublethal pulse cyanide exposure on several life stages of American flagfish Jordanella floridae. Can. Tech. Rep. Fish. Aquat. Sci. 975: 55-56.

Nous avons exposé Jordanella floridae à des doses sublétales de cyanure, à intervalles de 5 jours, pendant plusieurs stades de leur cycle biologique. Des paramètres morphologiques, histologiques et reproducteurs ont été utilisés pour étudier la sensibilité relative de ces stades.

Les expériences de développement comportèrent une exposition des oeufs à 0.065, 0.075, 0.087 et 0.15 mg/L de HCN depuis le moment de la fécondation jusqu'à l'éclosion dans une eau continuellement renouvelée à 25 ± 0.5°C. On voit, d'après les résultats, que la durée d'incubation augmente, variant de 140 h à 0.065 à 216 h à 0.15 mg/L de HCN, comparée à 114 h chez les témoins. Le succès de l'éclosion diminue, passant de 85.6% à 0.065 à 3% à 0.15 mg/L de HCN, comparé à 89% chez les témoins. De même, la survie des alevins diminue à mesure qu'augmente la concentration, avec valeurs de 96.96, 91.72, 83.92, et 0% à 0.065, 0.075, 0.087, et 0.15 mg/L de HCN respectivement, comparées à 7.8% chez les témoins.

Parmi les anomalies provoquées par le cyanure, on note une forte incidence de microphthalmie (altération des couches optiques de l'oeil) et de monophthalmie (désintégration complète de l'oeil), ainsi que des courbures du corps. A toutes les concentrations testées, l'hypophyse accuse une diminution marquée de taille.

Une exposition des oeufs du moment de la fécondation jusqu'à l'éclosion abaisse de façon marquée les niveaux de fécondité des femelles à la maturité sexuelle, et il se peut qu'il y ait une relation entre ce phénomène et la diminution de taille de l'hypophyse observée durant la période d'avant-éclosion.

Une exposition à HCN avant l'éclosion et de nouveau pendant 5 jours au stade juvénile abaisse encore davantage les niveaux de fécondité des femelles à la maturité et diminue également le succès de l'éclosion des oeufs qui avaient été pondus. Cette réduction supplémentaire pourrait résulter de dommages infligés aux ovocytes après exposition au stade juvénile. Les femelles exposées à HCN du moment de la fécondation jusqu'à l'éclosion et de nouveau pendant 5 jours alors qu'elles étaient matures ne diffèrent pas de façon marquée de celles qui avaient été soumises à une seule exposition avant l'éclosion. Chez tous les groupes traités au cyanure, la maturité sexuelle est retardée de 14±2 jours. On constate aussi, chez les poissons traités au cyanure, des cycles oestriens plus courts (passant de 12 à 8 jours), accompagnés de réductions du nombre maximal d'oeufs déposés au pic au cycle oestrien.

Selon ces résultats, de courtes expositions pulsées à de faibles niveaux de cyanure sont critiques, tant aux stades embryonnaires que juvéniles de développement, les effets se faisant sentir dans les niveaux de fécondité des adultes mûrs et aussi chez les embryons de la génération suivante.

TOXICITE SOUS-LETALE DU CUIVRE CHEZ LES OEUFS D'OMBLE DE FONTAINE: CARACTERISTIQUES BIOCHIMIQUES DE LA TOXICITE RETARDEE

R. Van Coillie, C. Thellen et R. Schoenert

Division des Services Biologiques,
Eco-Recherches Ltée (C-I-L),
Pointe-Claire, Québec, H9R 1E6

VAN COILLIE, R., C. THELLEN et R. SCHOENERT 1980 Toxicité sous-létale du cuivre chez les oeufs d'omble de fontaine, Salvelinus fontinalis: caractéeristiques biochimiques de la toxicité retardée. Can. Tech. Rep. Fish. Aquat. Sci. 975: 57-67.

Des oeufs embryonnés d'omble de fontaine ont été soumis à diverses concentrations sous-létales de cuivre dans le but de mettre en évidence l'effet toxique retardé d'un traitement continu sur l'embryon. La mortalité cumulée des oeufs est peu significative durant les vingt jours de traitement précédant l'éclosion. Par contre, un effet létal ultérieur est induit chez les alevins issus de ces oeufs. Au niveau des embryons, l'accumulation du cuivre a un effet inhibiteur sur les synthèses des ARN (acides ribonucléiques) pendant l'organogénèse. En conséquence, le non-remplacement des ribosomes maternels chez l'alevin pourrait expliquer le mécanisme de toxicité retardée observée après l'éclosion.

VAN COILLIE, R., C. THELLEN et R. SCHOENERT 1980 Toxicité sous-létale du cuivre chez les oeufs d'omble de fontaine, Salvelinus fontinalis: caractéeristiques biochimiques de la toxicité retardée. Can. Tech. Rep. Fish. Aquat. Sci. 975: 57-67.

Embryonated brook trout eggs were subjected to various sublethal concentrations of copper in order to observe the delayed toxic effect of a continuous treatment on the embryo. The cumulative mortality of the eggs is insignificant in the twenty days prior to hatching. However, a subsequent lethal effect is induced in the fry hatched from such eggs. In the embryonic stage, the accumulation of copper has an inhibiting effect on the ribonucleic acid (RNA) syntheses during organogenesis. Consequently, the non-replacement of maternal ribosomes in the fry could explain the mechanism of delayed toxicity observed after hatching.

Key words: Bioassays; embryology; developmental stages; trout, brook; algae; Chlorella vulgaris; copper; sylvicor; ribonucleic acid

INTRODUCTION

Depuis quelques années, il y a un intérêt grandissant pour la toxicité du cuivre chez les organismes aquatiques. Certaines études de toxicité létale ont d'une part montré que l'effet de ce métal lourd peut varier selon les conditions physico-chimiques (Howarth 1978; Sylva 1976). D'autres expériences se sont par contre orientées vers les effets sous-létaux du cuivre (McKim 1971, 1974) et leurs aspects physiologiques, histologiques, etc. (Sprague 1976). Suite à ces recherches, on s'oriente de plus en plus vers l'explication des mécanismes de la toxicité d'autant plus que l'introduction de techniques plus précises permettent d'explorer certains aspects autrefois ignorés.

Cette étude est présentée dans le but de mettre en valeur le phénomène de toxicité retardée observé après un traitement sous-létal de cuivre chez des oeufs de poissons. Elle veut en outre préciser un aspect du mécanisme de toxicité du cuivre à l'aide de techniques biochimiques.

APPROCHE EXPERIMENTALE

Des oeufs embryonnés d'omble de fontaine, *Salvelinus fontinalis*, obtenus de la pisciculture provinciale de Baldwin Mills (Québec) ont été placés dans quatre aquaria de 2.5 litres et conservés à l'obscurité dans une chambre à température contrôlée (5°C± 0.1). L'eau d'une rivière locale a été utilisée à un débit constant (10 ml/min) pour chaque bassin durant toute l'expérience. Ces conditions physico-chimiques principales ont été maintenues telles qu'identifiées au tableau 1.a et des traitements périodiques aux rayons ultra-violets ou à l'ozone assuraient sa pureté microbienne.

Après une acclimatation de 20 jours, les oeufs ont été soumis à un traitement continu de 0, 5, 10, 15 µg/l de cuivre ($CuSO_4.5H_2O$). Ces solutions de sulfate de cuivre étaient ajoutées à l'eau de dilution par un système de Bouteille Mariotte (Leduc 1966) et de pompe proportionnelle. Le dosage du cuivre était effectué par spectrophotométrie en absorption atomique avec et sans flamme (EPA 1974); tel qu'indiqué au tableau 1.b, une concentration naturelle d'environ 4 µg/L était présente dans l'eau de dilution.

Tableau 1: Conditions physico-chimiques et teneurs en cuivre dans les aquaria lors de l'incubation expérimentale.

1.a Physico-chimie de base

Température (°C)	5.0 ± 0.2*
pH	6.8 ± 0.2
Dureté (mg/L Ca Mg)	22.6 ± 1.7
Conductivité (µohms)	55 ± 4.3
Salinité (mg/L)	38.7 ± 2.8
Oxygène dissous (mg/L)	13.4 ± 0.3
Obscurité	sauf lors de l'échantillonnage

1.b Teneurs en cuivre (µg/L)

Nominales	Déterminées
0	4.3 ± 0.3*
5	8.9 ± 0.4
10	14.1 ± 0.8
15	19.6 ± 1.3

* Intervalles de confiance (t 0.05) pour N = 30 en a.
N = 20 en b.

Après l'éclosion des oeufs, d'une part, le traitement au cuivre était maintenu pour 100 alevins pendant 20 jours et, d'autre part, un groupe de 100 autres alevins était transféré dans des bassins de récupération pendant 30 jours afin de déceler les mortalités retardées.

ECHANTILLONNAGE

Aux jours 0, 10, 20 de l'incubation expérimentale, des oeufs étaient prélevés afin de procéder aux mesures de croissance embryonnaire et de mortalité et aux analyses biochimiques appropriées. La croissance des embryons était déterminée en mg de poids frais et de poids sec (après séchage durant 18 heures dans une étuve à 105° C). La mortalité cumulée (%) était évaluée à partir de prélèvements de 100 oeufs.

En vue de déterminer les effets d'excès sublétaux du cuivre sur l'anabolisme des oeufs au niveau de leurs synthèses d'ARN (acides ribonucléiques), 150 oeufs étaient enlevés et transférés dans une solution contenant un précurseur radioactif spécifique (5 µCide 14C uridine/ml; activité spécifique 490 mCi/mM) pendant 60 minutes à 5° C et à l'obscurité. Après plusieurs lavages à l'eau distillée, les oeufs étaient ouverts dans une solution de Ringer G4 glacée pour en retirer les embryons. On extrayait ensuite les ARN de ces derniers, au moyen de procédés biochimiques (Parish 1972; Clark 1964) résumés au tableau 2. Par la suite, le dosage de la radioactivité incorporée était effectué avec un scintillateur Picker Nuclear Liquimat afin de déterminer les valeurs anaboliques des ARN. Après l'extraction, des profils de sédimentation des ARN embryonnaires étaient aussi établis.

RESULTATS

1. Croissance des embryons

Durant les vingt jours de traitement des oeufs embryonnés, des mesures périodiques de croissance des embryons ont été effectuées en poids sec et poids frais (tableau 3). Une croissance de plus de 70% a été observée en conditions normales tandis que cet accroissement diminuait proportionnellement avec l'augmentation de la concentration de cuivre.

Tableau 2: Techniques d'extraction et de séparation des ARN d'embryons (Parish 1972).

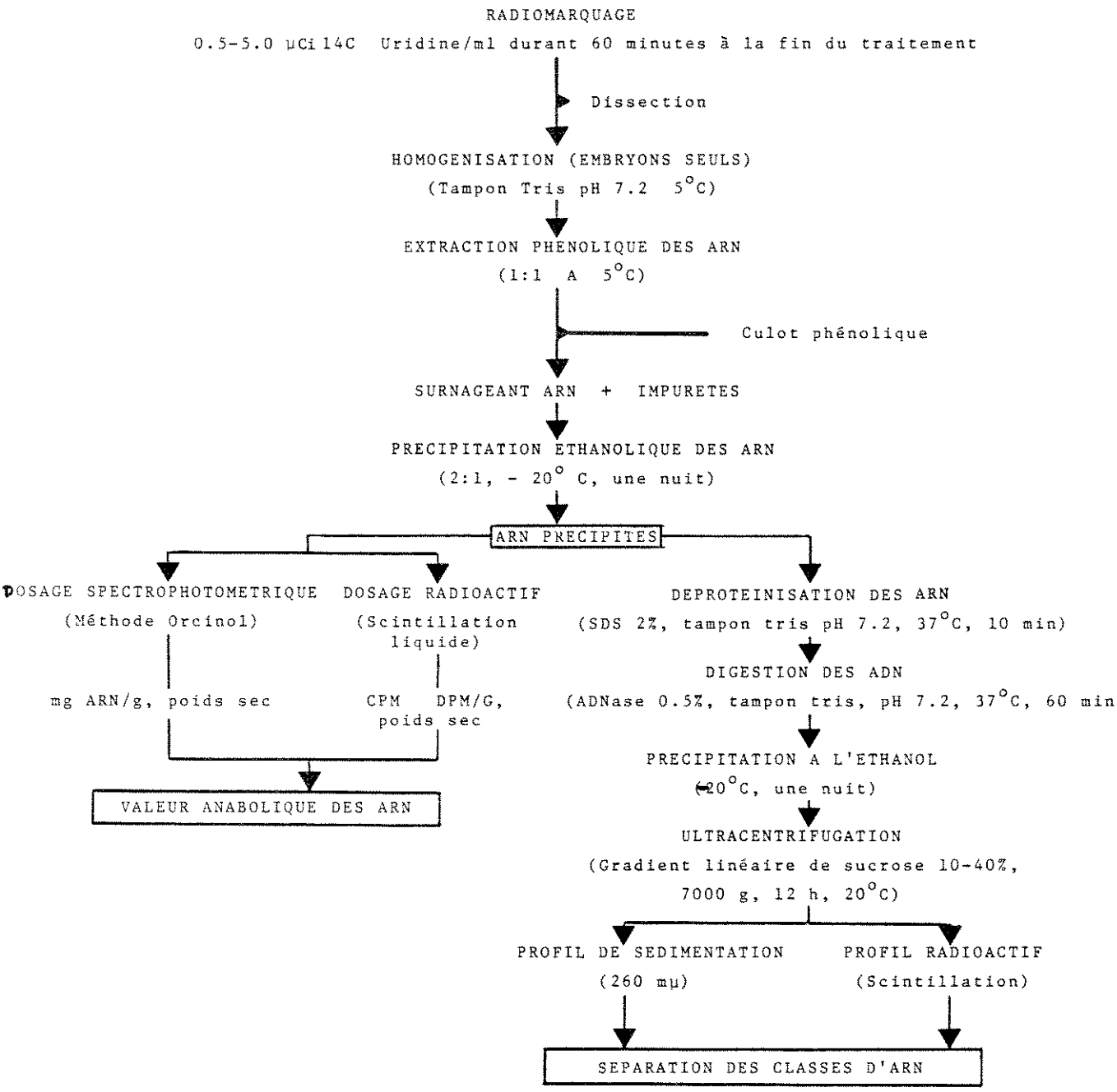


Tableau 3: Variations de la croissance lors de l'incubation expérimentale (mg, poids frais et poids sec) des oeufs.

	JOUR 0		JOUR 10		JOUR 20	
	Poids frais* (mg)	Poids sec* (mg)	Poids frais* (mg)	Poids sec* (mg)	Poids frais* (mg)	Poids sec* (mg)
CONTROLE	121.4 ± 16.5	18.1 ± 2.1	158.7 ± 17.8	22.5 ± 2.5	215.8 ± 24.1	30.8 ± 3.3
			Δ 31%	Δ 24%	Δ 78%	Δ 70%
5 µg/L, Cu^{++}	121.4 ± 16.5	18.1 ± 2.1	151.3 ± 13.1	21.0 ± 1.7	196.9 ± 25.6	27.9 ± 3.3
			Δ 25%	Δ 16%	Δ 62%	Δ 54%
10 µg/L, Cu^{++}	121.4 ± 16.5	18.1 ± 2.1	147.4 ± 16.2	19.7 ± 1.4	177.3 ± 19.7	24.5 ± 1.9
			Δ 21%	Δ 9%	Δ 46%	Δ 35%
15 µg/L, Cu^{++}	121.4 ± 16.5	18.1 ± 2.1	141.0 ± 10.8	19.0 ± 0.8	165.2 ± 21.8	22.9 ± 2.9
			Δ 16%	Δ 5%	Δ 36%	Δ 26%

* Intervalles de confiance (t 0.05) pour N = 50

Tableau 4: Mortalité cumulée (%) observée lors de l'incubation expérimentale des oeufs et alevins.

	TRAITE		TRAITE	NON TRAITE	
	20 jours		20 jours	20 jours	30 jours
CONTROLE	3%	ECLOSION	17%	14%	19%
5 µg/L, Cu^{++}	4%		55%	23%	84%
10 µg/L, Cu^{++}	6%		91%	40%	94%
15 µg/L, Cu^{++}	9%		100%	67%	100%

2. Mortalité cumulée

Le traitement continu au cuivre durant la période d'éclosion n'a pas donné lieu à un pourcentage de mortalité significatif (tableau 4). Par contre, après l'éclosion, la continuation du traitement pendant 20 jours a un effet létal direct sur les alevins, spécialement aux concentrations >10 µg/L où plus de 90% de mortalité cumulée est observé. Par ailleurs, les alevins non-traités mais issus d'oeufs traités au cuivre sont aussi mortellement affectés après 20-30 jours de délai. Ceci soutient que des synthèses essentielles au développement des alevins ont été inhibées par le cuivre chez les embryons d'omble de fontaine lors de leur traitement en conditions sous-létales avec ce métal.avant l'éclosion.

3. Effets métaboliques

Pour expliciter les mécanismes d'action toxique du cuivre dans l'embryon, les effets métaboliques de ce métal ont été étudiés au niveau de la synthèse des ARN. A cette fin, l'extraction des ARN radiomarqués des embryons nous a permis de déterminer les valeurs anaboliques des ARN. Les résultats, exposés au tableau 5, indiquent qu'en conditions normales, une augmentation de la synthèse des ARN est observée durant la période avant l'éclosion et que cette élaboration diminue proportionnellement avec la concentration et la durée du traitement au cuivre. Lors de l'expérimentation, nous avons en outre cherché à savoir, parmi les diverses classes d'ARN des embryons, quelles étaient celles dont l'élaboration était le plus affectée par le cuivre. Dans ce but, nous avons effectué des intégrations de profils de sédimentation des ARN embryonnaires radiomarqués et avons ensuite comparé les intégrations obtenues pour les embryons traités avec 15 µg/L de Cu^{++} avec celles précisées pour les embryons-témoins. Les résultats de cette comparaison figurent au tableau 6 et montrent que le cuivre inhibe principalement l'élaboration des pré-r-ARN (et conséquemment des r-ARN et ribosomes) parmi les catégories d'ARN.

DISCUSSION

En conditions sous-létales, l'ensemble des effets induits par le cuivre chez les oeufs embryonnés d'omble de fontaine ne provoque pas une hausse significative de la mortalité chez ces oeufs

Tableau 5: Effets du cuivre sur la synthèse des ARN chez les oeufs d'omble de fontaine.

TRAITEMENT	JOUR 0	JOUR 10	JOUR 20
CONTROLE	291 34*	372 31* Δ 28%	518 53* Δ 79%
5 µg/L, Cu^{++}	291 34	366 42 Δ 26%	399 41 Δ 37%
10 µg/L, Cu^{++}	291 34	352 36 Δ 22%	378 31 Δ 30%
15 µg/L, Cu^{++}	291 34	329 34 Δ 13%	343 36 Δ 18%

* DPM µCi ^{14}C uridine/ mg ARN (60" incorporation) (poids sec) ± Intervalles de confiance (t 0.05, N = 10)

Tableau 6: Réduction des synthèses des ARN suite à l'exposition au cuivre des oeufs d'omble de fontaine (Jour 20).

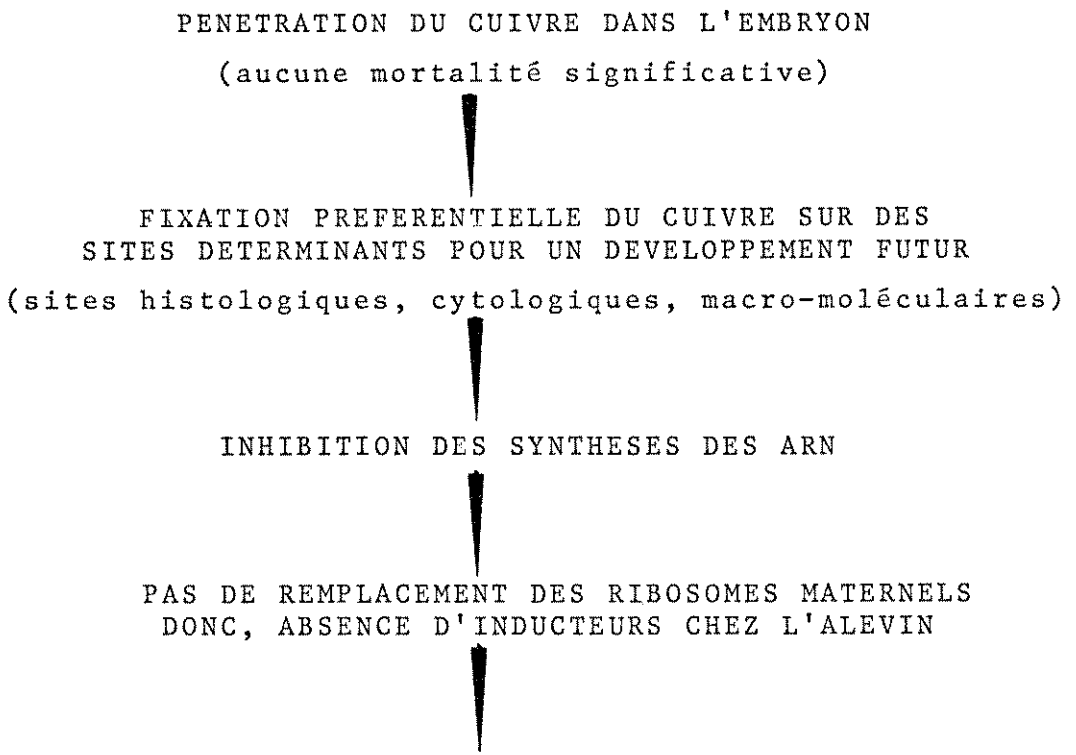
	4-7 S, ARN (t-ARN etc)	18-28 S, ARN (r-ARN)	35-38 S, ARN (Pré-r-ARN)	50-50 S, ARN (Pré-m-ARN)
CONTROLE	110*	410*	200*	140*
5 µg/L, Cu^{++}	105 -Δ 4%	385 -Δ 6%	185 -Δ 7%	130 -Δ 7%
10 µg/L, Cu^{++}	95 -Δ13%	310 -Δ24%	155 -Δ22%	120 -Δ14%
15 µg/L, Cu^{++}	80 -Δ26%	240 -Δ41%	110 -Δ46%	90 -Δ34%

* DPM µCi ^{14}C uridine/ mg de ARN (classe) (60" incorporation) (poids sec)

mais entraîne une augmentation prononcée de la mortalité des alevins issus de ceux-ci. Bien que le chorion capte la majorité du cuivre pénétrant dans les oeufs, il s'en accumule graduellement à l'intérieur de l'embryon (Van Coillie et al. 1975), ce qui y provoque une inhibition de la synthèse des ARN, principalement au niveau des ARN ribosomaux et préribosomaux. Durant l'organogenèse, ce sont surtout des ribosomes d'origine maternelle qui assurent l'élaboration des processus anaboliques (Hay 1968); après l'éclosion, le non-remplacement de ces ribosomes et l'insuffisance d'ARN inducteurs prêts défavorisent indirectement des nouveaux processus anaboliques et entraînent ainsi progressivement la mort des alevins (tableau 7).

Ces observations montrent que, plusieurs classes d'ARN étant des inducteurs de processus métaboliques essentiels décalés dans le temps, une inhibition de 40-60% de la synthèse des ARN par un agent chimique en concentration apparemment inoffensive suffit pour occasionner une toxicité retardée. On peut dès lors considérer que la détermination du degré de cette inhibition (taux d'incorporation de 14C uridine/h/mg ARN) chez des organismes-cibles est utile pour déceler un potentiel de toxicité retardée.

Tableau 7: Mécanismes de toxicité retardée engendrés par le cuivre chez l'embryon d'omble de fontaine.



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CHEMICAL METABOLIC REGULATORS: USEFUL OR HARMFUL STRESSORS?

G. Leduc

Concordia University, Montreal, Quebec

LEDUC, G. 1980 Chemical metabolic regulators: Useful or harmful stressors? Can. Tech. Rep. Fish. Aquat. Sci. 975: 68-70.

In water pollution toxicology there are two broad categories of chemicals which concern us: the synthetic chemicals such as domestic and industrial products and/or wastes, pesticides, etc., which can often readily be absorbed by organisms having little or no means of defense, because, evolutionarily speaking, these chemicals are entirely new to them.

The second category includes naturally occurring chemicals or compounds such as various dissolved gases of biological origin (NH_3, H_2S, HCN, CH_4) as well as a multitude of other organic substances. There is also a vast number of inorganic chemicals leached out of the earth crust and present in variable amounts in water. Aquatic life has evolved and "learned" to live with these "toxicants" of biological and/or weathering origin. Some elements however, like the heavy metals, have never been very abundant in water because they were locked in deep in the rock or present in an insoluble form. Due to their past scarcity they are today, most dangerous. Man, through various activities, creates toxic conditions by promoting excessive amounts of chemicals above and beyond what naturally existed in water.

Ecotoxicologists, protection agencies and legislators converge their efforts to achieve effective water quality objectives which are applicable, enforceable and ecologically sound. We have however, often adopted quite arbitrarily the principle of the Maximum is the Optimum to qualify the response of our test organisms to toxic conditions. We call "harmful" any treatment that will significantly "reduce" or "impair" such performance as growth, swimming, respiration or even egg and fry production. These poorer performances are not necessarily bad in nature where the Maximum is not always the Optimum.

Are we trying to do better than nature? In our quest for pollution control we strive for "clean" water but ignore the possibility of useful roles of many highly reactive natural chemicals in water acting as metabolic regulators. Also, testing at constant concentrations, we ignore the intermittent conditions that prevail in nature.

Chemicals such as NH_3, H_2S, HCN, NO_2^-, etc., can be very toxic yet they often occur in unpolluted waters sometimes at "toxic" levels. The harmful effects of these chemicals have long been studied and published but their roles in nature, at low intermittent (seasonal) levels have not. One of these however, free cyanide (HCN) has received a lot of attention in recent years and there is enough evidence to suggest a positive role of low intermittent levels.

Recent studies in Germany and in the Canadian Prairie Provinces show cyanide levels in streams of agricultural and wilderness watersheds to vary seasonally with peaks occurring during fall and winter in small streams but during summer in the larger ones. Fairly constant low levels of 0.005 mg/L were measured whereas occasional peaks of 0.03 to 0.06 mg/L were reported.

Acclimation of fish through continuous exposure to low levels of cyanide can enhance their resistance to potentially lethal concentrations. On the other hand, low levels of cyanide, harmful during continuous exposure may be harmless under intermittent exposure allowing the organism to recover and/or detoxify the poison. There is indeed laboratory evidence with several fresh species (cichlids, coho salmon, rainbow trout, brook trout, Atlantic salmon and flagfish fry) of a growth rebound during or after exposure to cyanide.

Remains the last question, could low seasonally varying levels of cyanide be valuable? Given the high biological reactivity of cyanide and its natural occurrence, it is unlikely that it would have had no evolutionary influence on aquatic organisms. Acting alone or in combination with other environmental factors on the metabolic rate through its action on the respiratory chain or indirectly through the endocrine system, cyanide could have a determining influence on physiological functions of ecological significance yet to be discovered.

The water quality objective of extremely low levels of cyanide required at all times to protect aquatic life is not entirely defendable in view of its frequent occurrence from natural origin.

Key words: Water quality; pollutants; organic compounds; bioassays; cyanides; metabolism; respiration; endocrine

LEDUC, G. 1980 Chemical metabolic regulators: Useful or harmful stressors? Can. Tech. Rep. Fish. Aquat. Sci. 975: 68-70.

Deux grandes catégories de substances chimiques nous intéressent dans la toxicologie de la pollution de l'eau : les substances chimiques synthétiques, telles que les produits ou déchets domestiques et industriels, les pesticides, etc., facilement absorbés par des organismes à peu près sans défense, puisque, en terme d'évolution, ces substances chimiques sont entièrement nouvelles pour eux.

La seconde catégorie comprend les substances ou composés chimiques produits naturellement, tels que les divers gaz dissous d'origine biologique (HN_3, H_2S, HCN, CH_4), ainsi qu'une multitude d'autres substances organiques. Il existe également un grand nombre de substances chimiques inorganiques extraites de la croûte terrestre par lessivage et présentes en quantités variables dans l'eau. Dans le cours de leur évolution, les organismes aquatiques ont "appris" à vivre avec ces "toxiques" d'origine biologique ou résultant d'une altération à l'air, ou des deux. Certains éléments, cependant, tels que les métaux lourds, n'ont jamais été abondants dans l'eau puisqu'ils étaient emprisonnés dans le roc ou présents sous une forme insoluble. Justement à cause de leur rareté passée, ce sont, aujourd'hui les plus dangereux. Par ses diverses activités, l'homme crée des conditions toxiques en produisant d'excessives quantités de substances chimiques, dépassant de beaucoup ce qui existait naturellement dans l'eau.

Les écotoxicologues, les organismes de protection et les législateurs combinent leurs efforts en vue de trouver des mesures efficaces de contrôle de la qualité de l'eau qui seront applicables, exécutoires et écologiquement saines. Nous avons toutefois adopté à maintes reprises, plutôt arbitrairement, le

principe que "maximum est optimum" pour décrire la réaction de nos organismes d'essai à des conditions toxiques. On qualifie de "nuisible" tout traitement qui "diminue" ou "ralentit" de façon marquée des performances telles que croissance, nage, respiration ou même production d'oeufs et d'alevins. Ces performances réduites ne sont pas nécessairement mauvaises dans la nature, où le maximum n'est pas toujours l'optimum.

Est-ce que nous essayons de surpasser la nature? Dans nos efforts visant à contrôler la pollution, nous avons comme objectif une eau "propre", mais nous ignorons les rôles que peuvent jouer plusieurs substances chimiques naturelles, fortement réactives, dans l'eau, qui agissent comme régulateurs métaboliques. En outre, le fait d'effectuer les essais à des concentrations uniformes ne tient pas compte des conditions intermittentes qui prévalent dans la nature.

Des substances chimiques telles que NH_3, H_2S, HCN, NO_2, etc., peuvent être très toxiques mais se trouver quand même dans des eaux non polluées à des niveaux "toxiques". On étudie depuis longtemps les effets nuisibles de ces substances, mais non leurs rôles dans la nature, à de bas niveaux intermittents (saisonniers). Il en est une, toutefois, le cyanure libre (HCN), auquel on a accordé beaucoup d'attention ces dernières années, et qui jouerait un rôle positif à de faibles niveaux intermittents.

De récentes études en Allemagne et dans les provinces des Prairies canadiennes démontrent que les niveaux de cyanure dans les cours d'eau de bassins agricoles et sauvages varient saisonnièrement. Des pics se produisent en automne et en hiver dans les petits cours d'eau, mais en été dans les grands. On a observé de faibles niveaux, assez uniformes, de 0.005 mg/L, mais des pics occasionnels de 0.03 à 0.06 mg/L ont été signalés.

Une exposition continue à de bas niveaux de cyanure, en permettant aux poissons de s'acclimater, peut accroître leur résistance à des concentrations potentiellement létales. D'autre part, de bas niveaux de cyanure, dangereux à une exposition continue, peuvent être inoffensifs à une exposition intermittente qui permet à l'organisme de se rétablir ou de se débarasser du poison, ou des deux. Des expériences en laboratoire démontrent en effet que chez plusieurs espèces de poissons en eau douce (cichlides, saumon coho, truite arc-en-ciel, omble de fontaine, saumon atlantique et alevins de Jordanella floridae), il y a rebondissement de la croissance durant ou après exposition au cyanure.

Dernière question, est-ce que de bas niveaux de cyanure, variant saisonnièrement, peuvent être utiles? Etant donné la forte réactivité biologique du cyanure et son occurrence naturelle, il est peu probable qu'il n'ait eu aucune influence sur l'évolution des organismes aquatiques. Soit qu'il agisse seul ou combiné à d'autres facteurs de l'environnement sur le taux métabolique par action sur la chaîne respiratoire, ou indirectement par le système endocrine, le cyanure pourrait avoir une influence déterminante sur des fonctions physiologiques d'importance écologique encore inconnues.

Parce qu'on trouve fréquemment du cyanure d'origine naturelle, il n'est pas sûr qu'on puisse justifier un objectif de qualité de l'eau avec niveaux extrêmement bas de cyanure en tout temps dans le but de protéger la vie aquatique.

AN EVALUATION OF CADMIUM TOXICITY TO OSMOREGULATORY FUNCTION IN RAINBOW TROUT (Salmo gairdneri)

M.A. Giles, H.S. Majewski, W.A. Macdonald and R.D. Danell

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

GILES, M.A., H.S. MAJEWSKI, W.A. MACDONALD and R.D. DANELL. 1980 An evaluation of cadmium toxicity to osmoregulatory function in rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci. 975: 71-72.

The effects of exposure of adult rainbow trout to 3.6 and 6.4 µg/L cadmium over a 178 day period on blood and urine levels of calcium, magnesium, sodium, potassium, chloride and total osmolality and on the rates of cadmium bioaccumulation in gill and kidney were investigated.

Cadmium accumulated rapidly to 25 µg/g dry weight in gill tissue (a 10^2 increase over controls) during the initial 23 days of exposure and remained constant at this level for the remainder of the exposure period. Cadmium increased rapidly (Δlog Cd = 0.033 µg/g/day) in kidney tissue during the first 23 days of exposure and continued to increase in a linear manner (Δlog Cd = 0.008 µg/g/day) for the remaining 155 days of exposure. In both gill and kidney tissues the rate and magnitude of cadmium accumulation was identical in fish exposued to 3.6 and 6.4 µg/L cadmium.

With the possible exception of plasma calcium, exposure to 3.6 µg/L cadmium did not induce any changes in the concentrations of blood or urine electrolytes. Exposure to 6.4 µg/L cadmium, however, induced a consistent reduction in plasma Ca^{+2} ($\bar{x}$ = 84.7 of control), Na^{+} ($\bar{x}$ = 96.9% of control), K^{+} ($\bar{x}$ = 87.9% of control) and Cl^{-} ($\bar{x}$ = 95.4% of control), and an increase in plasma Mg^{+2} ($\bar{x}$ = 119.2% of control). Although the plasma osmolality of exposed fish was not significantly different from control fish the contribution of the major electrolytes (Ca^{+2}, Mg^{+2}, Na^{+}, K^{+} and Cl^{-}) to total plasma osmolality was consistently less in cadmium treated fish.

Increased urinary loss of sodium, sufficient to account for the decrease in plasma sodium was observed in cadmium exposed fish. Urinary loss of both calcium and magnesium was reduced for the first 52 days of cadmium exposure but increased to rates above that of controls upon continued exposure. Chloride excretion by the kidney was not significantly changed in cadmium exposed fish. Analyses indicated that some non-identified osmotically active constituent(s) of the urine, possibly bicarbonate or phosphate, progressively increased in concentration over the period of exposure to cadmium and accounted for approximately 80% of the difference in calculated and measured loss of osmotic constituents in the urine.

Key words: Sublethal; bioassays; bioaccumulation; cadmium; trout, rainbow; osmoregulation

GILES, M.A., H.S. MAJEWSKI, W.A. MACDONALD and R.D. DANELL 1980 An evaluation of cadmium toxicity to osmoregulatory function in rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci. 975: 71-72.

On trouvera dans l'article qui suit les résultats d'une étude menée en vue de déterminer les effets d'une exposition de truites arc-en-ciel adultes à 3.6 et 6.4 µg/L de cadmium durant une période de 178 jours sur les niveaux sanguins et urinaires de calcium, magnésium, sodium, potassium, chlorures et osmolalité totale, ainsi que sur les taux de bioaccumulation de cadmium dans la branchie et le rein.

Le cadmium s'accumule rapidement et atteint 25 µg/g de poids sec dans le tissu branchial (une augmentation de 10^2 par rapport aux témoins) dans les 23 premiers jours d'exposition et se maintient à ce niveau jusqu'à la fin. Le cadmium augmente rapidement (Δlog Cd = 0.033 µg/g/jour) dans le tissu rénal dans les 23 premiers jours d'exposition et continue à augmenter linéairement (Δlog Cd = 0.008 µg/g/jour) durant les 155 jours qui restent. Dans les tissus branchial aussi bien que rénal, le taux et l'ampleur de l'accumulation de cadmium est identique chez des poissons exposés à 3.6 et 6.4 µg/L de cadmium.

Sauf possiblement pour le calcium plasmatique, une exposition à 3.6 µg/L de cadmium ne déclenche aucun changement de concentration des électrolytes sanguins ou urinaires. Cependant, une exposition à 6.4 µg/L de cadmium déclenche une réduction uniforme, dans le plasma, de Ca^{+2} ($\bar{x}$ = 84.7% du témoin), Na^+ ($\bar{x}$ = 96.9% du témoin), K^+ ($\bar{x}$ = 87.9% du témoin) et Cl^- ($\bar{x}$ = 95.4% du témoin), et une augmentation de Mg^{+2} plasmatique ($\bar{x}$ = 119.2% du témoin). Bien que l'osmolalité plasmatique des poissons exposés ne diffère pas de façon marquée de celle des témoins, la contribution des électrolytes majeurs (Ca^{+2}, Mg^{+2}, Na^+, K^+ et Cl^-) à l'osmolalité plasmatique est uniformément moindre chez les poissons traités au cadmium.

Chez les poissons exposés au cadmium, nous avons observé une perte de sodium accrue dans l'urine, en quantité suffisante pour expliquer une diminution du sodium plasmatique. La perte dans l'urine tant du calcium que du magnésium diminue dans les 52 premiers jours d'exposition au cadmium mais, avec exposition continue, augmente à des taux supérieurs à ceux des témoins. L'excrétion rénale des chlorures ne change pas significativement chez les poissons exposés au cadmium. On constate, après analyses, que certain constituant ou constituants osmotiquement actifs de l'urine, possiblement bicarbonates ou phosphates, augmentent de concentration durant la période d'exposition au cadmium et rendent compte de 80% environ de la différence entre la perte calculée et mesurée de constituants osmotiques dans l'urine.

THE EFFECTS OF TEMPERATURE ON THE ACUTE TOXICITY OF LEAD AND CADMIUM TO *Daphnia magna* AND THREE NATURALLY-OCCURRING SPECIES OF GREAT LAKES CRUSTACEAN ZOOPLANKTON

J.B. Wilson

Department of Zoology, College of Biological Sciences, University of Guelph, Guelph, Ontario, N1G 2W1

WILSON, J.B. 1980 The effects of temperature on the acute toxicity of lead and cadmium to *Daphnia magna* and three naturally-occurring species of Great Lakes crustacean zooplankton. Can. Tech. Rep. Fish. Aquat. Sci. 975: 73-80.

Very little research has been directed towards the effects of toxicants on the naturally-occurring crustacean zooplankters which form vital trophic links in lake ecosystems. Many experiments have been based on laboratory-cultured *Daphnia magna*, but these have generally featured static bioassays, arbitrary temperature selection, and/or animal concentrations far exceeding in situ levels. This study includes a representative of each of the three groups of Great Lakes crustacean zooplankton, as well as *Daphnia magna*, in a comparative examination of the acute effects of lead and cadmium. The lake species include *Cyclops bicuspidatus thomasi* (a cyclopoid copepod), *Diaptomus sicilis* (a calanoid copepod), and *Daphna galeata mendotae* (a cladoceran). All are abundant throughout the Great Lakes. Experiments utilized a 10-stage (one order-of-magnitude) micro-dilutor, five reps./concentration, ten animals/rep., and holding chambers which provided close to 100 ml/animal while permitting concentration for ease of counting. Lead and cadmium 96hr.LC50's were determined at a minimum of three temperatures for each species spanning normal in situ ranges. Experiments were also run separately by sex where applicable. Cadmium was 2 to 20 times more toxic than lead depending on species and temperature. While the responses of males and females differed significantly in a few cases, the magnitudes of the differences were small. Species differences were highly significant with *Daphnia magna* generally having the greatest resistance and *Diaptomus sicilis* the lowest resistance to the two metals. *Daphnia magna* may therefore be a poor indicator of crustacean plankton response to heavy metals. Significant variation in LC50's was also observed across temperature and indicated that the selection of a bioassay temperature can have very important effects on the results obtained.

Key words: Ecosystems; toxicity tests; zooplankton; bioassays; heavy metals; lead; cadmium

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Jusqu'à maintenant, on a fait très peu de recherches sur les effets des toxiques sur les crustacés zooplanctoniques d'un milieu naturel, représentant des liens trophiques essentiels dans les écosystèmes lacustres. Des cultures en laboratoire de *Daphnia magna* ont été à la base de plusieurs expériences, mais il s'agissait généralement d'essais biologiques statiques, avec choix arbitraire de températures ou avec des concentrations d'organismes beaucoup plus grandes que

dans la nature. La présente étude, comportant un examen comparatif des effets aigus du plomb et du cadmium, inclut un représentant de chacun des trois groupes de crustacés planctoniques des Grands Lacs ainsi que Daphnia magna. Les espèces lacustres comprennent Cyclops bicuspidatus thomasi (un copépode cyclopoïde), Diaptomus sicilis (un copépode calanoïde) et Daphnia galeata mendotae (un cladocère). Ces espèces sont toutes abondantes dans les Grands Lacs. Nous avons utilisé un microdilueur à 10 phases (un ordre de grandeur), cinq rep./concentration, dix animaux/rep. et des cellules assurant à chaque animal près de 100 mL, tout en permettant une concentration suffisante pour le comptage. Nous avons déterminé les CL50 après 96 h de plomb et de cadmium à au moins trois températures pour chaque espèce, couvrant les gammes normales in situ. Autant que possible, nous avons fait des essais séparés par sexe. Le cadmium est de 2 à 20 fois plus toxique que le plomb, selon l'espèce et la température. Les réactions des mâles et des femelles sont nettement différentes dans quelques cas, mais l'ordre de grandeur est petit. Les différences spécifiques sont hautement significatives, Daphnia magna étant généralement l'espèce la plus resistante et Diaptomus sicilis, la moins résistante aux deux métaux. Il se peut donc que Daphnia magna soit un mauvais indicateur de la réaction de crustacés planctoniques à des métaux lourds. Nous avons également observé une variation importante de la CL50 dans la gamme des températures testées. Le choix de la température dans les essais biologiques peut donc influer beaucoup sur les résultats.

This study was initiated in order to address five common weaknesses inherent in the literature on the toxicity of heavy metals to crustacean zooplankton. These weaknesses include: little research on naturally-occurring species; a lack of information on temperature effects; a preponderance of static bioassays; extreme overcrowding in test chambers; and a lack of replication during experiments. This study therefore utilized: three field species (Daphnia galeata mendotae, Diaptomus sicilis, Cyclops bicuspidatus thomasi) as well as Daphnia magna; three different test temperatures for each of the field species and four different test temperatures for Daphnia magna, with temperatures selected to bracket the in situ range; a ten stage, one order of magnitude micro-dilutor with five-way flow splitters delivering 100 mL of toxicant to each 1.5L holding tank every 5 minutes; one L plexiglass and 64μ mesh experimental chambers providing 100 mL/individual while allowing concentration for examination of animals; and five replications within each experiment. All acute toxicity experiments were carried out over a 96 hr period with interim monitoring, male and female animals were tested separately for Diaptomus and Cyclops, and two metals, lead as $Pb(NO_3)_2$ and cadmium as $Cd(NO_3)_2.4H_2O$ were used separately for each species at each temperature. Dilution water was moderately hard (130-140 mg/L as $CaCO_3$) with a pH between 8.0 and 8.2.

Over the temperature tolerance range of any species, it might be expected that at the extreme limits of temperature tolerance, the LC50 for any toxicant

would be very close to zero concentration, and that it might rise to a maximum value at some temperature between the two extremes. To describe such a temperature-toxicity relationship would require at least a second-order polynomial with an inflection point indicating a maximum LC50. In fact, in these experiments, one half of all temperature-toxicity relationships showed a significant variance reduction with a quadratic term fitted by means of orthogonal polynomials. While linear transformations also reduced variation about regression lines, the transformation of best fit varied between species and metals. Therefore, in all cases where the variation between temperatures was significant, a quadratic polynomial was fitted to the data points, even in those cases where the quadratic term did not add significant variance reduction.

Acute temperature-toxicity relationships might also be illustrated by the use of incipient lethal levels. This approach would recognize the effects of temperature on the metabolism of ectothermal organisms. In this study, however, there were no indications of an incipient lethal level after 96 hrs. Subsequent 16 day experiments also failed to indicate the presence of an incipient response. Therefore, 96 hr LC50s were used throughout as the basis for temperature comparisons.

Both lead and cadmium toxicity to Daphnia magna showed highly significant ($P<0.001$) variation between temperatures, as well as highly significant MSs (mean squares) for both linear and quadratic regression. The maximum lead 96 hr LC50 at the inflection point (16.5°C) was 998 µg/L, while the maximum cadmium 96 hr LC50 at 10°C was 270 µg/L (fig.1). The Pb/Cd LC50 ratio varied from 1.9 (10°C) to 16.4 (20°C).

The toxicity of lead to Cyclops bicuspidatus thomasi revealed no significant difference ($P>0.05$) between male and female responses, but a significant ($P<0.05$) variation between temperatures, and a very significant ($P<0.01$) MS for linear regression. The fitted quadratic regression for females (fig.1) revealed a maximum lead 96 hr LC50 at 20°C of 1135 µg/L. Cadmium toxicity was treated separately by sex since there was a significant interaction between sex and temperature. Female cadmium toxicity showed no significant variation between temperatures (fig.1) with a 96 hr LC50 averaging 319 µg/L. Male cadmium toxicity, however, showed a very significant variation between temperatures, as well as a significant MS for linear regression and a very significant MS for quadratic regression. The maximum cadmium 96 hr LC50 for males at 20°C was 441 µg/L. The Pb/Cd LC50 ratio varied from 2.4 (10°C) to 3.6 (20°C).

Lead toxicity to Daphnia galeata mendotae showed no significant difference between temperatures, with an average 96 hr LC50 of 714 µg/L. Cadmium toxicity, however, showed a highly significant variation between temperatures, and a very significant MS for both linear and quadratic regression. The fitted quadratic regression (fig.1) showed a maximum cadmium 96 hr LC50 at 10°C of 74 µg/L. The Pb/Cd LC50 ratio varied from 9.5 (10°C) to 17.4 (20°C).

Lead toxicity to Diaptomus sicilis was treated separately by sex since there was a highly significant difference between sexes as well as a highly significant interaction between sex and temperature. Both males and females of this species showed a significant variation in toxicity between temperatures as well as a significant MS for linear regression. The fitted quadratic regression for males showed a maximum lead 96 hr LC50 at 5°C of 275 µg/L. The fitted quadratic regression for females showed a maximum lead 96 hr LC50 at

5°C of 460 µg/L (fig.1). The toxicity of cadmium revelaed no significant difference between male and female responses. There was, however, a highly significant variation between temperatures and a highly significant MS for linear regression. The fitted quadratic regression for females (fig.1) showed a maximum 96 hr LC50 at 5°C of 86 µg/L. The Pb/Cd LC50 ratio varied from 3.2 (5°C) to 6.5 (10°C).

The fitted curves for the females of all species (fig.1) indicated an apparent variability in temperature effects on metal toxicities, although all curves could logically be extended to fit the theoretical second-order polynomial. The two temperatures common to all four species in this study were 10 and 15°C, and the data from these two temperatures were compiled in a three-wasy ANOVA detailing species, temperature, and metal effects.

The ANOVA (Table 1) showed that both species and metal differences were highly significant. The presence of very significant interactions, however, indicated that the temperature-toxicity curves were not in phase, due either to the different modes of action of the two metals, or to the different temperature ranges and optima of the different species.

Because there was no significant variation between temperatures in the ANOVA, the results for the 10 and 15°C LC50s were grouped for each species and metal (fig.2). The same species order of sensitivity applied to both lead and cadmium LC50s with Cyclops being the most resistant species and Diaptomus being the most sensitive species. The relative toxicities for each species, however, were different for the two metals. There was a slight overlap between the Diaptomus Pb LC50 and the Cyclops Cd LC50, and the Pb/Cd LC50 ratios ranged from 2.7 for Cyclops to 11.7 for Diaptomus.

Several conclusions can be drawn from this study on the basis of the comparative results:

(1) Daphnia magna is not a particularly useful indicator species, although the range of results indicated that no single species would be suitable as a general crustacean zooplankton representative. Daphnia magna could be classified as neither the most resistant, nor the most sensitive of the species studied. In fact, the results for magna did not correlate consistently with those of the cogeneric galeata. As well, magna appeared to have a somewhat narrow temperature tolerance range when compared with the field species.

(2) The most sensitive species are probably the cold deep hypolimnetic species such as the Diaptomus used in this study, or other large glacial relict species such as Limnocalanus macrurus, Senecella calanoides, or Mysis relicta.

(3) The sex of an animal may have an effect on its sensitivity to heavy metals, but the effect is probably small compared to other environmental factors.

(4) Pb/Cd toxicity ratios are not consistent. A constant ratio between the toxicities of these two metals might be more closely approximated by extending the series of experimental temperatures beyond the in situ ranges in order to establish LC50 peaks for each species. The definition of maximum LC50s might provide a convenient theoretical basis for comparisons between both metals and species. The location of toxicity minima outside in situ temperature ranges, however, would largely negate the predictive value of such results.

(5) Temperature is an extremely important factor affecting toxicity in crustacean zooplankton, and the selection of a bioassay temperature (or bioassay temperatures) should be done very carefully. The variety of temperature-toxicity patterns within the temperature ranges studied indicates that the temperature tolerance zones of different species vary widely, both in breadth and in overall shape.

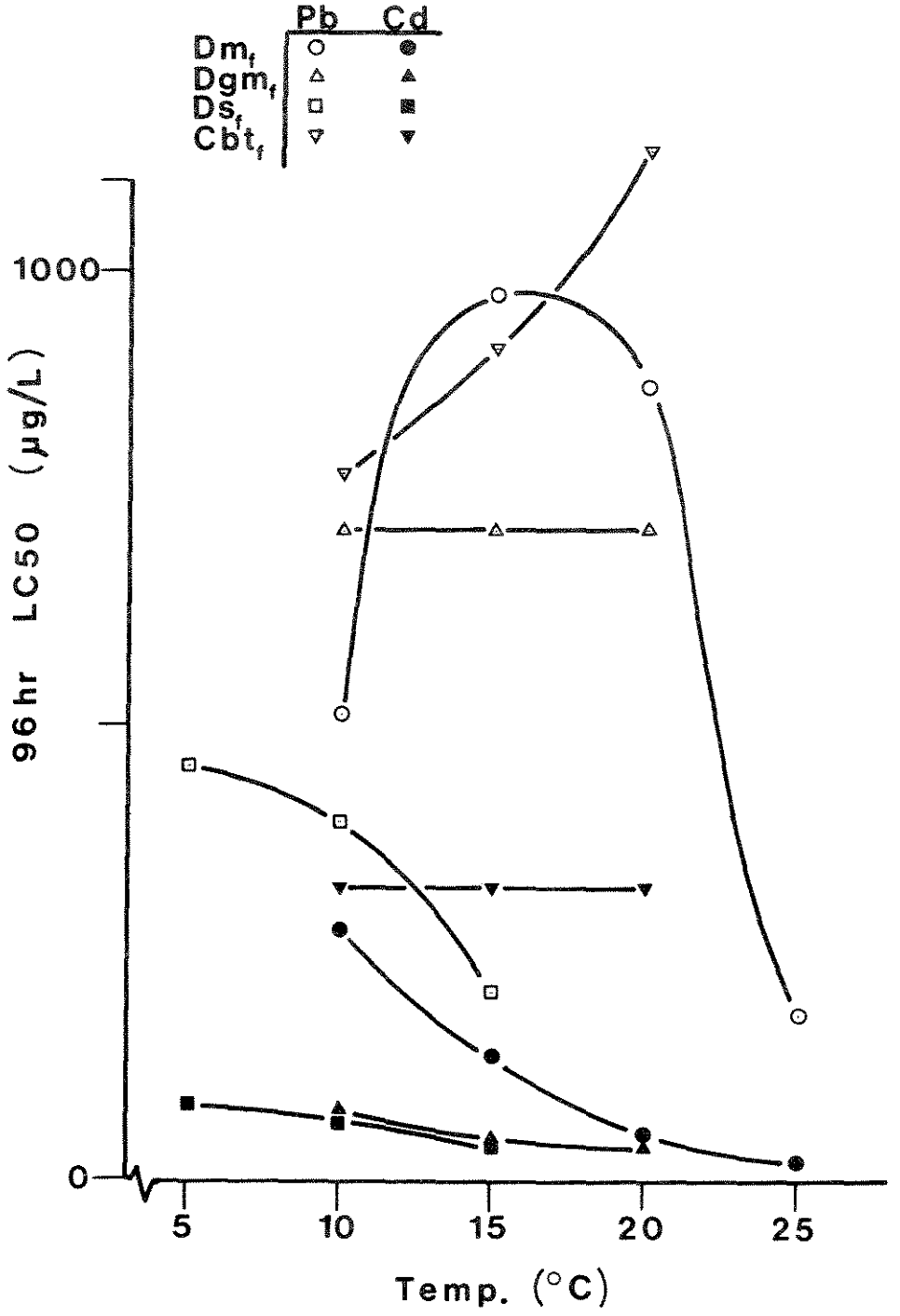

Figure 1. The relationship between temperature and the lead and cadmium acute 96hr. LC50s for the females of each of four zooplankton species: Daphnia magna (Dm_f); Daphnia galeata mendotae (Dgm_f); Diaptomus sicilis (Ds_f); and Cyclops bicuspidatus thomasi (Cbt_f). Each point is the average of five replications, and regression lines represent the best fit of a quadratic polynomial.

4 species (females only), 2 temperatures (10'C, 15'C), 2 metals (Pb, Cd), 5 replications

Source	d.f.	S.S.	M.S.	F_s
Between species	3	1609963	536654	39.48 ***
Between temperatures	1	3634	3634	0.27 n.s.
Between metals	1	4661181	4661181	342.89 ***
Species X Temperature	3	168046	56015	4.12 **
Species X Metal	3	475019	158339	11.65 ***
Temperature X Metal	1	123781	123781	9.11 **
Species X Temp. X Metal	3	381273	127091	9.35 ***
Error	64	870004	13594	
Total	79	8292902		

Table 1. ANOVA table for species, temperature, and metal effects on acute toxicity. Data are for female animals at 10 and 15°C.

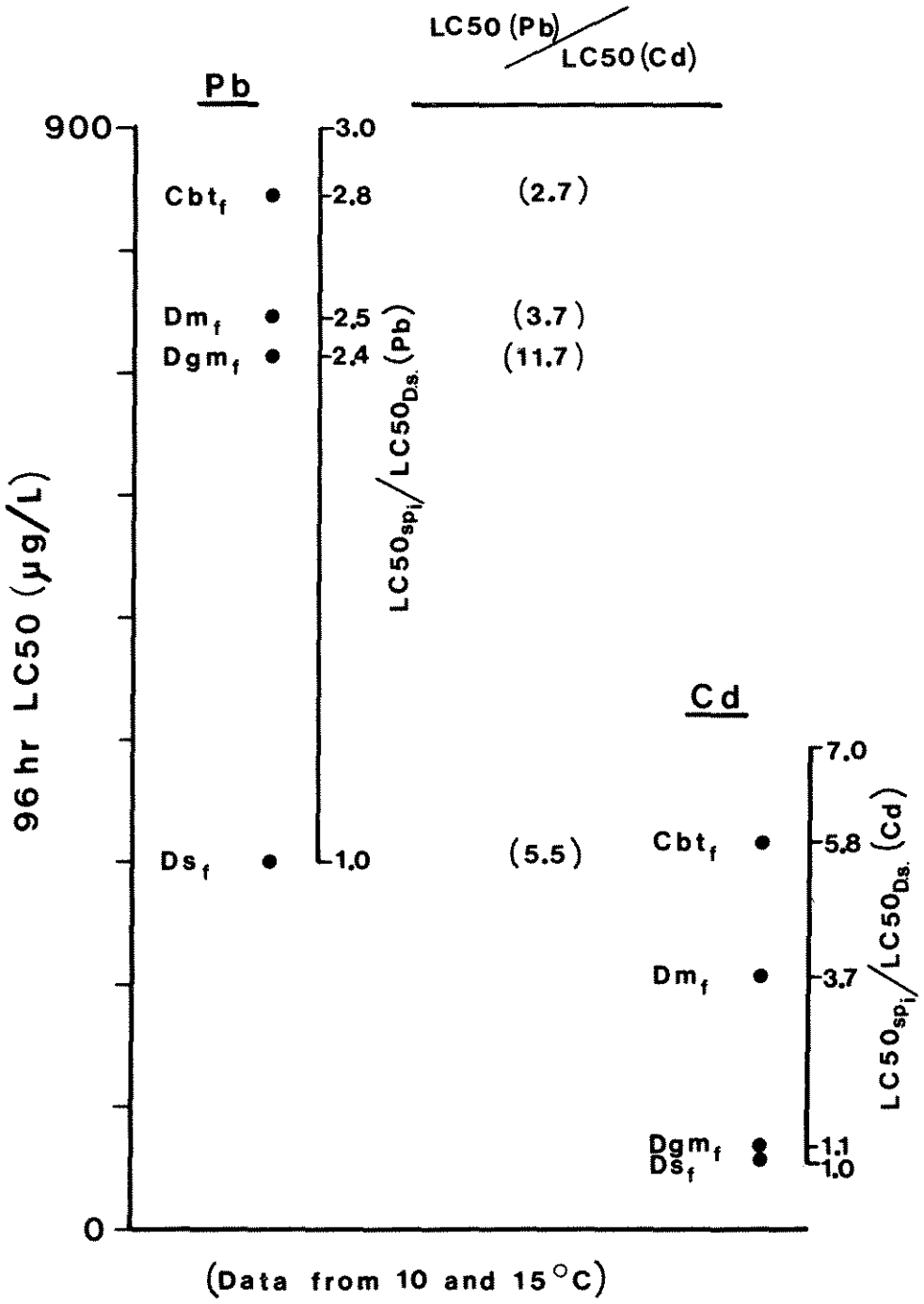

Figure 2. The absolute and relative 96hr LC50s for each of the four zooplankton species. Each point is the average of ten replications from both 10 and 15°C data. The left ordinate gives the absolute LC50 values while the right ordinates give the ratio of each species' LC50 relative to that of the most sensitive species (Diaptomus sicilis for both lead and cadmium). The numbers in brackets represent the Pb/Cd LC50 ratios for each species.

THE EFFECTS OF ACID AND CADMIUM ON IMPOUNDED ZOOPLANKTON IN A CANADIAN SHIELD LAKE

S.G. Lawrence

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

LAWRENCE, S.G. 1980 The effects of acid and cadmium on impounded zooplankton in a Canadian Shield Lake. Can. Tech. Rep. Fish. Aquat. Sci. 975: 81-90.

The effects on plankton invertebrate populations of added acid and of cadmium were monitored in seven 1.5 x 10^5 L impoundments placed over sediments in a lake being artificially acidified. Two enclosures were acidified to pH 5 and pH 4 and immediate effects on the zooplankton community studied. 1, 3, 10, and 30 µg cadmium/L were added to each of four enclosures in 1977 and residual effects of 1-10 µg Cd/L on the zooplankton community studied in 1978. The system containing 30 µg Cd/L was acidified to pH 4 in 1978 and effects on the zooplankton monitored.

The biomass of the control impoundment was dominated by *Diaptomus minutus*. In all but one manipulated system the average total biomass was lower than in the control. The zooplankton biomass of manipulated systems were dominated by *Tropocyclops prasinus mexicanus*, *Bosmina longirostris* or rotifers.

Key words: Toxicity; biomass; *Bosmina*; *Tropocyclops*; *Diaptomus*; Rotifera.

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Nous avons suivi les effets, sur des populations d'invertébrés planctoniques, d'une addition d'acide et de cadmium dans sept enceintes de 1.5 x 10^5 L placées sur les sédiments d'un lac artificiellement acidifié. Après avoir acidifié deux enceintes à des pH de 5 et de 4, nous avons étudié les effets immédiats sur la communauté zooplanctonique. A chacune de quatre enceintes nous avons ajouté 1, 3, 10 et 30 µg/L de cadmium en 1977 et observé, en 1978, les effets résiduaires de 1-10 µg/L de Cd sur la communauté zooplanctonique. Le système contenant 30 µg/L de Cd fut acidifié à un pH de 4 en 1978, et nous avons suivi les effets sur le zooplancton.

La biomasse de l'enceinte témoin est dominée par *Diaptomus minutus*. Dans tous les systèmes manipulés, sauf un, la biomasse totale est inférieure à celle du témoin. La biomasse zooplanctonique des systèmes manipulés est dominée par *Tropocyclops prasinus mexicanus*, *Bosmina longirostris* ou des rotifères.

INTRODUCTION

The effects of acid precipitation on soft water, oligotrophic ecosystmes are known to be multiple rather than simple. According to Schindler (1979) acid can exchange in watershed soils and in lake sediments for vital materials such as calcium and potassium and for toxic materials such as heavy metals.

Various studies in Scandanavia and North America on acid precipitation (e.g. Almer et al. 1974; Leivestad et al. 1976; Sprules 1975; Roff and Kawiatowski 1977) have shown that the number of zooplankton species in lakes with pH below 5.5 is less than in lakes with higher pH. Yan and Strus (1980) have shown similar results in heavy metal contaminated lakes of low pH. Various studies on effects of metals and low pH on various invertebrates conducted in the laboratory (e.g. Davis and Ozburn 1969; Terhaar et al. 1977 and Marshall 1978) and in enclosures of less than 40 L in lakes (Marshall and Mellinger 1978) have provided data about short term effects on various zooplankton populations and communities and indicated direction for more long term studies. Marshall and Mellinger (1980) described effects on zooplankton density of single additions of various concentrations of cadmium added to 1.5×10^5 L impoundments set into the sediments of Lake 223 located near Kenora, Ontario.

This paper describes the composition and biomass of the zooplankton in the same impoundments during the next field season, i.e., after the impoundments had overwintered *in situ*. Further, the effects on zooplankton of reducing the pH of two impoundments to pH 5 and 4 and of reducing the pH of the impoundment which received the highest concentration of cadmium in the previous year to pH 4 are described.

METHODS AND MATERIALS

Seven enclosures, 10 m in diameter and open to the sediment, were placed in 2 m water in a bay of the lake. During 1977, the hydrogen ion concentration of two enclosures was lowered by addition of sulfuric acid in one to pH 5 and in the other to pH 4. Cadmium (as chloride) was added to four other enclosures in concentrations of 1, 3, 10, or 30 µg Cd/L. By late fall cadmium had been transferred quantitatively to the sediments in all enclosures (Schindler et al. 1978). The pH of the lake was lowered from approximately 6.25 to 6.0 by addition of sulfuric acid and the pH of the control enclosure was allowed to equilibrate with that of the lake.

During 1978, the impoundments which received acid were again lowered and maintained at pH 5 and 4, the pH of the enclosure which received 30 µg Cd/L was lowered and maintained at pH 4 and pH of the lake and control impoundment lowered to about 5.75. The enclosures which received 1, 3, or 10 µg Cd/L in 1977 were not manipulated again in 1978, but were monitored for long term effects of cadmium. These systems are referred to as Cd-1, Cd-3 and Cd-10.

Zooplankton samples were taken every 2-4 weeks from mid-July to early October from all enclosures using a net sampler with 54 µ mesh, towed vertically through the water column from just above the sediment to the surface

of the enclosure. The lake epilimnion was sampled with the same net. Samples were preserved in 4% (final concentration) formalin. Zooplankton contained in two 1 mL aliquots were identified to life stage and counted using a Sedgewick Rafter cell and the values averaged. Biomass as dry weight was calculated by assigning a geometric form to each species, measuring the major dimensions and calculating the volume obtaining a wet weight value from volume by assuming that 1 mm^3 weighs 1 mg and estimating dry weight at 7% of wet weight (K. Patalas, Freshwater Institute, pers. comm.). Average proportional biomass figures were calculated by dividing the average biomass of population or group by the average total biomass for the period in question.

The data are presented for various time periods: 17 July - 9 October for the cadmium series and control; 24 July - 9 October (or 4 September) for the acid and acid/cadmium series and control because manipulation of these impoundments began after on 19 July and the pH 4 impoundment was damaged after September 4.

RESULTS AND DISCUSSION

CONTROL SYSTEM AND LAKE EPILIMNION

Findley and Saesura (1980) showed that in 1977 all the enclosure systems were dominated by chrysophytes and small chlorophytes. In 1977 and 1978, these same trends were pronounced in the epilimnion of L. 223.

The lake epilimnion was dominated by the following zooplankton species over the period examined: the calanoid *Diaptomus minutus*, the cyclopoid copepod *Tropocyclops prasinus mexicanus*, the cladoceran *Bosmina longisrostris* and a group of rotifers of which *Keratella taurocephala*, *Kellicottia longispina* and *Polyarthra vulgaris* were prominent members. The copepods *Epichura lacustris* and *Mesocyclops edax*, both predators in late phases of their life cycles, occasionally became dominant; *Diaphanosoma brachyurum* and *Daphnia galeata mendotae* were prominent at various times.

In the control enclosure, *D. minutus*, *T. prasinus mex.*, *B. longirostris* and the rotifers *K. taurocephala* and *P. vulgaris* contributed significantly to the biomass over the period examined. *D. galeata mendotae*, *D. brachyurum* and *M. edax* were found occasionally and sometimes accounted for a significant proportion of the biomass. *E. lacustris* did not appear in the sub-samples of zooplankton taken from the impoundments.

Zooplankton communities in the lake epilimnion and the control impoundment were thus somewhat similar. Figure 1, their total biomass, shows that the curves are similar, but the impoundment appears to lag three weeks in development. Comparison of proportional average biomass in the lake epilimnion and control (Table 1) shows that herbivorous crustacea dominate both systems over the season. Carnivorous crustacea are present in somewhat different proportions in these systems possibly because the carnivore *E. lacustris* was not found in the impoundments. The more prominent rotifer population in the control system may be the result of enclosure. We have found while using small continuously flowing enclosures in lakes, rotifer biomass often rises. Apparently, the high numbers of organisms occurring in the control

enclosure compared with that in the lake epilimnion in 1977 (Marshall and Mellinger 1980) were the indirect result of enclosure which stimulated greater phytoplankton production. The numbers of crustaceans in the control enclosure in 1978 more closely resembles those in the lake epilimnion (Fig. 2).

Table 1. Average proportional biomass expressed as mg dry weight/m^3 and as % of the total biomass in the lake epilimnion and in the control impoundment. 24 July - 26 September, 1978.

	Lake Epilimnion		Control Impoundment	
	dry wt.	%	dry wt.	%
Herbivorous crustacea	24.7	73	18.0	81
Carnivorous crustacea	8.1	26	2.2	10
Rotifers	1.0	3	2.0	9

CADMIUM CONTAINING SYSTEMS

Figure 3 shows the total zooplankton biomass from 17 July - 9 October 1978 in the control and cadmium containing enclosures. The zooplankton biomass of all systems containing cadmium is less than that of the control system. Table 2 shows the average total zooplankton dry weight and the average proportional biomass of the important groups over the period of study. The only plankter which is consistently lower in biomass when compared with the control is *D. minutus*, although its biomass in the Cd-3 system is about two times that in either Cd-1 or Cd-10 systems.

The average proportional biomass of *T. prasinus mex.* and of *B. longirostris* rose in the Cd-1 system but fell in the Cd-3 and Cd-10 enclosures. The rotifer biomass rose dramatically as the apparent cadmium concentration increased so that in the Cd-3 and Cd-10 systems they accounted for 51 and 68% of the biomass, respectively.

The other members of the community may be adjusting to the apparent reaction of *D. minutus* to cadmium in the Cd-1 system, i.e. *T. prasinus mex.*, *B. longirostris*, and the rotifers may have responded to the lowered biomass of *D. minutus* by successfully competing for space and food. However, the biomass of *Tropocyclops* and *Bosmina* decreased in the other two cadmium-containing systems; *D. minutus* responded by successfully competing in these impoundments, but at lower levels than in the control. The zooplankton in the Cd-1 system had a very reduced biomass compared to the control and to other cadmium containing systems and these changes in biomass may be related to that fact more than any other.

Marshall and Mellinger (1980) predicted as a result of their work on these enclosures in 1977 that long term effects of cadmium would probably be greater than those observed after a few weeks enclosure. These results confirm this prediction.

Table 2. Proportional biomass expressed as mg dry weight/m^3 and % of total biomass of species in control and cadmium treated impoundments. 17 July - 9 October, 1978.

	Control		Cd-1		Cd-3		Cd-10	
	dry wt.	%	dry wt.	%	dry wt.	%	dry wt.	%
Total crustaceans	20.1	91	3.9	76	5.7	49	2.2	32
Total rotifers	2.0	9	1.2	24	6.0	51	7.0	68
D. minutus	11.9	54	0.7	14	3.7	32	2.1	20
T. prasinus mex.	2.7	12	2.1	42	1.6	14	0.9	9
B. longirostris	2.7	12	1.0	20	0.4	4	0.2	2
Other crustaceans	2.9	13	0	0	0.1	1	0.1	1
K. taurocephala	0.2	1	0.3	6	1.4	12	1.5	14
K. cochlearis	<0.1	<1	<0.1	<1	1.2	10	1.3	12
P. vulgaris	0.8	4	0.3	5	1.9	16	1.8	17
G. stylifer	0.2	1	<0.1	<1	0.4	3	0.7	7
K. longispina	0.2	1	0.3	5	0.1	1	0.1	1
Other rotifers	0.5	2	0.3	6	1.1	9	1.6	16

ACIDIFIED SYSTEMS

Figure 4 shows the time course of acidification in the lake and in the three enclosures being acidified. This process was terminated for the pH 4 impoundment shortly after 4 September when a large hole developed as the result of a storm.

The zooplankton biomass in systems which were acidified to pH 5 and pH 4 are presented in Fig. 5. Table 3 shows the average proportional biomass in each acidified system from 24 July - 9 October. *D. minutus* nearly disappeared, the biomasses of *T. prasinus mex.* and the rotifers rose in pH 5 and fell in pH 4, but *B. longirostris* and the rotifers rose in pH 5 and fell in pH 4. Apparently, *D. minutus* does not withstand sudded changes in pH at least in impoundments. According to Carter (1970) this species occurred in ponds in which the pH was above 6.0. However, Sprules (1975) and Roff and Kwiatkowski (1977) found that *D. minutus* was sometimes the only crustacean zooplankter in Precambrian shield lakes where the pH had dropped to as low as pH 4.2 over a period of years.

At pH 5 biomass in all the major groups has changed significantly from the control. *T. prasinus mex.* biomass increased at pH 5 because of conditions favorable to its growth or because *D. minutus* nearly disappeared soon after these systems were manipulated. Carter (1970) did not find *T. prasinus mex.* below pH 6 and Sprules (1975) notes that it did not occur in the La Cloche Lakes below pH 5 so its success in this situation is probably due to the lack of competition from *D. minutus* which figures prominently in the ponds and lakes examined by Carter and Sprules. *B. longirostris* responded

Table 3. Proportional biomass expressed as mg dry weight/m^3 and % of total biomass of species found in control, acid and acid/cadmium treated impoundments. 24 July - 9 October, 1978.

	Control		pH 5		pH 4*		Cd-30 - pH4		Cd-30 - pH 4*	
	dry wt.	%	dry wt.	%	dry wt.	%	dry wt.	%	dry wt.	%
Total crustaceans	21.6	91	8.0	59	16.8	93	15.6	82	21.0	81
Total rotifers	2.1	9	5.5	41	1.1	6	3.4	18	4.9	19
D. minutus	13.5	57	0.5	4	0.5	3	0.2	1	0.3	1
T. prasinus mex.	2.4	10	3.9	29	0.9	5	0.8	4	1.0	4
B. longirostris	3.1	13	3.2	24	15.4	86	14.2	75	19.2	74
Other crustaceans	2.6	11	0.3	2	0	0	0.4	2	0.5	2
K. taurocephala	0.2	1	4.6	34	0.7	4	2.7	14	4.1	16
K. cochlearis	<0.2	<1	<0.1	<1	<0.2	<1	<0.2	<1	<0.3	<1
P. vulgaris	0.9	4	0.4	3	<0.2	<1	<0.2	<1	<0.3	<1
G. stylifer	0.2	1	<0.1	<1	<0.2	<1	0.2	1	0.3	1
K. longispina	0.2	1	<0.1	<1	<0.2	<1	<0.2	<1	<0.3	<1
Other rotifers	0.5	2	0.4	3	0.2	1	0.4	2	0.3	1

* 24 July - 4 September

well to acid; at pH 4, it was the dominant zooplankter accounting for 86% of the biomass. This is in agreement with the work of Carter (1970) who found *B. longirostris* in great abundance in acid ponds and with that of Sprules who found it in high numbers in lake waters as acidic as pH 3.8. *K. taurocephala* formed 34% of the biomass and 79% of the density at pH 5, but only 4.5% of the biomass and 61% of the density at pH 4, in agreement with the data of Roff and Kwiatkowski who found that this organism formed 67% of the zooplankton numbers in a lake of pH 4.4.

CADMIUM-ACID INTERACTION

Figure 6 shows the zooplankton biomass in Control, pH 4 and pH 4 - Cd-30 systems from 24 July to 4 September. Table 3 shows mg/m^3 and proportional biomass for all systems treated with acid and with acid and cadmium. *D. minutus* did not occur in the pH 4 - Cd-30 system after manipulation was started. Over the whole period, *T. prasinus mex.* maintained its biomass at about the same proportion as in the control. The biomass of the rotifers rose in this environment as did that of *B. longirostris*, although the proportional biomass was not so high as in pH 4 alone, possibly in response to somewhat elevated cadmium levels. Between 24 July and 4 September, the average biomass in this system was the highest of those measured due almost entirely to the extremely high numbers of large *B. longirostris* present at one sampling date. *Bosmina* biomass was lower in systems containing cadmium where pH was not altered, but this effect was apparently overcome by acid conditions.

SUMMARY

1. *D. minutus* populations decreased with sudden lowering of pH and in all concentrations of cadmium.

2. *B. longirostris* biomass increased with extreme lowering of pH but appeared to be sensitive to competition from other zooplankton under less acid conditions.

3. *T. prasinus mex.* is an opportunistic competitor not sensitive to pH above 5 or to low concentrations of cadmium. It is apparently sensitive to somewhat higher concentrations of cadmium and to pH 4.

4. Rotifers as a group do not appear to be adversely affected by either lowered pH or by residual cadmium. They probably respond to increased or decreased grazing pressure or competition for food. *K. taurocephala* is able to compete successfully at pH 5, less well at pH 4.

5. The effects of low concentrations of cadmium on zooplankton community structure are long term under these circumstances as predicted by Marshall and Mellinger (1980).

6. Extrapolation of data obtained from impoundments to the natural situation should be tentative. Such data can be used as part of a data pool to predict effects on aquatic ecosystems, to plan larger scale experimentation and to lead to more defined experimentation using impounded natural waters.

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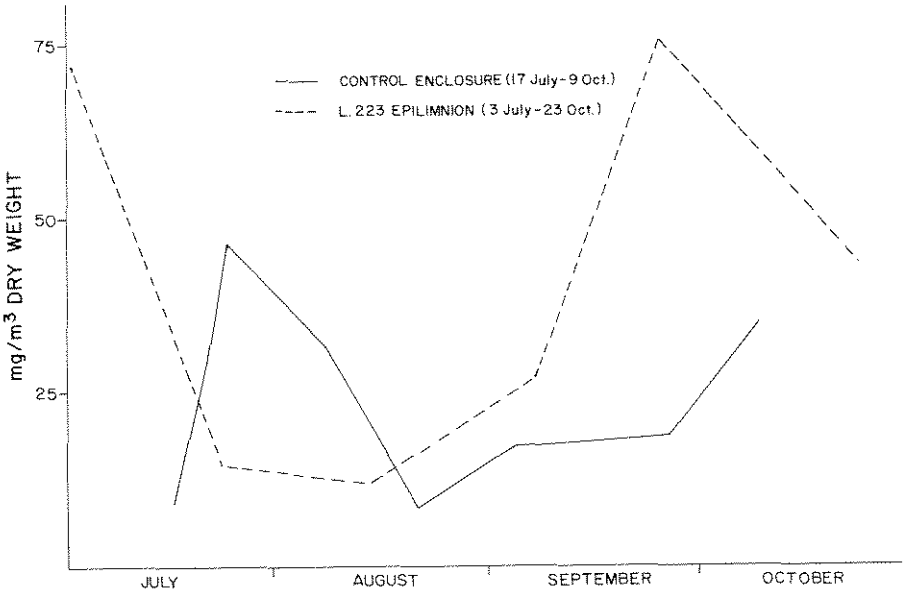

FIGURE 1. TOTAL ZOOPLANKTON BIOMASS, CONTROL SYSTEMS.

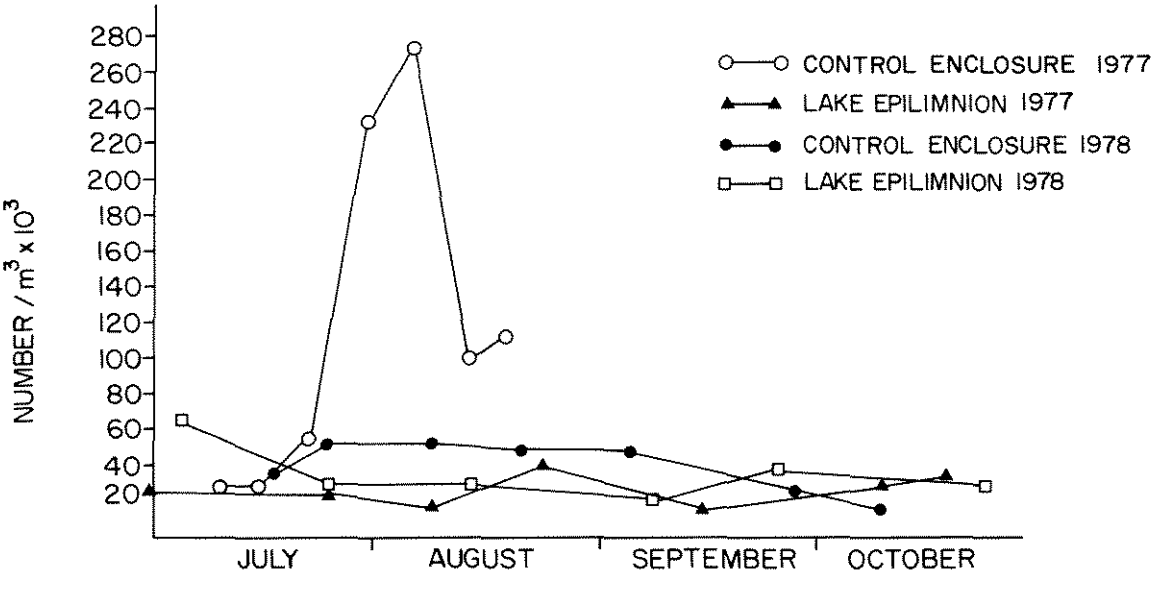

FIGURE 2. TOTAL CRUSTACEAN DENSITY, L. 223 AND CONTROL ENCLOSURE.

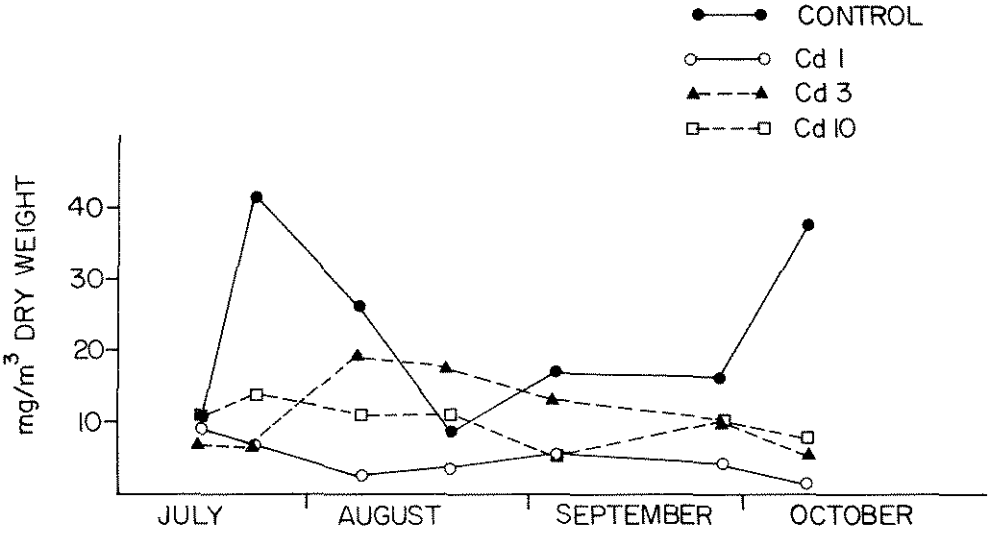

FIGURE 3. TOTAL ZOOPLANKTON BIOMASS, CADMIUM ENCLOSURES.

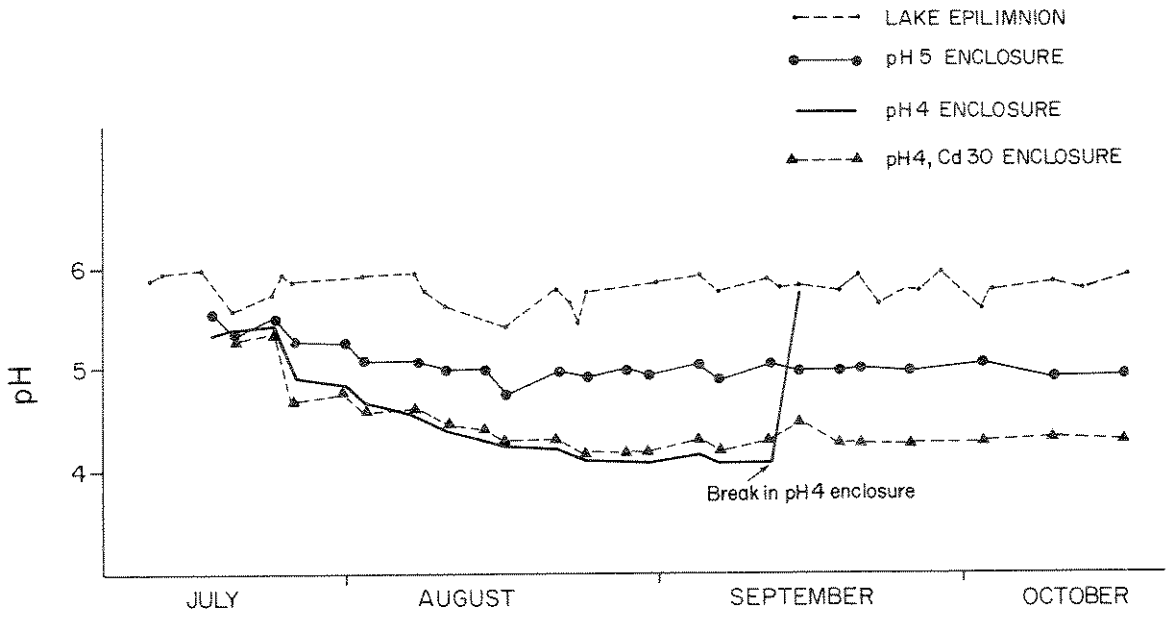

FIGURE 4. ACIDIFICATION OF L. 223 AND ENCLOSURES, 1978.

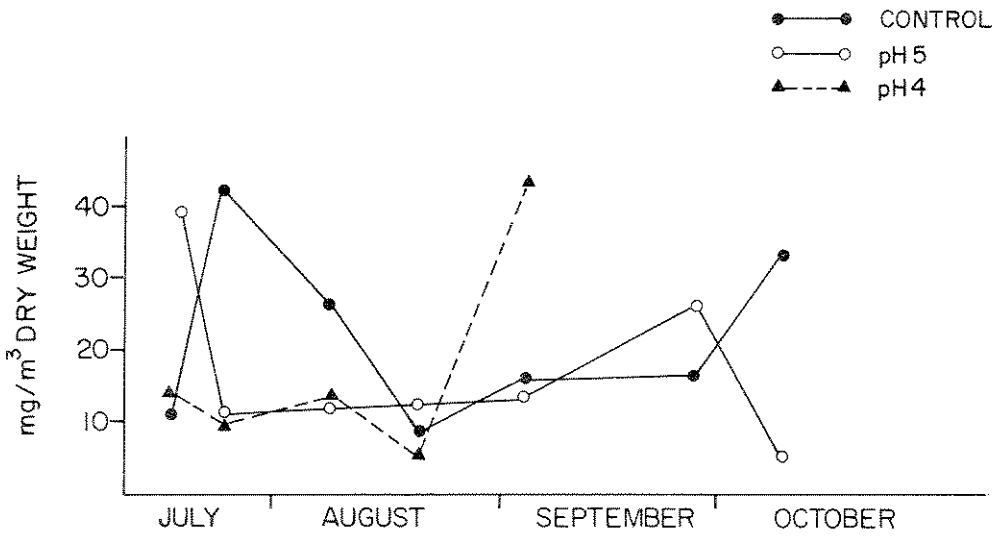

FIGURE 5. TOTAL ZOOPLANKTON BIOMASS, ACID ENCLOSURES.

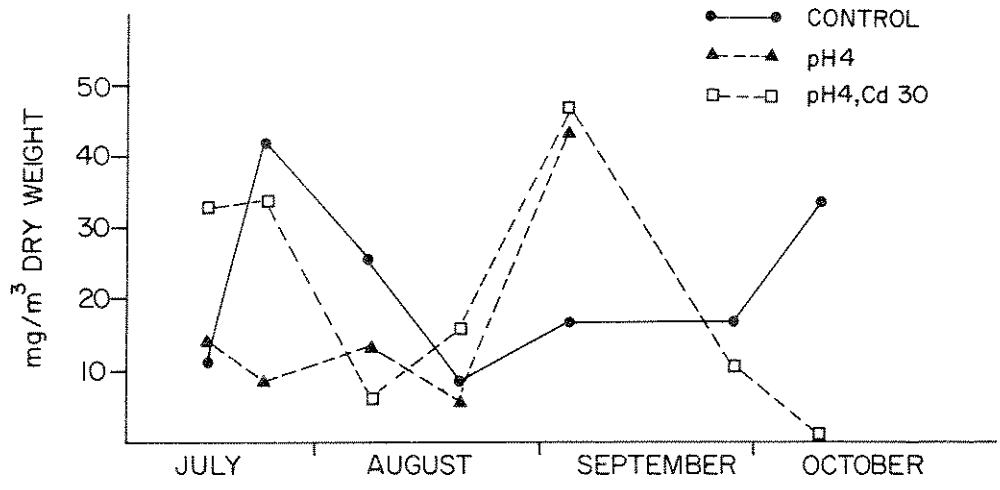

FIGURE 6. TOTAL ZOOPLANKTON BIOMASS, ACID-CADMIUM INTERACTION.

METAL ION TOXICITY TO DAPHNIA MAGNA : A NOVEL APPROACH TO DATA PLOTTING

C.R. Macdonald[1], T.R. Jack[2] and J.E. Zajic[1]

[1] Faculty of Engineering Science, University of Western Ontario, London, Ontario, Canada N6A 5B9

[2] Physical Sciences Division, Scarborough College, University of Toronto, West Hill, Ontario, Canada M1C 1A4

MACDONALD, C.R., T.R. JACK and J.E. ZAJIC 1980 Metal ion toxicity to Daphnia magna : A novel approach to data plotting. Can. Tech. Rep. Fish. Aquat. Sci. 975: 91-107.

The acute toxicity of transition metal ions to Daphnia magna is analyzed in terms of a simple model in which death arises as

$$M^{2+}_{(aq)} + \text{organism} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{M bound to organism} \xrightarrow{k_2} \text{death}$$

a consequence of metal ion binding to unspecified sites in the organism. The form and kinetics of this model are identical to those generated for Michaelis-Menton enzyme kinetics and a plot analogous to the Lineweaver-Burk plot for enzyme kinetics can be used to characterize the acute toxicity response in terms of two constants, a dissociation constant for metal ion/organism complex formation and the minimum median survival time induced by the metal ion.

Key words: Toxicity; freshwater Crustacea; Cladocera

MACDONALD, C.R., T.R. JACK and J.E. ZAJIC 1980 Metal ion toxicity to Daphnia magna : A novel approach to data plotting. Can. Tech. Rep. Fish. Aquat. Sci. 975: 91-107.

Le présent article décrit une analyse de la toxicité aiguë d'ions métalliques de transition chez Daphnia magna, en termes d'un simple modèle dans lequel la mort se produit comme suit :

$$M^{2+}_{(aq)} + \text{organisme} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{M lié à l'organisme} \xrightarrow{k_2} \text{mort,}$$

une conséquence de la fixation des ions métalliques sur des sites non spécifiés dans l'organisme. La forme et la cinétique de ce modèle sont identiques à celles qu'engendre la cinétique enzymatique de Michaelis-Menton. On peut utiliser un tracé analogue à celui de Lineweaver-Burk pour la cinétique enzymatique quand il s'agit de caractériser la réaction de toxicité aiguë en termes de deux constantes, une constante de dissociation pour la formation du complexe ion métallique/organisme et le temps de survie médian minimal provoqué par l'ion métallique.

Introduction

There is some evidence that the toxicity of transition metal ions can be related to the ability of the free ion to form stable complexes with sensitive sites in the biological systems. Thus Shaw (1961) noted a strong correlation between the log of the complex formation constants for Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} with general chelating ligands such as ethylenediamine and the negative log molar concentration for the LD_{50} of the same metal ions for a wide variety of organisms. This correlation has been further refined by Jones and Vaughn (1978) to incorporate the effects of hard-soft acid-base theory on the stability of metal complex formation in biological systems.

Various effective sites of binding have been suggested to explain the toxic action of these metal ions for aquatic organisms.

$$nM^{2+} + \text{gill surface} \rightleftharpoons \text{"}M_n\text{-gill surface"} \qquad (1)$$

$$nM^{2+} + \text{enzyme} \rightleftharpoons \text{"}M_n\text{-enzyme"} \qquad (2)$$

$$nM^{2+} + \text{membrane} \rightleftharpoons \text{"}M_n\text{-membrane"} \qquad (3)$$

In eq. (1), the acute toxicity results from the interference of metal ions bound to the gill surface with oxygen transfer (Lloyd 1960). In eq. (2), the binding of metal ions to enzymes, inactivates a vital catalyst (Ochiai 1977; Shaw 1954). In eq. (3), the binding of metal ions to cell membranes results in the entry of the metal ions into the cell where various necessary functions

are disturbed (Mount 1966). All of these postulated mechanisms are similar in that the extent of metal ion toxicity depends on the ability of the metal ion to form stable complexes with the various donor atoms likely to be present in a biological site; oxygen, nitrogen or sulfur. Hence it is not surprising that Shaw (1961) found that for a wide range of organisms the LD_{50} for divalent cations of the first transition series is inversely related to the order of the stabilities expected for metal ion complex formation as predicted by simple crystal field theory; $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ (Hanzlik 1976). This correlation suggests that it is valid to establish a model for metal ion induced acute toxicity without specifying the site of metal binding or the actual mechanism involved in the toxic action. In such a model the metals can be simply viewed as binding to the "organism" even though the response of the organism may indeed involve metal ion binding sites of various types.

Without specifying the site of metal binding and toxic action explicitly it is possible then to propose the following general model consistent with Shaw's observations.

$$M^{2+}_{(aq)} + \text{organism} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{M bound to organism} \xrightarrow{k_2} \text{death} \qquad (4)$$

The form and kinetics of this model are identical to those described as Michaelis-Menton enzyme kinetics or membrane transport kinetics and a plot analogous to the Lineweaver-Burk plot can be used to characterize the metal toxicity in terms of two constants;

a "dissociation constant", K_d, for the metal ion and the minimum median survival time obtainable with that metal ion.

In this paper, we undertake to analyze the acute toxicity response of an aquatic organism, Daphnia magna, to the metal ions, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} from the first transition series using this model.

MATERIALS AND METHODS

The procedure used in obtaining the acute toxicity data employed herein have been outlined in detail elsewhere (Macdonald et al., to be published). A brief summary will be given here.

A stock culture and a breeding culture of Daphnia magna were maintained in 2/45 l aqueous and 80/200 ml square sided jars respectively. The water in both cultures was filtered pond water collected from a local conservation area which contained an indigenous population of Daphnia.

All toxicity tests were conducted on neonates (<24 hours old) which were separated daily from the breeding culture using a filtering device described by Behie et al. (1977). The dilution medium used for all the tests was an "acetate" test medium consisting of $Ca(OAc)_2$ (211 mg/l), K_2SO_4 (26 mg/l), NaOAc (200 mg/l) and $CaCl_2$ (14 mg/l) made up in distilled deionized water. This medium was specifically designed to minimize the complexation of the metal ions so that the principal solution species in each case is the free aquated metal ion (Macdonald et al., in press). The solution chemistry of the test solutions was modelled using the

computer program REDEQL2 (McDuff and Morel 1973), made available to us through Professor Ann Zimmerman of the University of Toronto. In all cases, the free aquated metal ion represents more than 55% of the total metal present in solution. All metal ions were added in the form of sulphate salts.

The tests were conducted in triplicate in 30 ml beakers with 12 neonates per beaker and the results analyzed on the basis of both individual beakers and sets of three at each concentration tested. The toxic response was taken to be death on the basis of complete internal and external immobility and was expressed in terms of the medium survival time (MST), the geometric mean of the survival times of the neonates in each test population.

RESULTS

The acute toxicity of the selected metal ions to Daphnia magna in the artificial test medium is summarized in Figure 1 where the negative log molar concentrations of the metal ions required for the threshold of toxicity and for the 8-hr LC_{50} are plotted against the first transition series of metals. The shape of the plot in either case resembles that noted by Shaw (1961) for a variety of organisms.

The median survival time (MST) calculated for each metal ion is an expression of toxicity which is inversely proportional to the rate of mortality. Thus a plot of the reciprocal of median survival time as shown in Figure 2 for nickel(II) is in effect a

plot of the rate of mortality against the concentration of metal ion present and takes the form expected for a phenomenon which follows saturation kinetics. As such, this plot is analogous to that expected for other familiar processes such as the adsorption of ions into a monolayer on a surface, the kinetics of enzyme activity or the mediated transport of metal ions across a cell membrane.

Treating the model eq. (4) in a manner analogous to the Michaelis-Menton treatment of enzyme kinetics leads to eq. (5) which can be rearranged to yield eq. (6), a linear transformation of eq. (5).

$$MST^{-1} = \frac{MST_m^{-1}\ [M^{2+}]}{K_d + [M^{2+}]} \qquad (5)$$

where MST, median survival time

MST_m, minimum median survival time

$[M^{2+}]$, metal concentration

$$K_d = \left(\frac{k_{-1} + k_2}{k_1}\right)$$

$$MST = MST_m K_d \left(\frac{1}{[M]}\right) + MST_m \qquad (6)$$

Plotting eq. (6) as MST against $[M]^{-1}$, Figure 3, is analogous in enzyme kinetics to the Lineweaver-Burk plot and allows the derivation of two constants; the minimum median survival time (MST_m) and the constant (K_d).

The actual plots obtained from the toxicity data for zinc(II) and nickel(II) are given in Figure 4 where the concentration of metal ion has been taken as the total metal ion concentration. Computer modelling (REDEQL2) of the bioassay solutions (prior to introduction of the test organism) has shown that the principal solution species for each metal tested was the free aquated ion and that in all cases the free ion concentration is linearly proportional to the total metal concentration over the concentration range tested. Thus the linear form of this plot is not altered by use of free metal ion concentrations in place of total metal ion concentrations as is illustrated for copper(II) in Figure 5. The data obtained for all the metal ions tested are summarized in Table 1 in terms of the characteristic constants, K_d and MST_m.

Again by analogy with the Michaelis-Menton treatment for enzyme kinetics, the constant, K_d should approximate the dissociation equilibrium constant for the metal/organism complex in eq. (4) (since k_2 will be much smaller than k_1 and k-1). Thus a plot of -log K_d against the first transition series metals should correlate with that predicted by crystal field theory for the stability of metal ion complex formation. Figure 6 shows this to be the case and compares the negative log of K_d with the log of the formation constants for the complexation of the same metal ions with two general chemical ligands, ammine and malonate.

CONCLUSIONS

The model proposed herein eq. 4 provides a simple rational method for characterizing the acute toxicity effects induced by metal ions in terms of the parameters; K_d, related to the ability of the metal ion to form stable coordination complexes at biological binding sites and MST_m, the minimum median survival time obtainable with the test metal ion. With these two parameters, the median survival time at any toxic concentration of the metal ion can be determined directly through eq. 6 or through the plot shown in Figure 3.

In the present case the method has been applied to acute toxicity data obtained on D. magna but should be generally useful in a wide range of acute toxicity testing particularly where aquatic organisms are involved provided that complexing agents are not a significant factor in the test medium. The model is of course a gross oversimplification of the actual mechanism of toxicity and is best applied to short term toxicity tests where subtleties in the exact nature of the effective binding sites are of minimal importance. The data employed herein arise from static bioassay procedures and it would be of interest to extend the application of the model to acute toxicity data obtained by continuous flow bioassay techniques using other organisms.

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TABLE 1; Summary of metal ion toxicities in terms of K_d and MST_m

Metal Ion		MST_m (hr)	K_d (mg/l)
Copper(II)	Total Metal	0.29	0.25
	Free Metal	0.27	0.14
Manganese(II)	Total Metal	1.74	833.3
	Free Metal	~0	10,000
Cobalt(II)	Total Metal	5.82	76.92
	Free Metal	5.01	76.92
Nickel (II)	Total Metal	2.71	52.6
Zinc(II)	Total Metal	3.39	213
	Free Metal	1.95	303

Free metal ion concentrations were obtained by computer modelling, REDEQL2 (McDuff and Morel 1973).

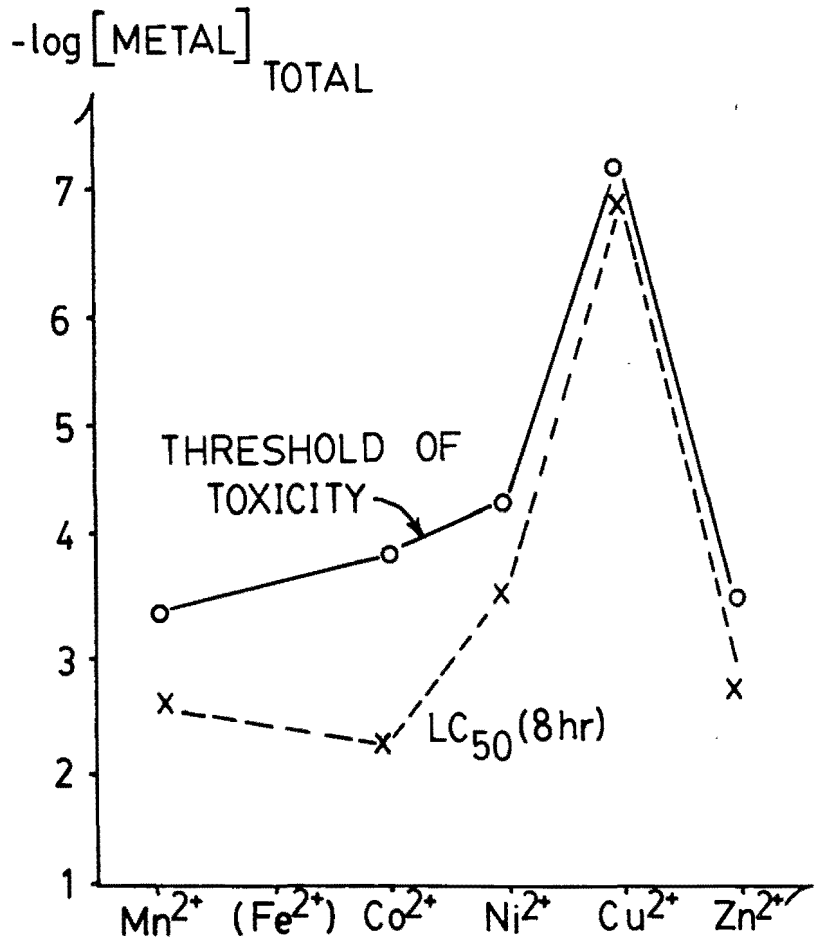

Figure 1. The toxicity of metal ions to Daphnia magna.

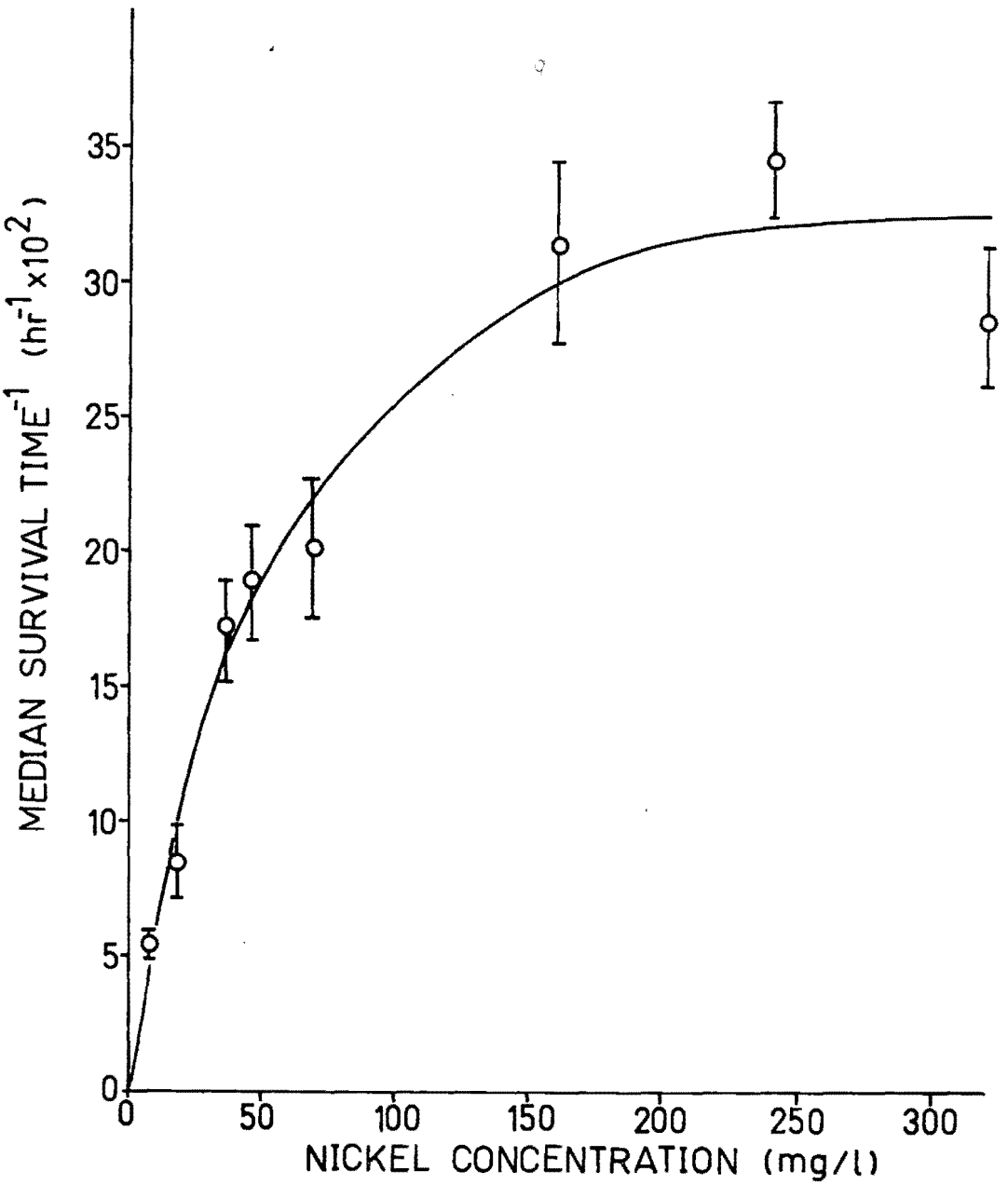

Figure 2. Toxicity of nickel(II) to Daphnia magna expressed by the reciprocal of median survival time (or rate of mortality) as a function of nickel(II) concentration.

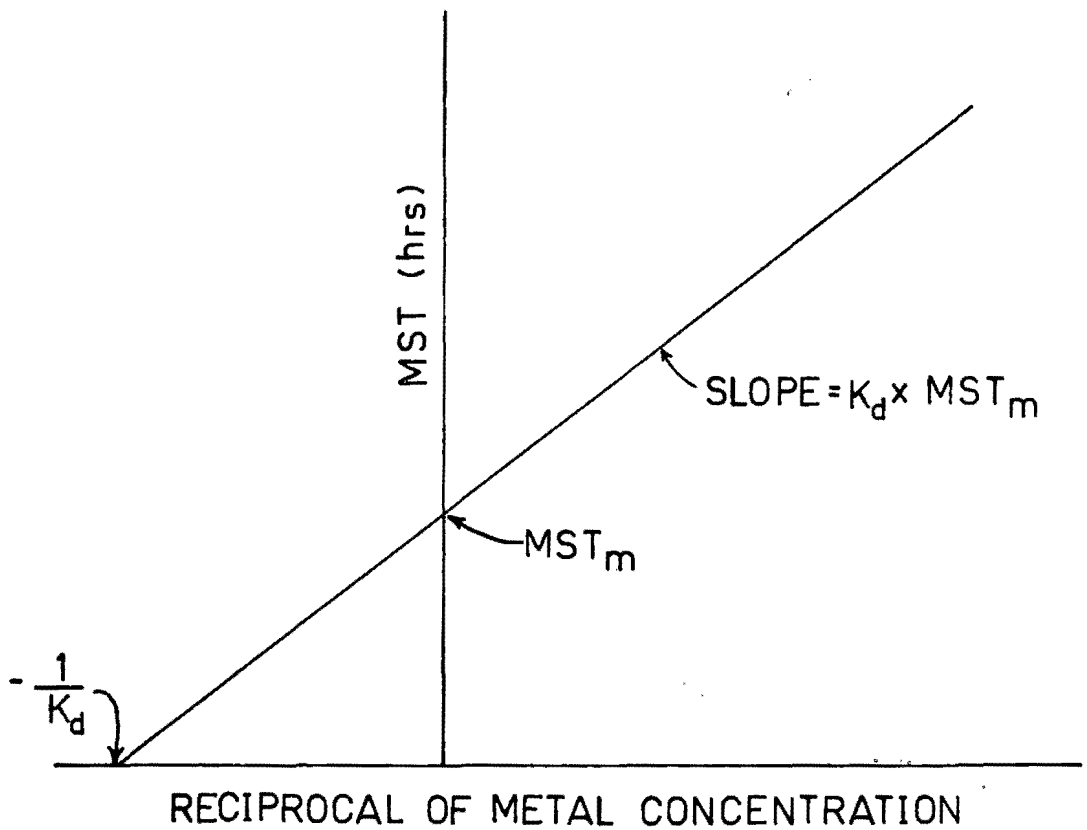

Figure 3. General form of the plot for obtaining the minimum median survival time (MST_m, hrs.) and the dissociation constant (K_d) from eq. 6.

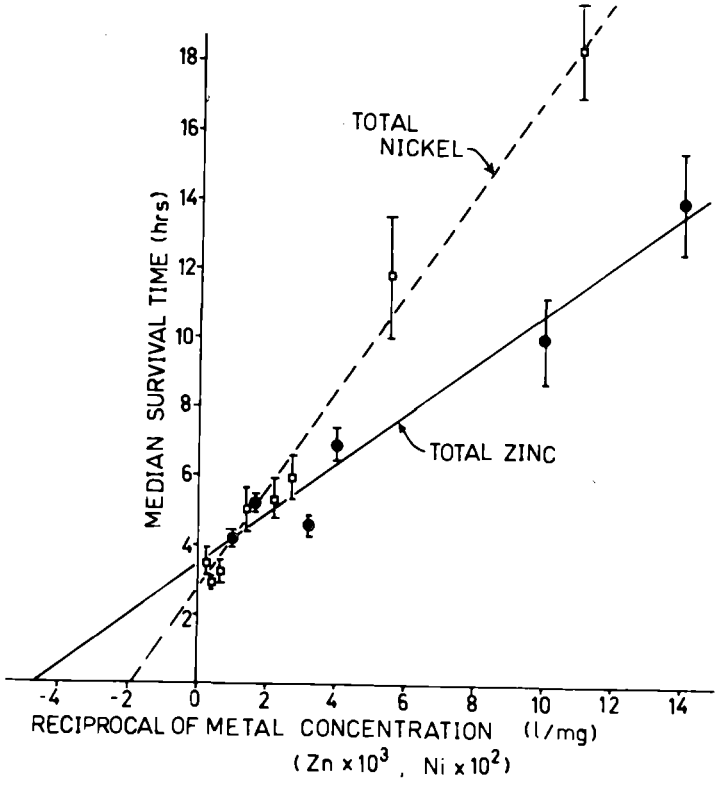

Figure 4. Toxicity data for Daphnia magna plotted on the basis of eq. 6 for zinc(II) and nickel(II).

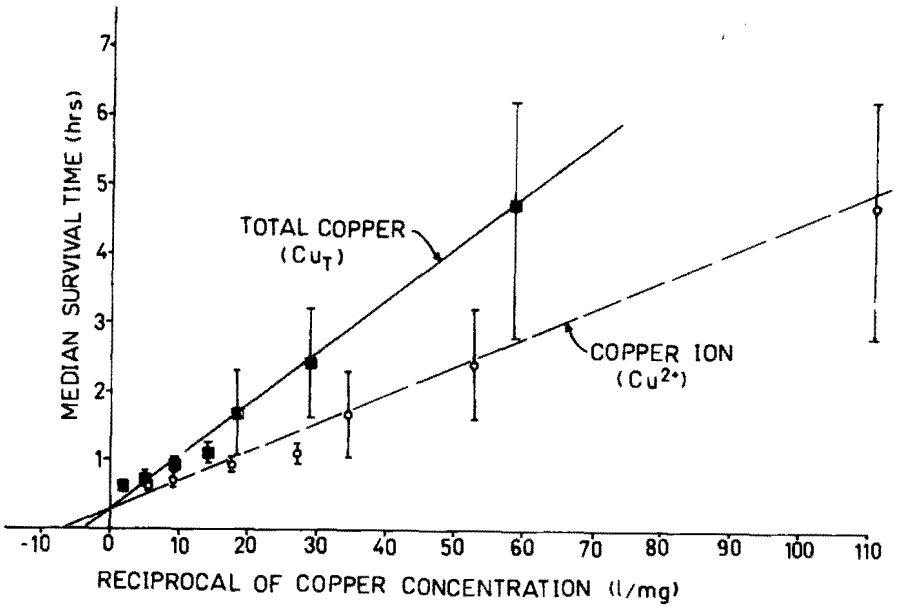

Figure 5. Toxicity data for Daphnia magna plotted on the basis of eq. 6 for copper(II) as the total copper ion (■) and as the calculated free aquated copper ion (O) concentrations.

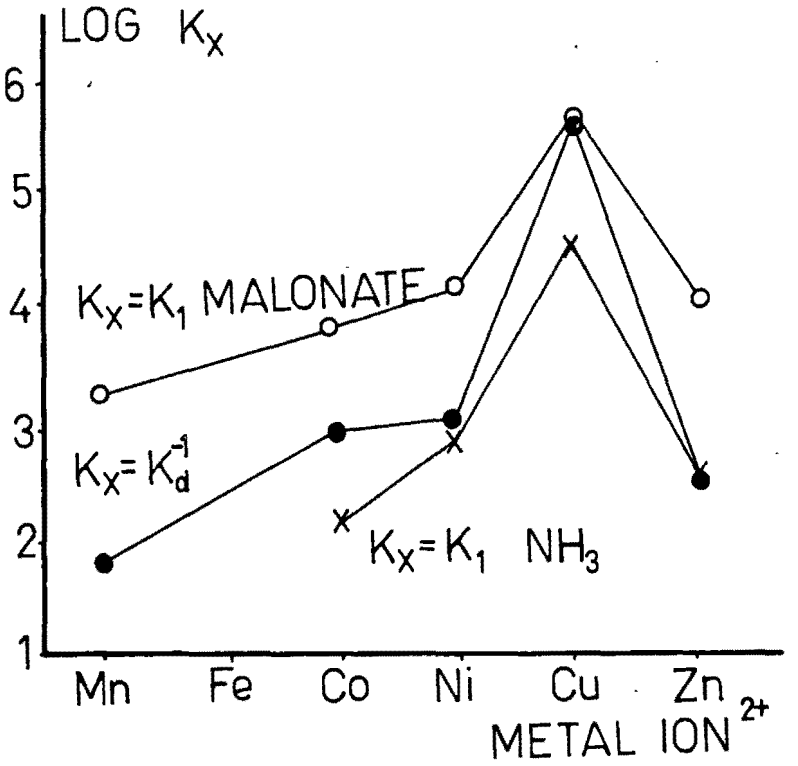

Figure 6. A comparison of the negative log of the dissociation constant (K_d) (●) obtained from toxicity plots as shown (Figures 4 and 5) with the log of the formation constants for the corresponding metal ion complexes with the ligands, NH_3 (X) (MacKay and MacKay 1972) and malonate (O) (Angelici 1975).

INDUCED TOLERANCE TO CADMIUM IN WHITE SUCKERS (*Catostomus commersoni* Lacépède) BY EXPOSURE TO SUBLETHAL CONCENTRATIONS OF HEAVY METALS

D.A. Duncan[1] and J.F. Klaverkamp[2]

[1]Department of Zoology, University of Manitoba, Winnipeg, Manitoba, R3T 2N2
[2]Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, R3T 2N6

DUNCAN, D.A. and J.F. KLAVERKAMP 1980 Induced tolerance to cadmium in white suckers (*Catostomus commersoni* Lacépède) by exposure to sublethal concentrations of heavy metals. Can. Tech. Rep. Fish. Aquat. Sci. 975: 108-109.

An organism's potential to exhibit increased tolerance to metals could have very significant consequences in the environment and in using laboratory toxicity data to establish water quality criteria. Reports of apparently healthy fish populations existing in contaminated habitats are appearing in the scientific literature indicating the need for more knowledge of effects due to sub-lethal contamination.

This investigation determines the ability of white suckers (*C. commersoni*) to acquire tolerance to cadmium (Cd) when exposed to sub-lethal concentrations of Cd, zinc (Zn), mercury (Hg), or selenium (Se) before exposure to lethal Cd concentrations. The 96 hour LC50 values of Cd, Zn, Hg and Se for white suckers (exposed to background metal levels only) in soft water at 12°C are 0.82, 1.66, 0.60 and 28.2 mg/L respectively. Mortality curves ($\log_{10}$ median survival time vs $\log_{10}$ Cd concentration) were obtained from fish exposed for one week to metal concentrations (ranging from background levels to 0.89 of their 96 hr LC50), and the corresponding median survival times (MSTs) were compared.

One week exposure to 3.6 µg Cd/L resulted in decreased MSTs, indicating a synergistic effect of the exposure. No change in MSTs was observed after one week exposure to 6.5 µg Zn/L or 0.07 µg Hg/L. Similar exposures to 178, 410, and 730 µg Cd/L, 193 and 890 µg Zn/L, 1.03 and 220 µg Hg/L, or 99.39 and 1935 µg Se/L resulted in increased MSTs, indicating development of induced tolerance. For example, 7 days exposure to 410 and 730 µg Cd/L, 890 µg Zn/L, or 220 µg Hg/L resulted in the MST (at 5 mg Cd/L) increasing 34, 50, 33, o4 376% respectively. The induced tolerance to Cd resulting from sub-lethal Cd exposure at concentrations of 178, 410, or 730 µg Cd/L produced a concentration increase of MST values. Clearly the induction phenomenon is non-specific as cross tolerance was observed. That is, not only was Cd tolerance induced by previous exposure to Cd, but also by previous exposure to Zn or Hg and, ot a lesser extent, Se. This acquired tolerance may result from an increased synthesis, such as in the liver or gills, of a small molecular weight protein, metallothionein, which is rich in SH groups and has a metal-detoxification function. Experiments will be conducted to examine the relationship of metallothionein concentration, to increased tolerance to Cd in white suckers.

Key words: Sublethal; toxicity; cadmium; zinc; mercury; selenium; LC50; detoxification

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L'aptitude d'un organisme à acquérir une plus grande tolérance aux métaux pourrait avoir de très importantes conséquences dans l'environnement et dans l'usage de données de toxicité obtenues en laboratoire pour fixer des critères de qualité de l'eau. Les publications scientifiques mentionnent l'existence de populations de poissons apparemment en bonne santé dans des habitats contaminés, signe qu'il nous faut connaître davantage les effets d'une contamination sublétale.

La recherche décrite dans le présent article permet de déterminer l'aptitude des meuniers noirs (*C. commersoni*) à acquérir une tolérance au cadmium (Cd) après avoir été exposés à des concentrations sublétales de Cd, zinc (Zn), mercure (Hg) ou sélénium (Se) avant une exposition à des concentrations létales de Cd. Les valeurs de CL50 après 96 h de Cd, Zn, Hg et Se pour les meuniers noirs (exposés seulement à des niveaux naturels de métaux) dans de l'eau douce à 12°C sont 0.82, 1.66, 0.60, et 28.2 µg/L respectivement. Nous avons établi des courbes de mortalité (log décimal du temps de survie médiane c. le log décimal de la concentration de Cd) à partir de poissons exposés durant une semaine à des concentrations de métaux (variant de concentrations naturelles à 0.89 de leur CL50 après 96 h), et ensuite comparé les temps de survie médiane correspondants (MST).

Une exposition d'une semaine à 3.6 µg/L de Cd cause une diminution des MST, signe d'une effet de synergie de l'exposition. A 6.5 µg/L de Zn ou 0.07 µg/L de Hg, nous n'observons aucun changement de MST après exposition de une semaine. Des expositions semblables à 178, 410 et 730 µg/L de Cd, 193 et 890 µg/L de Zn, 1.03 et 220 µg/L de Hg ou 99.39 et 1,935 µg/L de Se causent une augmentation de MST, indiquant que se développe une tolérance induite. Par exemple, une exposition de 7 jours à 410 et 730 µg/L de Cd, 890 µg/L de Zn ou 220 µg/L de Hg résulte en une augmentation de MST (à 5 µg/L) de 34, 50, 33 ou 376% respectivement. Les MST augmentent en fonction de la concentration par suite de la tolérance provoquée au Cd résultant d'une exposition sublétale à Cd à des concentrations de 178, 410 ou 730 µg/L de Cd. Le phénomène d'induction n'est pas spécifique, une tolérance réciproque ayant été observée. C'est-à-dire que non seulement la tolérance au Cd est provoquée par une exposition préalable au Cd, mais aussi au Zn, Hg et, à un degré moindre, au Se. Cette tolérance acquise peut résulter d'une synthèse accrue, comme dans le foie ou les branchies, d'une protéine de faible poids moléculaire, la métallothionéine, riche en groupes SH et capable de détoxifier les métaux. Nous poursuivrons des expériences en vue d'étudier la relation entre la concentration de métallothionéine et la tolérance accrue au Cd chez les meuniers noirs.

ALBERTA OILS SANDS ENVIRONMENTAL RESEARCH PROGRAM (AOSERP) TOXICOLOGY AND RELATED PROJECTS

A. Avramenko, B.A. Munson, R.T. Seidner

AOSERP, Edmonton, Alberta

AVRAMENKO, A., B.A. MUNSON and R.T. SEIDNER 1980 Alberta oil sands environmental research program (AOSERP) toxicology and related projects. Can. Tech. Rep. Fish. Aquat. Sci. 975: 110.

The Alberta Oil Sands Environmental Research Program (AOSERP) is currently completing its fifth year. This program, initiated under a joint Federal-Provincial agreement, has been operating with the primary function of providing an adequate information base to environmental managers so they could ensure the quality of the environment as development of the oil sands proceed. To date, environmental research has been primarily directed towards delineating baseline states. (e.g., a description of habitats and species relationships, and air and water quality monitoring). Definitions of current industrial impact and projections of probable impacts have been conducted. For example, approximately 300 Tonnes of sulphur per day will be expelled to the environment in addition to quantities of metals present in oil sands - primarily: titanium. In addition, process water discharges and release of mine depressurization will affect surface waters.

Key words: Environmental research; impact; sulphur; titanium

AVRAMENKO, A., B.A. MUNSON and R.T. SEIDNER 1980 Alberta oil sands environmental research program (AOSERP) toxicology and related projects. Can. Tech. Rep. Fish. Aquat. Sci. 975: 110.

Le Programme de recherches sur l'environnement des sables bitumineux de l'Alberta (AOSERP) en est actuellement à sa cinquième année. Le but principal de ce programme, entrepris selon un accord fédéral-provincial, est de fournir aux gestionnaires de l'environnement une banque d'information adéquate qui leur permettra d'assurer la qualité de l'environnement à mesure que se développe l'exploitation des sables bitumineux. Jusqu'à maintenant, la recherche sur l'environnement a porté surtout sur la définition des conditions de référence fondamentales (p. ex., une description des habitats et des relations interspécifiques, ainsi que la surveillance de la qualité de l'air et de l'eau). On a défini les répercussions industrielles actuelles et projeté les répercussions probables pour l'avenir. C'est ainsi par exemple qu'on estime à 300 t de soufre par jour les quantités qui seront déversées dans l'environnement, en plus des quantités de métaux présents dans les sables bitumineux, principalement le titanium. On prévoit en outre que le rejet des eaux usées et la libération des produits de décompression des mines affecteront les eaux de surface.

STANDARDIZATION OF AQUATIC BIOASSAYS : COMPROMISES BETWEEN BIOLOGICAL AND ECONOMICAL CRITERIA

G. Persoone

Laboratory for Biological Research in Aquatic Pollution,
State University of Ghent, J. Plateaustraat 22, B-9000 Ghent, Belgium

PERSOONE, G. 1980 Standardization of aquatic bioassays : Compromises between biological and economical criteria. Can. Tech. Rep. Fish. Aquat. Sci. 975: 111-122.

A variety of ecotoxicological methods have been worked out and are described in the scientific literature to experimentally predict the impact of chemical substances on aquatic terrestrial biota.

Only during recent years have serious efforts been made at both the national and international level to select a few bioassays which should be standardized for general applicability.

The choice for a standard bioassay, of the test species as well as of the criterion to assess the dose-effect relationship is a matter of the compromises which one is willing to make between the factors ecological representativeness and sensitivity on one hand, and manageability in the laboratory and cost of the test on the other hand.

This assertion is demonstrated for the aquatic environment through a proposal for standardization of a short-term toxicity test with Artemia nauplii; this bioassay has but a relatively low ecological representativeness and sensitivity, but on the other hand an inherent potential for universal application as an extremely cheap reference yard-stick test.

Key words: Bioassays; invertebrates; Crustacea; Artemia; intercalibration; ecotoxicology

PERSOONE, G. 1980 Standardization of aquatic bioassays : Compromises between biological and economical criteria. Can. Tech. Rep. Fish. Aquat. Sci. 975: 111-122.

La littérature scientifique contient une variété de méthodes écotoxicologiques permettant de prédire expérimentalement l'impact de substances chimiques sur les biocoenoses aquatiques et terrestres.

Ce n'est que récemment que de sérieux efforts ont été faits, tant au niveau national qu'international, afin de choisir quelques essais biologiques pouvant être normalisés pour application générale.

Le choix, dans un essai biologique standard, tant des espèces expérimentales que du critère servant à évaluer la relation dose-effet, est après tout matière à compromis. On doit considérer, d'une part, la représentativité écologique et la sensibilité des facteurs et, d'autre part, leur facilité de manipulation en laboratoire et le coût de l'essai.

Nous illustrons, dans l'environnement aquatique, par un projet de normalisation d'un test de toxicité à court terme avec des nauplii d'Artemia; cet essai biologique a une représentativité écologique et une sensibilité relativement faibles, mais peut, par contre, s'appliquer universellement comme essai de référence très peu dispendieux.

The endeavours to experimentally predict the impact of chemicals on biotic systems, has led to the elaboration of ecotoxicological laboratory tests, the variety and the complexity of which almost match the variety of groups of species existing in nature and the complexity of biological phenomena at the different levels of biotic organization.

In their recent attempt to ..."establish which tests should be used for assessing potential effects of chemicals in the environment and to advise the Step Sequence Group on the testing that should be carried out at the different levels of a tiered system" (Hueck 1979) the Chemicals Testing Programme Ecotoxicology Group of the Organization for Economic Cooperation and Development (OECD) first tried to gather the existing methodological knowledge on bio-assays by collecting the descriptions of "accepted" tests.

The result is an impressive synopsis containing the formats of 122 ecotoxicological tests, which according to the authors of the report "represent only a sample of possible methodologies".

In view of the regulatory measures which are now taken in some member countries of the O.E.C.D. and the European Economic Communities (E.E.C.) to reduce the adverse effects of chemicals on man and on the ecosystem, the most difficult step still remains, however, to determine from this synopsis which methods should be recommended for inclusion in the different levels of testing which are usually considered for the evaluation of the potential toxicity of chemicals ; namely the basic, the confirmatory and the definitive.

According to Hueck (1979) who supervised the activities of the specific OECD Working Group mentioned above "the choice of the test methods actually to be used, should be made on a few rather self-evident criteria :

1. representativeness of the test model
2. sensitivity
3. manageability in the laboraotry
4. cost of execution.

If it is obvious that these four points are indeed the crucial parameters on which a bio-assay should be based, one must, however, unfortunately notice that these factors "interact negatively" when it comes to practice!

We shall try to demonstrate hereunder the basic antagonism between the first two criteria and the latter two, with regard to the elaboration and the subsequent application of a particular test procedure.

Representativeness (including ecological function) and sensitivity constitute the essence of an "in vitro" assay since the results need to be extrapolated for predictive purposes to the environment.
Bio-assays must, from this point of view, be as much as possible a blue print of existing natural situations.

Manageability in the laboratory and cost of execution on the other hand are economical imperatives which determine the practical applicability of bio-assays at different scales.

We have tried to visualize in a chart (Fig. 1) the inter-relations and the interdependence of the factors mentioned above in the development of a particular test procedure (or the acceptance of an existing one).

This chart illustrates the antagonism between :

1) the cost factor (the economical choice) represented by the equipment needed, the duration of the test, the degree of expertise of the personnel and last but not least the recruitment of the test organisms needed and/or the maintenance of the stock ;

2) the biological imperatives representativeness and sensitivity (biological choice).

Looking into the history of ecotoxicological tests it appears that the first "bio-assays" were short-term tests carried out with the animals "at hand" and only considered the most simple criterion, namely the life-death dose-response relationship.

It was very soon objected that for predictive purposes the ecological representativeness of the test-species which were used was not considered satisfactory in many cases ; the tests had to be carried out with more "typical" organisms representative for the different categories of the trophic chain.

This of course, led to increased biological and technological difficulties in recruiting appropriate test-species and maintaining a stock in the laboratory, and as a consequence, to a sensible rise of the costs of the bio-assay.

At the same time the short-term life-death criterion was rejected because it immediately expresses the "maximum" biological damage which can be done to an organism.

More sensitive criteria were thus proposed and long-term experiments were worked out leading to a steadily increasing complexity of the test procedures and to an almost exponential increase of the costs.

When we consider the scientific literature on ecotoxicological testing todate we have the choice among a wealth of methods ranging from the most simple, short-term life-death test on species which can be cultured or recruited very easily, to the most sophisticated long-term microcosm bio-assay or multispecies accumulation test which can only be carried out in a few specialized centers in the world.

The basic principles outlined in our scheme are applicable for all these bio-assays : each increase in ecological representativeness and sensitivity automatically results in increased costs of the test (by virtue of more sophisticated equipment, a longer duration of the experiment, a need for more qualified personnel and last but not least more expenses for the recruitment of the test-species or their long-term maintenance in the laboratory).

It is to be hoped that at any advisory or decision making level, this intrinsic relationship "representativeness-sensitivity" versus "costs" will be clearly understood and taken into consideration in the final choice of particular ecotoxicological tests, not at least for the practical implementation at a broad level.

In this regard it looks quite obvious that the tests selected for the basic (range finding) levels should be much simpler and practical, which means cheaper, but as a result less representative and sensitive, than those withheld for the confirmatory and definitive levels.

Although not expressed in terms of economical repercussions the sequence of

1) simple short-term tests at the basic level, on a few functionally important types of test-species ;
2) bio-assays "of a more complicated nature" at the confirmatory level ;
3) tests at the definitive level with "multi-species systems"

is the approach advised by the OECD working group in its "Report on the assessment of potential environmental effects of chemicals".

It is the opinion of this author that at whatever level of testing, it will nevertheless always remain necessary to make compromises between biological imperatives and economical possibilities not the least when the factor standardization is taken into account.

Todate indeed contrary to human toxicology where tests are standardized to a very high degree through the use of rats and mice as universally accepted test-species, very little has been accomplished in the standardization of aquatic ecotoxicological tests.

The basic and obvious reason is the complexity of aquatic ecosystems and the difficulty of selecting even a few candidate species which are available or can be made available (and kept in culture) worldwide.

We shall not reopen here the discussion on this topic which has been the subject of countless hours of debate between bio-assay experts all over the world during the last years.

Regardless of all that has been said, it is nonetheless obvious that the use at the world-level of one and the same simple standardized aquatic bio-assay with the same species would be very welcome as a common "yardstick".

As we defined earlier, simplicity and practicality automatically mean low cost but also lower representativeness and sensitivity of the bio-assays.

Based on these premises there is, in the present state of our knowledge, one aquatic invertebrate which fulfills the prerequisites for a universal applicability of a simple and very inexpensive ecotoxicological test, namely the brine shrimp Artemia.

The unique advantage of this species is that it produces cysts, which are very small (0.3 mm), and which can be dried and stored for years without losing their hatchability.

Secondly the larvae can be hatched out very easily from the cysts, within 24 hours.

This means that bioassays can be started wherever and whenever required from biological material stored "on the shelf".

One of the most burdensome factors (from the biological, technological as well as the economical point of view) of any type of ecotoxicological test proposed so far, namely, the recruitment and the maintenance of the stock, is completely eliminated in the case of utilization of Artemia.

One could object here that Artemia is certainly not the only aquatic invertebrate known to produce cysts. There are indeed several groups of animals - protozoa, rotifers, cladocerans, ... to quote only a few - which are known to reproduce by cryptobiotic eggs or cysts when the environmental conditions become unfavourable.

When it comes, however, to commercial availability of the cysts, for application of the bio-assay at the world level, it appears that only brine shrimp cysts can be found on the market.

The reason for this is that Artemia cysts are used extensively in commercial aquaculture, because the nauplii can be hatched out very easily and constitute an excellent live food for larval fishes and crustaceans. The economic value of brine shrimp is now so well established that tons of cysts are harvested yearly from the saline biotopes where this particular animal thrives, for processing and sale to aquaculture hatcheries and aquarium hobbyists.

Since the quantity of cysts needed for one bioassay is extremely low (in comparison to those needed for aquaculture purposes) the costs of the biological material only make up a few cents, a price which will be hard to beat with whatever other species...!

Bioassays with Artemia have been proposed for many years by different authors.

We have endeavoured, at the Artemia Reference Center at the State University of Ghent in Belgium, to work out a standard procedure, based on the pros and the cons of the Artemia tests described in the literature, and completed by our own findings.

The results have been published in two papers: "Research on the development of a short term standard toxicity test with Artemia nauplii" (Vanhaecke et al. 1980a) and "Proposal for a short term standard toxicity test with Artemia larvae" (Vanhaecke et al. 1980b).

During the course of development of this particular test we endeavoured to standardize all parameters to a maximum, keeping constantly in mind, however, that the test procedure should be made as simple as possible, in order to be useful and practical even for laboratories with modest equipment and personnel.

To check the reproducibility of the dose-effect relationship and the conformity with the experimental procedure we decided to organize an intercalibration exercise among interested laboratories.

After a call for participation to which more than 100 answers were received, the exercise is now in progress in North-America under the joint supervision by the Artemia Reference Center at the State University of Ghent in Belgium and the Toxicology Section of the Freshwater Institute in Winnipeg, Canada.

A similar exercise will be started shortly in Europe, sponsored by the Commission of the European Economic Communities, and supervised by the Artemia Reference Center.

We would like to underline very strongly here that this test does not aim at replacing any other ecotoxicological test at whatever level.

The Artemia test on the contrary basically aims at providing a very simple and handy tool for the broad assessment of toxicity of chemicals, a first universal yard stick at the pre-basic level.

The sensitivity of the Artemia bio-assay, at least with the criterion life-death is not very high, (though it is not much inferior to that of short-term Daphnia test.) ; the reproducibility equals at least that of the range-finding tests with other species.

As far as the ecological representativeness is concerned it is clear that Artemia, like any other existing species, is by definition but a poor candidate for particular "local" situations.

In this regard we refer once again to our scheme to remind that the choices which we made in devising this test all started from economical considerations (to work out one of the most handy and certainly the cheapest standard bioassay procedure ever proposed); this choice of course had to pay its toll to ecological representativeness and sensitivity.

The possibilities and limitations of the Artemia test can be summarized as follows : due to its euryhaline character and a salinity tolerance ranging from brakish water (10 °/₀₀) to hypersaline water (280 °/₀₀), Artemia is an excellent test-species for effluent bio-assays in brackish and coastal waters.

On the other hand, Artemia is not suited basically for freshwater effluent testing since brine shrimp do not thrive in freshwater biota.

As said earlier, this test is most convenient and can be used very easily as a universally applicable common yard-stick for the first rough assessment of the toxicity of new chemicals.

To conclude it is clear that despite the wealth of information available we still have a very long way to go in the development

of the predictive tools for the "in vitro" assessment of the damage which our advanced chemical and industrial technology can bring to the natural environment.

In this regard more attention should be paid to the relationship between "biological"and"economical factors" since it is this relation which will determine in first instance the applicability of the bio-assays and thus their practical implementation at the national and international level.

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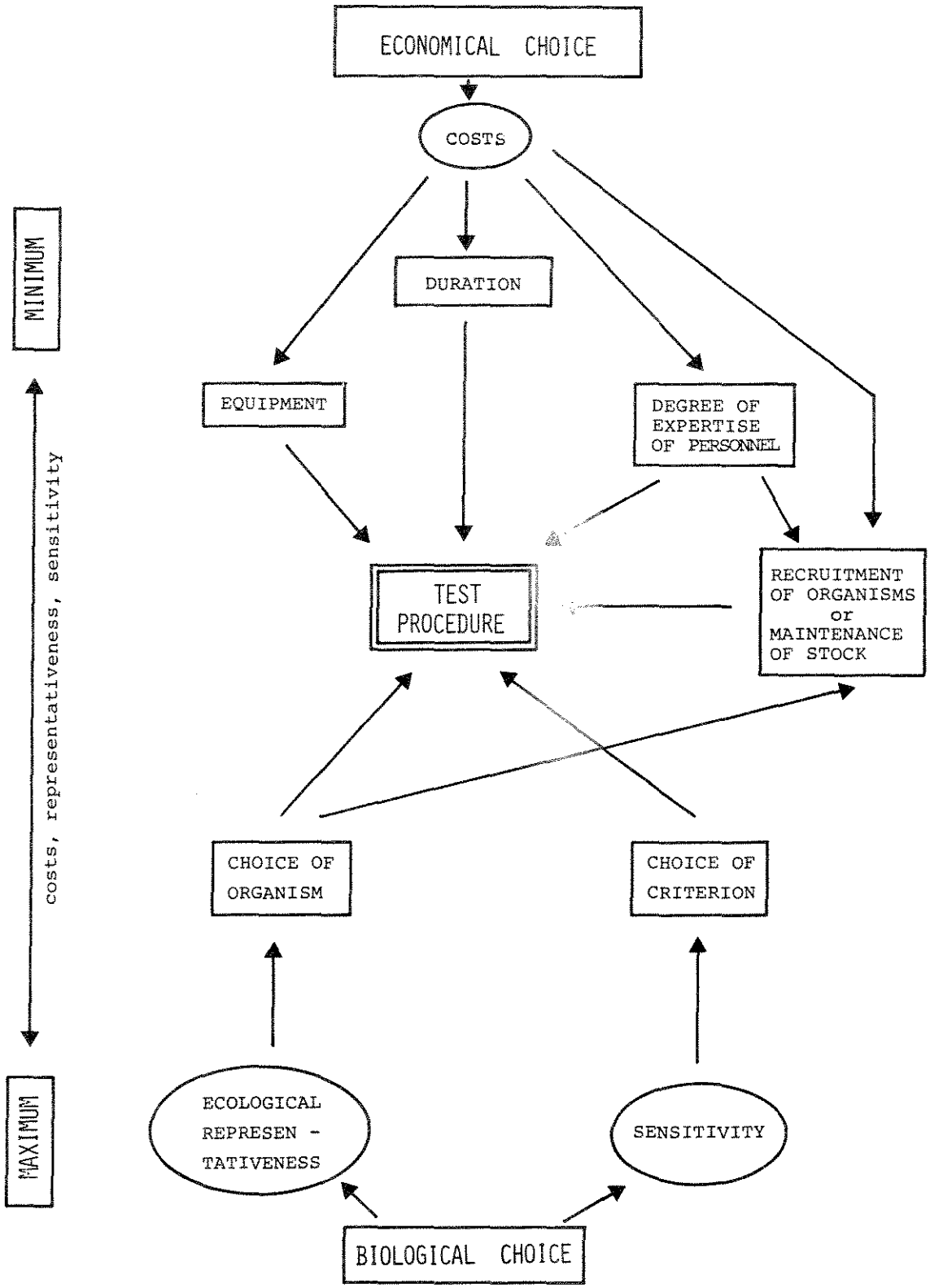

Figure 1. Interelationships of the basic factors determining the choice of bioassay test methods.

INTERACTIONS OF PESTICIDES AND SOLVENTS IN MICROBIAL SENSITIVITY TESTS

R.E. Burrell, G.W. Stratton and C.T. Corke

Department of Environmental Biology, University of Quelph,
Guelph, Ontario, N1G 2W1

BURRELL, R.E., G.W. STRATTON and C.T. CORKE 1980 Interactions of pesticides and solvents in microbial sensitivity tests. Can. Tech. Rep. Fish. Aquat. Sci. 975: 123-130.

In this study solvent-pesticide interactions were determined for two fungicides, benomyl and captan, and an insecticide, permethrin, with the solvent acetone. The solvent-fungicide interactions resulted in synergistic and antagonistic effects for each fungicide, respectively, when the test organism was the aquatic fungus Pythium ultimum. The solvent insecticide-interaction was additive when tested on the cyanobacterium Anabaena inaequalis.

It was concluded that solvent-pesticide interactions must be determined to obtain a true evaluation of toxicity.

Key words: Acetone; toxicity; interactions

BURRELL, R.E., G.W. STRATTON and C.T. CORKE 1980 Interactions of pesticides and solvents in microbial sensitivity tests. Can. Tech. Rep. Fish. Aquat. Sci. 975: 123-130.

L'étude décrite ci-dessous a servi à déterminer les interactions solvant-pesticide de deux fongicides, bénomyl et captan, et d'un insecticide, permethrine, avec l'acétone comme solvant. Les interactions solvant-fongicide ont des effets synergiques et antagonistes avec chaque fongicide respectivement, quand l'organisme expérimental est le champignon aquatique Pythium ultimum. L'interaction solvant-insecticide est cumulative quand elle est testée sur la cyanobactérie Anabaena inaequalis.

Nous concluons que, pour obtenir une évaluation réelle de la toxicité, il faut d'abord déterminer les interactions solvant-pesticide.

INTRODUCTION

Manten et al. (1950) reported that 2% (v/v) acetone (2-propanone) had no perceptible effect on the growth of various fungi tested. To the present, no one has questioned this observation. This has led to the general use of acetone in microbial assays when water-insoluble compounds are added to growth media. This solvent is usually incorporated into the medium to give a final concentration of 1% (Edgington et al. 1971).

Recently it has been shown that 1% acetone severely affects viral replication (Ghendon and Samoilova 1968; Chernos et al. 1972) and the ultrastructure of algal cells (Parasher et al. 1978). We have shown with soil fungi that a 1% concentration of acetone (v/v) can cause an inhibition of radial growth in excess of 60%, depending upon the species (unpublished results).

Theoretically, acetone can interact synergistically, antagonistically or additively with a pesticide in a microbial sensitivity assay. The interaction found will be dependent upon the concentration of acetone, the pesticide and the test organism.

In this paper interactions of the solvent acetone with the fungicides benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) and captan (N-(trichloromethyl)thio-4-cyclohexene-1,2-dicarboximide) on the aquatic fungus Pythium ultimum, were examined. The solvent interactions with the insecticide permethrin (3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate) on the cyanobacterium Anabaena inaequalis, were also studied. A rapid screening procedure to ascertain the type of response due to solvent-pesticide combinations is outlined together with necessary methodology to minimize these interactions.

MATERIALS AND METHODS

CULTURES

A culture of Pythium ultimum was supplied by the Department of Environmental Biology, University of Guelph, and maintained on potato dextrose agar (Difco). The culture of Anabaena inaequalis is fully described elsewhere (Stratton and Corke 1979).

FUNGAL ASSAY

Acetone was added to 100 mL volumes of potato dextrose agar (PDA) to give concentrations of 0 (control), 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 and 1.5 percent (v/v). These were shaken for two minutes on a rotary shaker and referred to as solvent controls. Two additional series consisting of acetone (as above) plus one concentration of selected fungicide were prepared. The fungicides and concentrations used were benomyl, 50 ppm (µg/mL), and captan, 1 ppm. The agar was dispensed as 10 mL aliquots into Petri plates, allowed to solidify and then inoculated with an 8 mm mycelial disc, taken from the outer edge of a six day old culture, of the aquatic fungus P. ultimum. The plates were placed in plastic boxes (27 cm x 19 cm x 10 cm) and incubated at 30° C in the dark. After 30 hours of incubation the diameters of all treatments and controls were measured. The effect of acetone

on P. ultimum was determined by comparing the radial growth on acetone-treated agar to that on untreated agar. The solvent plus fungicide effect was similarly determined. A net fungicide effect was determined by comparing the radial growth on the solvent plus fungicide treated agar to the growth on the corresponding solvent controls. All determinations were then plotted as percent inhibition versus the logarithm of solvent concentration.

ALGAL ASSAY

The test criterion in the algal assay was nitrogenase activity as determined by the acetylene reduction technique (Hardy et al. 1973). Ten mL volumes, of a 7 day algal culture of A. inaequalis (1.2×10^7 cells), were transferred to tissue culture flasks which had an internal volume of 74 mL and a surface area of 25 cm^2 (Stratton and Corke 1979). Acetone was added to one series of flasks to give the following solvent concentrations; 0 (control), 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 percent (v/v). A second series was made with acetone (as above) and 100 ppm permethrin. The flasks were sealed with serum stoppers and a 10% volume of air was removed and replaced with acetylene. Ethylene production was measured after 5 hours of incubation and assayed by gas chromatography (Stratton and Corke 1979). Ethylene peaks were identified by retention time and quantitated by comparing peak heights to a standard curve.

STATISTICS

Significant differences between controls and treatments were determined using Dunnett's t-test, as outlined by Winer (1971).

RESULTS AND DISCUSSION

If, as Manten et al. (1950) have suggested, there is no effect from acetone, or if the effect is strictly additive, then the measured toxicity of a pesticide will not change through the spectrum of solvent concentrations employed. However, if there is an increase or decrease in the toxicity with the combination, then a synergism or antagonism has occurred. Theoretically, those responses which can be obtained are summarized in Figure 1.

When acetone was used at concentrations greater than 0.4% (v/v) with the fungicide benomyl and the fungus P. ultimum a synergistic response was noted (Fig. 2). This synergistic response occurred at solvent levels far below those used in standard fungicide assays by other researchers (Edgington et al. 1971). At 1.0% acetone (v/v) the net fungicide effect was a 58% inhibition. This effect was 40% higher than the calculated toxicity at solvent concentrations less than 0.4% (v/v). This type of interaction can lead to erroneous estimations of toxicity when standard assay methods are used.

This same solvent, when used with the same organism (P. ultimum), and the fungicide captan caused an antagonistic response (Fig. 3). The response was additive from 0.1 to 0.8% acetone (v/v) but at higher concentrations the combination was significantly antagonistic. The net fungicide effect at 1.0% acetone was a 4.5% inhibition while at concentrations below 0.8% the inhibition observed was 32%. This 27.5% difference was indicative of antagonism. This was further substantiated by the observation that growth on the experimentals treated with 1.5% acetone and 1 ppm captan was greater

than that on the solvent controls. Such interactions can lead to extreme underestimations of fungicide toxicities and the rejection of potentially good compounds from screening trials.

Over the spectrum of solvent concentrations used with A. inaequalis, the calculated toxicity of 100 ppm permethrin did not change significantly (Fig. 4) denoting an additive response. Therefore, the response obtained at any of the acetone concentrations used would be an accurate indication of permethrin's inherent toxicity.

When the response is additive the effect of the solvent can be corrected with an appropriate solvent control. If the response is antagonistic or synergistic then a correction is difficult, if not impossible. It is for this reason that interactions must be determined for each solvent-pesticide combination used with each test organism. A simplified screening procedure and the methodology needed to determine and minimize these interactions is given in Figure 5.

SUMMARY

Solvent-pesticide interactions are varied and unpredictable, and this makes the determination of interactions occurring in each test system important. Once the interactions have been assessed, a solvent concentration can be selected which will minimize undesirable effects. Such testing should prevent the rejection of good compounds, which appear poor, due to antagonism. It will also prevent the field testing of compounds that are in fact poor toxicants unless they are synergized with a solvent.

ACKNOWLEDGEMENTS

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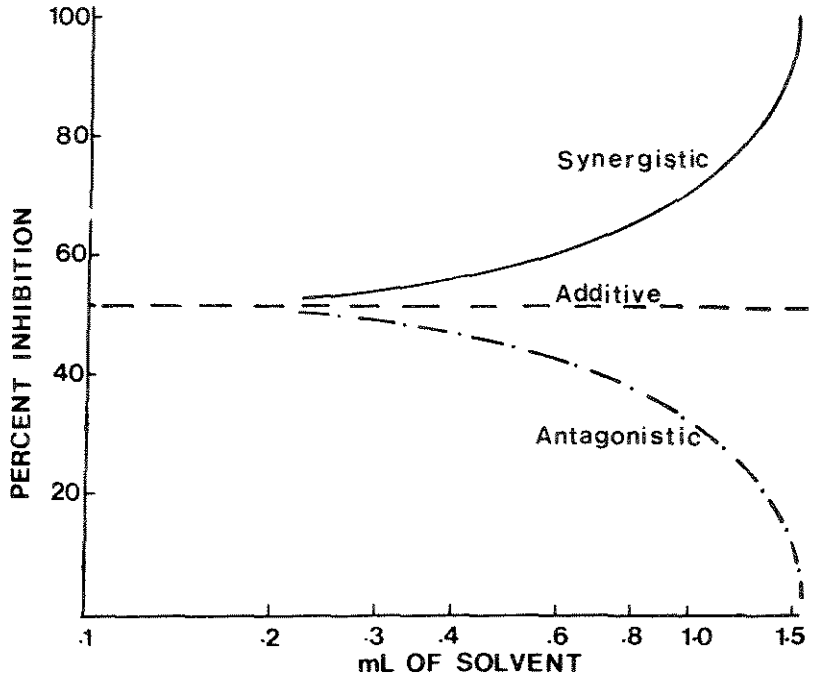


Fig. 1. Theoretical pesticide toxicity data representing one concentration of a pesticide used in combination with various concentrations of a solvent.

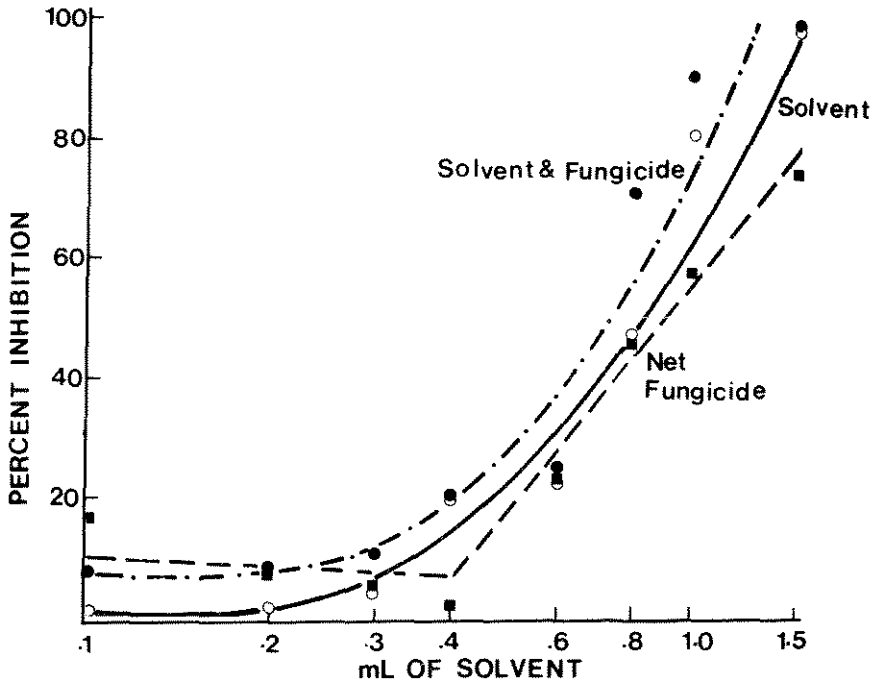


Fig. 2. The synergistic response obtained by combining various concentrations of acetone with 1 concentration of benomyl (50 ppm) and P. ultimum.

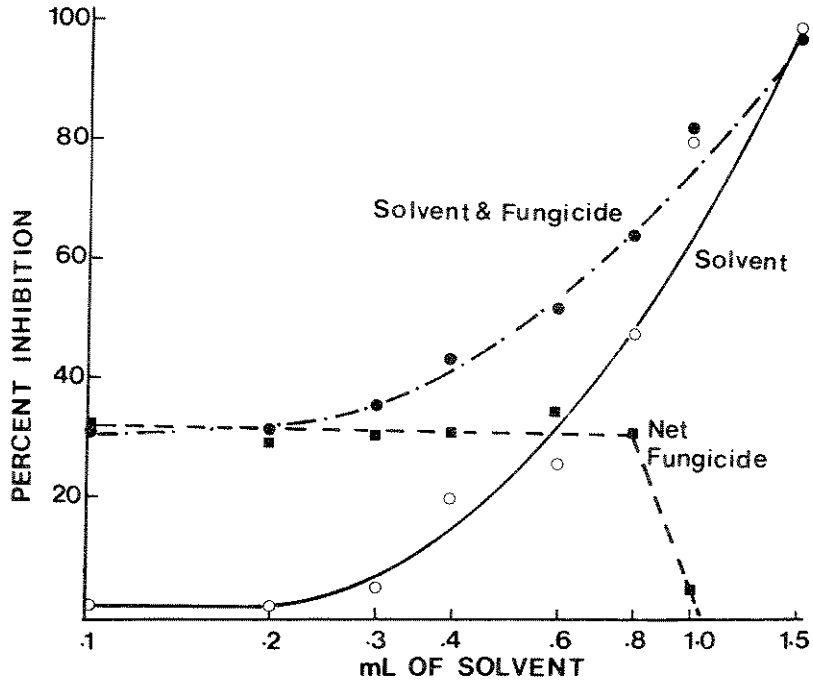


Fig. 3. The antagonistic response obtained by using various concentrations of acetone in combination with 1 concentration of captan (1 ppm) and P. ultimum.

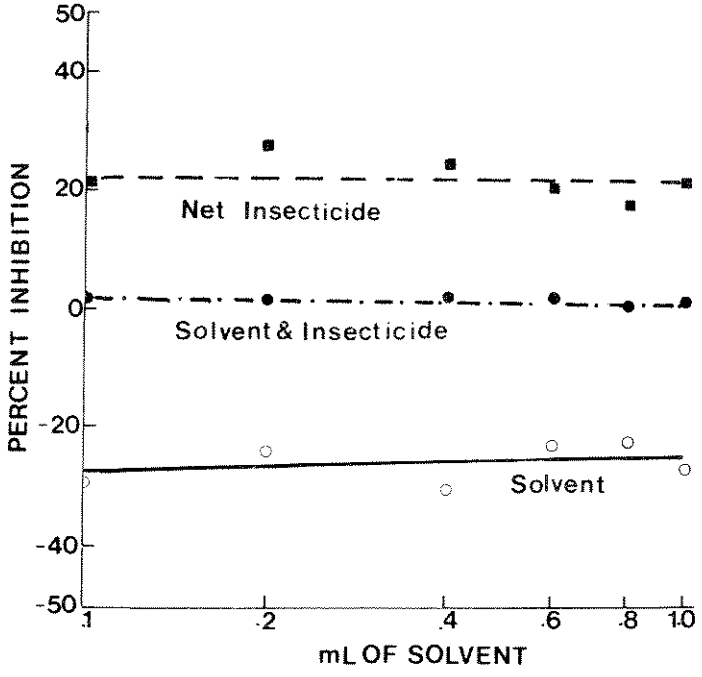
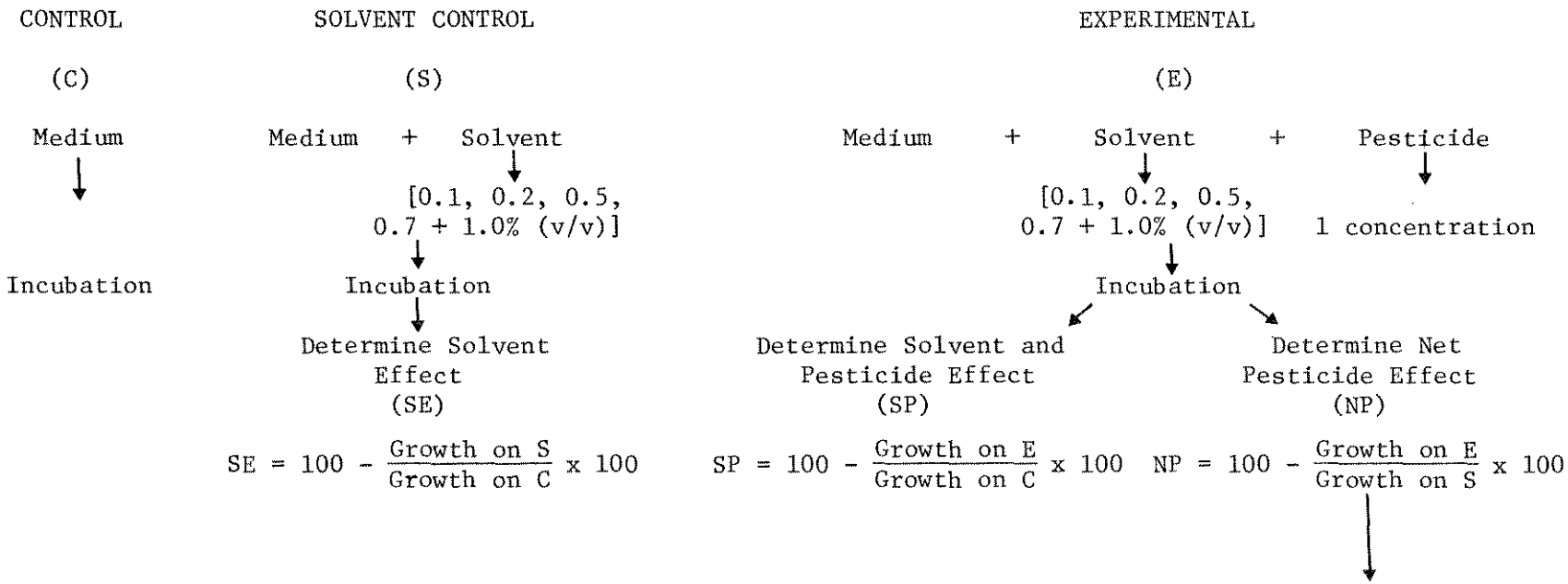


Fig. 4. The additive response obtained by using various concentrations of acetone in combination with 100 ppm permethrin and A. inaequalis.

Fig. 5. A rapid screening technique for determination of solvent pesticide interactions.



* Additive effect can be verified by the Gowing Equation (Gowing, 1960)

$$\text{Expected Additive} = A + \left(\frac{100 - A}{100} \times B\right)$$

$$\text{In this case } SP = NP + \left(\frac{100 - NP}{100} \times SE\right)$$

If both sides of equation are equal, then the effect is additive.

THE EFFECT OF PESTICIDES AND THEIR METABOLITES, ALONE AND IN COMBINATION, ON ALGAL PROCESSES

G.W. Stratton, A.L. Huber and C.T. Corke

Department of Environmental Biology, University of Guelph, Guelph, Ontario, N1G 2W1

STRATTON, G.W., A.L. HUBER and C.T. CORKE 1980 The effect of pesticides and their metabolites, alone and in combination, on algal processes. Can. Tech. Rep. Fish. Aquat. Sci. 975: 131-139.

The effects of the insecticide permethrin, and the herbicides atrazine and diuron, on the blue-green alga Anabaena inaequalis are considered. Decomposition of permethrin results in the formation of metabolites which are from 3-50 times more toxic than permethrin towards photosynthesis and acetylene reduction. Permethrin is more toxic than its metabolites to algal growth. Both synergism and antagonism were observed against algal growth when permethrin was combined with some of its metabolites. Diuron and atrazine are 2-20 and 5-100 times more toxic, respectively, than their metabolites towards growth, photosynthesis, and acetylene reduction.

Key words: Permethrin; atrazine; diuron; pyrethroid

STRATTON, G.W., A.L. HUBER and C.T. CORKE 1980 The effect of pesticides and their metabolites, alone and in combination, on algal processes. Can. Tech. Rep. Fish. Aquat. Sci. 975: 131-139.

Nous examinons les effets, sur l'algue bleu-vert Anabaena inaequalis, de l'insecticide permethrine et des herbicides atrazine et diuron. En se décomposant, la permethrine forme des métabolites de 3 à 50 fois plux toxiques que la permethrine elle-même quant à la photosynthèse et à la réduction de l'acétylène. La permethrine est plus toxique que ses métabolites vis-à-vis de la croissance algale. Nous avons observé à la fois du synergisme et de l'antagonisme dans la croissance des algues quand la permethrine est combinée avec certains de ses métabolites. Le diuron et l'atrazine sont de 2 à 20 et 5 à 100 fois plus toxique, respectivement, que leurs métabolites quant à la croissance, la photosynthèse et la réduction de l'acétylène.

INTRODUCTION

As the useage of pesticides increases, so does the concern over the effects of these compounds on non-target processes. Algae are an important group of non-target organisms as the hydrosphere is the ultimate reservoir for many environmental toxicants (Livingston 1977). Algae form part of the primary producer trophic level in aquatic ecosystems and, in the case of blue-green algae, may also play a significant role in the nitrogen cycle.

Research regarding the effects of pesticides on algae has been extensively reviewed by Butler (1977). Most data concern the toxicity of parent compounds and available data on the interaction of pesticide metabolites with algae deal mostly with DDT and the metabolite DDE, which are similar in toxicity to marine and freshwater algae (Bowes and Gee 1971; Mosser et al. 1974; Powers et al. 1979). Propanil is more toxic to blue-green algae than the metabolite 3,4-dichloroaniline (Wright et al. 1977), while the degradative products of aldrin, dieldrin, and endrin are similar in toxicity to the parent compounds (Batterton et al. 1971).

A pesticide and its degradation products can be present in any given ecosystem at the same time, therefore the toxicity of combinations of these compounds should be evaluated, since synergistic and antagonistic interactions may occur. DDT and DDE, at concentrations of 0.5 ppm, have little effect on algal growth when used alone or in combination (Mosser et al. 1974). Information on other compounds are absent.

This communication considers the toxicity of the pyrethroid insecticide permethrin, the symmetrical triazine herbicide atrazine, and the substituted urea herbicide diuron, and some of their metabolites, on photosynthesis, nitrogen fixation and growth of the blue-green alga Anabaena inaequalis. As well, the toxicity of combinations of permethrin and its metabolites are evaluated.

MATERIALS AND METHODS

CULTURE

Anabaena inaequalis is fully described elsewhere (Stratton and Corke 1979) and was maintained in a liquid, nitrogen-free medium (Stratton and Corke 1979). All experimental systems were incubated at a temperature of 22°C and a light intensity of 7000 lux.

TEST CHEMICALS

The chemicals used in this study are described in Table 1. Diuron and its metabolites were added to all experimental flasks as aqueous solutions, while permethrin, atrazine, and their metabolites were added as acetone solutions, which were 0.1% acetone (v/v) for growth studies and 1.0% for photosynthesis and acetylene reduction experiments. Acetone controls were included in all appropriate experiments.

GROWTH

Algal growth was assessed by following the change in absorbance at 600 nm, with time, on a Bausch and Lomb Spectronic 20 spectrophotometer (Stratton and Corke 1979). Sidearm flasks of 500 mL capacity, containing 95 mL of medium,

and 1 mL of test compound(s), were inoculated with 5 mL of a 7 day algal culture to yield an initial cell concentration of approximately 6.5×10^4 cells per mL. For interaction studies, the compounds were added in the order in which they appear in the degradation pathway (refer to Table 1).

PHOTOSYNTHESIS

Photosynthesis was studied by following the uptake of $^{14}CO_2$ from $NaH^{14}CO_3$ (Stratton and Corke 1979). Tissue culture flasks (74 mL internal volume) were used in replicates of 5 for the permethrin and atrazine series of compounds. Each contained 9.8 mL of cells, 0.1 mL of radioisotope and 0.1 mL of test compound(s) to give an initial concentration of about 6.5×10^4 cells and 0.1 µCi of radioactivity per mL. Test tubes were used in replicates of 5 for the diuron series of compounds and each contained 5 mL of cells, an equal volume of test solution, and 0.1 mL of radioisotope, to give an initial concentration of approximately 1×10^6 cells and 0.2 µCi of activity per mL. The cells were incubated for 2 h and harvested by filtration through 0.45 µm membrane filters. The amount of radioactivity incorporated into the cells was determined using a liquid scintillation counter (Stratton and Corke 1979). Counts were corrected for counting efficiency and those obtained for dark-incubated cells were subtracted from values obtained for corresponding light-incubated systems. Percent inhibition was plotted against the logarithm of test compound concentration and EC_{50} values determined.

NITROGEN FIXATION

Nitrogenase activity was assayed using the acetylene reduction technique. Tissue culture flasks were used in replicates of 5 for the permethrin and atrazine series of test compounds. Each contained 9.9 mL of cells and 0.1 mL of the test compound, to give an initial cell concentration of about 6.5×10^4 cells per mL. Glass serum vials (73 mL total volume) were used for experiments involving the diuron series of chemicals and contained 5 mL of cells and an equal volume of test solution to give an initial concentration of approximately 1×10^6 cells per mL. After the addition of a 10% atmosphere of acetylene, the cells were incubated for 5 h and the ethylene produced was assayed by gas chromatography (Stratton and Corke 1979). Data were expressed as nmoles ethylene produced per 10^5 cells and percent inhibition was plotted against the logarithm of test chemical concentration and EC_{50} values determined.

STATISTICS

Throughout this communication the term significance refers to conclusions made following a Dunnett's test for comparison of means at $\alpha = 0.05$ (Winer 1971).

RESULTS AND DISCUSSION

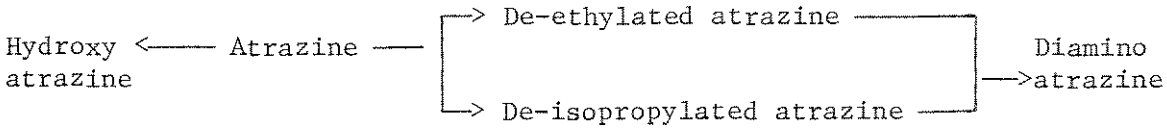
Data on the effect of diuron and its metabolites on Anabaena inaequalis are outlined in Table 2. The EC_{50} of diuron towards growth, photosynthesis

and acetylene reduction was 0.02, 0.04, and 1.5 ppm (μg/mL), respectively. These data are similar to those recorded for other algae. The EC_{50} of diuron for the growth and photosynthesis of various green algae varies from 0.01-0.17 ppm (Walsh 1972; Virmani et al. 1975). Growth of the blue-green alga Anabaena variabilis is significantly inhibited by 0.01 ppm diuron (Goulding et al. 1978) while photosynthesis by Anabaena is completely inhibited at concentrations ranging from 2.3-7.0 ppm (Stewart and Pearson 1970).

Diuron follows a linear degradation pathway (Dalton et al. 1966) as follows: Diuron ——> DCMMU ——> DCU ——> 3,4 DCA. For all three assay criteria, the metabolites of diuron were significantly less toxic than the parent compound. The difference in toxicity ranged from 2-20 times, depending on the particular compound involved. The one exception was DCMMU which did not differ significantly from diuron in its toxicity towards growth and photosynthesis. The order of toxicity for photosynthesis and acetylene reduction was diuron $\geq$ DCMMU > DCU > 3,4 DCA. For growth, the order of DCU and 3,4 DCA in the toxicity series was reversed. Diuron degradation is an example of environmental pesticide detoxication or inactivation where the metabolites show reduced toxicity towards the test organism.

The effects of atrazine and its metabolites are presented in Table 3. The EC_{50} of atrazine towards growth yield, growth rate, photosynthesis, and acetylene reduction was 0.03, 0.10, 0.28 and 55 ppm, respectively. The EC_{50} for the growth of green algae ranges from 0.06-0.16 ppm atrazine (Walsh 1972). Concentrations of 1.0 ppm cause almost complete growth inhibition in Chlorella and Scenedesmus (Gramlich and Frans 1964; Virmani et al. 1975). However, levels of 2-20 ppm are needed for the inhibition of photosynthesis in both green and blue-green algae (Rohwer and Fluckiger 1979). Greater than 90 ppm is needed for complete inhibition of acetylene reduction in lichens (Kallio and Wilkinson 1977), while the EC_{50} for this process in Anabaena cylindrica is only 0.22 ppm (Rohwer and Fluckiger 1979).

Atrazine follows a branched degradation pathway (Kaufman and Kearney 1970) as follows:



Atrazine was significantly more toxic than any of its metabolites for all assay criteria. The difference in toxicity ranged from 5-100 times, depending upon the metabolite involved. This is another example of pesticide detoxication, or inactivation. The toxicity series was atrazine > de-ethylated atrazine > de-isopropylated atrazine > diamino atrazine and hydroxy atrazine. Although de-ethylated and de-isopropylated atrazine share the same place in the degradation pathway, the former was always more toxic than the latter. The presence of the isopropyl group apparently contributes to phyto-toxicity.

The effects of permethrin and some of its metabolites are outlined in Table 4. The EC_{50} of permethrin towards growth yield, growth rate, photosynthesis and acetylene reduction was 1.6, 5.0, >100 and >100 ppm, respectively. No other data have been published on the effects of permethrin towards algae.

The metabolites of permethrin studied form a linear degradation pathway (Holmstead et al. 1978) as follows: Permethrin ——> PBAlc ——> PBAld ——> PBAc. For growth, permethrin and PBAlc did not differ significantly in their toxicity. For this assay criterion the metabolites PBAld and PBAc were sig-

nificantly less toxic than the parent compound. However, for photosynthesis and acetylene reduction the metabolites were 3-50 times more toxic than permethrin. The toxicity series was PBAld > PBAc > PBAlc > permethrin. This is an example of the environmental activation of pesticides.

Permethrin and the three phenoxy-based metabolites were interacted and these combinations assayed for their toxicity towards algal growth. Data were analyzed for synergism and antagonism using the Gowing equation (Gowing 1960). All combinations containing three or four compounds induced synergistic interactions. Results for combinations of two chemicals are presented in Table 5. All combinations containing PBAc gave an antagonistic response, while all others induced synergism. The levels of PBAc used in these studies were too low to cause a pH change and more detailed studies are required to evaluate the mechanisms involved in these interactions. However, the fact that pesticide metabolites can interact to induce synergistic and antagonistic responses is of great environmental significance, especially when we have examples of environmental pesticide activation.

Based upon the data presented here, future studies on the environmental toxicity of pesticides should be concerned with the toxicity of known metabolites and the effects of combinations of these metabolites with parent compounds. Researchers should also be concerned with the effects of combinations of various unrelated pesticides and/or their metabolites.

ACKNOWLEDGEMENTS

Research funds for this study were provided by the Ontario Pesticide Advisory Board and the National Research Council of Canada. The authors wish to acknowledge the technical assistance provided by M.L. Kurp.

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Table 1. A description of the compounds used in this study.

Chemical Name	Designation	Conversion Factor
	Diuron Series	
3-(3,4-dichlorophenyl)-1,1-dimethyl urea	Diuron[a]	4.3[e]
3-(3,4-dichlorophenyl)-1-methyl urea	DCMMU[a]	4.6
3,4-dichlorophenylurea	DCU[a]	4.9
3,4-dichloroaniline	3,4 DCA[a]	6.2
	Atrazine Series	
2-chloro-4-ethylamino-6-isopropylamino-s-triazine	Atrazine[b]	4.6
2-chloro-4-amino-6-isopropylamino-s-triazine	De-ethylated Atr[b]	5.3
2-chloro-4-ethylamino-6-amino-s-triazine	De-isopropylated Atr[b]	5.8
2-chloro-4,6-diamino-s-triazine	Diamino Atr[b]	6.9
2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine	Hydroxy Atr[b]	5.1
	Permethrin Series	
3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate	Permethrin[c]	2.6
3-phenoxybenzyl alcohol	PBAlc[d]	5.0
3-phenoxybenzaldehyde	PBAld[d]	5.1
3-phenoxybenzoic acid	PBAc[d]	4.7

[a] obtained from E.I. Dupont de Nemours & Co., Wilmington, Delaware, U.S.A.
[b] obtained from Ciba-Geigy Canada Ltd., Cambridge, Ontario, Canada
[c] obtained from Chipman Chemicals Ltd., Stoney Creek, Ontario, Canada
[d] purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.
[e] The EC_{50} values in this paper are quoted as ppm; to convert ppm into μmolar values, multiply by this conversion factor.

Table 2. The effect of Diuron and some of its metabolites on A. inaequalis[a].

Compound	Growth Yield	$^{14}CO_2$ Uptake	Acetylene Reduction
Diuron	0.02	0.04	1.5
DCMMU	0.04	0.07	3.0
DCU	>1.0	50	-
3,4 DCA	0.15	>100	35

[a]Table values are mean EC_{50} in ppm (µg/mL).

Table 3. The effect of Atrazine and some of its metabolites on A. inaequalis[a].

Compound	Growth Yield	Growth Rate	$^{14}CO_2$ Uptake	Acetylene Reduction
Atrazine	0.03 ± 0.01	0.1	0.28 ± 0.07	55 ± 25
De-ethylated Atr	1.0 ± 0.2	4.0	2.5 ± 0.7	>100
De-isopropylated Atr	2.5 ± 0.5	7.0	9.0 ± 1.2	>100
Diamino Atr	>10	>10	>100	>100
Hydroxy Atr	>10	>10	>100	>100

[a]Table values are mean EC_{50} ± standard deviation in ppm (µg/mL).

Table 4. The effect of Permethrin and some of its metabolites on A. inaequalis[a].

Compound	Growth Yield	Growth Rate	$^{14}CO_2$ Uptake	Acetylene Reduction
Permethrin	1.6 ± 0.8	5.0	>100	>100
PBAlc	2.5 ± 0.5	3.5	30 ± 3	48 ± 6
PBAld	55 ± 25	>50	2.0 ± 0.5	12 ± 5
PBAc	25 ± 5	>50	20 ± 2	35 ± 7.5

[a]Table values are mean EC_{50} ± standard deviation in ppm (μg/mL).

Table 5. The effect of combinations of Permethrin and its metabolites on the growth of A. inaequalis[a].

	PBAc	PBAld	PBAlc	PER
PER	ANT	SYN	SYN	-
PBAlc	ANT	SYN	-	SYN
PBAld	ANT	-	SYN	SYN
PBAc	-	ANT	ANT	ANT

[a]A-antagonistic, S-synergistic. The concentrations used in this experiment were those inducing a 50% reduction in growth, as outlined in Table 4.

BIOACTIVITY AND DEGRADATION OF PERMETHRIN IN ARTIFICIAL POOLS

G.P. Rawn[1], G.R.B. Webster[1], and D.C.G. Muir[2]

[1] Pesticide Research Laboratory, Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2

[2] Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, R3T 2N6

RAWN, G.P., G.R.B. WEBSTER and D.C.G. MUIR 1980 Bioactivity and degradation of permethrin in artificial pools. Can. Tech. Rep. Fish. Aquat. Sci. 975: 140-149.

Outdoor artificial pools (15 m^2 x 0.37 m deep) were treated with permethrin emulsifiable concentrate containing ^{14}C-permethrin at 0.028 kg a.i./ha (15 ppb). Water, hydrosoil, and fish (fathead minnows) samples collected over a period of 28 days following treatment were monitored for residues of permethrin and degradation products by GLC, TLC-autoradiography and liquid scintillation counting. Determination of permethrin bioactivity in the water, using 4th instar Aedes aegypti larvae, showed 100% mortality immediately and for 12 hours post-treatment; only 24% mortality was found after 24 hours and none was observed at 72 hours. Radiotracer data indicated a rapid loss of permethrin in the water which corroborated the bioassay results. A photolysis study indicated that photochemical loss of permethrin in pond water was rapid. Considerable photoisomerization of the cis- and trans-isomers of permethrin was observed. The cis-isomer disappeared more slowly than the trans-isomer.

Key words: Bioassays; permethrin; water; hydrosoil; fish; mosquitos; Aedes aegypti; radioactivity; photolysis

RAWN, G.P., G.R.B. WEBSTER and D.C.G. MUIR 1980 Bioactivity and degradation of permethrin in artificial pools. Can. Tech. Rep. Fish. Aquat. Sci. 975: 140-149.

Les auteurs ont traité des bassins artificiels extérieurs (15 m^2 x 0.37 m de profond) avec un concentré émulsifiable de permethrine contenant ^{14}C-permethrine à 0.028 kg a.i./ha (15 µg/L). Ils ont recueilli des échantillons d'eau, d'hydrosol et de poissons (têtes-de-boule) sur une période de 28 jours après le traitement. Les résidus de permethrine et les produits de dégradation ont été mesurés par chromatographie en phase gazeuse, chromatographie en couche mince-autoradiographie et par comptage à scintillation liquide. La bioactivité de la permethrine dans l'eau, déterminée sur des larves d'Aedes aegypti au 4^e stade, démontre qu'il y a mortalité de 100% immédiatement et durant 12 h après le traitement. Après 24 h, on n'a constaté que 24% de mortalité et aucune après 72 h. D'après le radiotraceur, il y a perte rapide de permethrine dans l'eau, ce qui confirme les résultats d'essais biologiques. Une étude de photolyse indique que la perte photochimique de la permethrine dans l'eau des étangs est rapide. Nous avons observé une forte photoisomérisation des isomères cis et trans de la permethrine. L'isomère cis disparaît plus lentement que l'isomère trans.

INTRODUCTION

The synthetic pyrethroid insecticides, a promising new family of insect control products, are of very low toxicity to mammals, birds and plants, but are of very high toxicity to insects and fish. Several of the recently developed synthetic pyrethroids, permethrin, cypermethrin, fenvalerate, and decamethrin, provide superior control of insect pests compared to the older organophosphorus, carbamate, and chlorinated hydrocarbon insecticides. In addition, while they are contact insecticides and stomach poisons, their action as nerve poisons does not involve interaction with acetylcholinesterase as do the organophosphates and carbamates (Elliott et al. 1978). All pyrethroids are lipophilic, and in this respect, resemble the chlorinated hydrocarbons; however, pyrethroids are readily metabolized by mammals and birds and readily photodegraded.

Wide use of permethrin (NRDC 143, FMC 33297, WL 43479, PP 550, Ambush, Ectiban) for insect pest control in Canadian agriculture and forestry is anticipated within the next few years; experimental results to date indicate that permethrin effectively controls many agricultural and forestry pests and is an effective mosquito larvicide.

Permethrin is known to degrade readily in soil, largely through the action of microorganisms, with a "half-life" of 0.5-6 weeks (ICI 1977, Williams and Brown 1979). Identified degradation products include 3-phenoxybenzyl alcohol, 3-phenoxy benzoic acid, and cis- and trans-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylic acids, all of which eventually degrade further. Cis-trans photoisomerism of permethrin occurs most readily with the ultimate products of photolysis being similar to those formed in soil; monodechlorinated species are also formed (Holmstead et al. 1978).

Analysis of residues of permethrin itself may be carried out using gas liquid chromatography (GLC) (Williams 1976; Chapman and Simmons 1977; Fujie and Fullmer 1978). Quantitation of degradation products, which are somewhat polar, can be carried out using radiotracer techniques with ^{14}C-labelling at specific locations in the molecule, in this case, thin layer chromatography (TLC) followed by autoradiography and liquid scintillation counting (LSC) (Shono et al. 1979; Glickman et al. 1979).

To date, no detailed quantitative appraisal of the bioavailability and residue levels of permethrin in treated aquatic systems has been reported.

OBJECTIVES

Since permethrin is toxic to many aquatic organisms, there are several questions to be answered regarding the compatibility of insecticidal uses of permethrin with maintenance of aquatic environmental quality:

1. What is the bioavailability of permethrin when it is used as a mosquito larvicide in artificial pools? How long will efficacious levels last?
2. What is the rate of loss of permethrin in treated pond water by photochemical processes?
3. To what degree is permethrin lost through degradation and adsorption in treated pond water? What residue levels of permethrin and degradation

products will be encountered in water, hydrosoil, and fish?
4. What are the bioaccumulation rates of permethrin and degradation products in fish?

METHODS

Artificial pools (5 x 3 m) have been maintained for four years at the Glenlea Research Station south of Winnipeg. The pools were excavated, lined with polyethylene, and covered with sand and 15 cm of sod; they were maintained for the present study at a depth of 38 cm using water from a nearby dugout. Natural flora and fauna were allowed to develop in and around the pools and have been kept under control by periodic cutting and pond maintenance.

Two pools were treated with permethrin emulsifiable concentrate at the rate of 0.028 kg a.i./ha or 15 µg/L. The formulated material (*cis*-*trans* ratio 40:60) was spiked with 69 or 88 µCi of (^{14}C)-permethrin labelled either in the methylene or cyclopropyl group. Pool 7 was treated with methylene labelled permethrin and pool 3 with cyclopropyl labelled permethrin. A third pool served as an untreated control.

Fathead minnows were added to each pool at 72 h post-treatment.

Samples of pond water and hydrosoil were taken one day pre-treatment and 2, 4, 8, 12, 24, 36, 48, 72, 96, 168, 264, 336, 504, and 696 h post-treatment. Samples of fathead minnows were also taken at 96, 264, 336, 504, and 696 h.

1. PHOTODEGRADATION

Two sets of nine 1000 ml erlenmeyer flasks were filled with 800 ml of pond water. To each flask in one set, 9.11 µg of *cis*-permethrin in 1.0 ml acetone was added to give a concentration of 11.4 µg/L. One flask served as a darkened control. To each flask in the other set was added 8.10 µg of *trans*-permethrin yielding a concentration of 10.1 µg/L. Immediately following treatment, the flasks were partially submerged in an outdoor artificial pool so that the bottom 5-8 cm of each flask was immersed in water. At 0, 1, 6, 12, 24, 48, 96, and 144 h (light) and 144 h (dark) post-treatment, one flask from each set was removed and the water extracted using methylene chloride. At 144 h, the contents of both the light and darkened flasks were analyzed for residues of *cis*- and *trans*-permethrin.

GLC analysis of the *cis*- and *trans*-permethrin were performed using a Tracor 220 gas chromatograph fitted with a ^{63}Ni electron capture detector. A 1.2 m Pyrex, 4 mm i.d. column was packed with 100-120 mesh Chromosorb W-HP coated with 5% OV 210. Temperatures (^{o}C): injector, 235; detector, 350; column, 220; flow rate: 5% methane in argon carrier, 40 ml/min; retention time: *cis*/*trans*, 4.0 min/4.6 min, respectively.

2. BIOAVAILABILITY

At each sampling time, a 100 ml aliquot of pond water was taken from the bulk sample (see 3) and a 24 h bioassay conducted using 25 lab-reared fourth instar larvae of *Aedes aegypti*. At 72 h post-treatment, when the fathead minnows were added, residues of permethrin had diminished to levels

no longer sufficient for mosquito larval mortality. Fish are known to concentrate residues of lipophilic compounds from water, and as such provide a further measure of bioavailability of permethrin. At each sampling time, three fish were taken per pool and analyzed for uptake of permethrin residues.

3. RESIDUE LEVELS, RATE OF LOSS OF PERMETHRIN AND DEGRADATION PRODUCTS

(a) Water

Pond water samples were collected by compositing ten 100 ml random dip samples from each pool. The 900 ml remaining following the bioassay (see 2) was preserved and subsequently extracted with methylene chloride. An aliquot of the extract was removed and combined with PCS (Amersham) and xylene (Caledon) for total ^{14}C-analysis by a Beckman LS-7500 liquid scintillation counter. The samples were counted for 20 min and quench corrections were made using an internal channels ratio method. The remaining extract was divided into three and analyzed by TLC, autoradiography, and LSC. The three solvent systems used for the TLC were hexane/ether (10/1), cyclohexane saturated with formic acid/ether (3/2), and chloroform/ethyl acetate/methanol (6/3/1).

(b) Hydrosoil

Four days prior to treatment, 48-455 ml wide-mouth jars were filled with ambient soil and placed on the bottom of each pool. At each sampling time, four jars per pool were removed to provide samples of hydrosoil for residue analysis. From each set of four jars, the top 0.5 cm of materials was taken and combined. A subsample of this hydrosoil was removed for oxidation and LSC for total ^{14}C levels.

Hydrosoil samples (0.5 g) and 0.3 ml Combustaid (Packard Instruments) were combusted for 1.5 min on a Packard Model 306 oxidizer and $^{14}CO_2$ was collected in CO_2-M-Met (Amersham) and PCS (Amersham). Counting procedures followed those outlined in 3(a).

(c) Fish

Fathead minnows were captured at each sample period using a minnow trap and analyzed using oxidation and LSC (see 3(a) and (b)).

(d) Metabolites

Analysis of extracts of water and hydrosoil samples by TLC enabled the separation and quantitation of cis- and trans-permethrin and major metabolites. The solvent systems used for TLC and silica gel plates have already been described by Shono et al. 1979 and Glickman et al. 1979; autoradiography and LSC were used to locate and quantitate residues.

Samples of technical permethrin, ^{14}C-cyclopropyl permethrin, ^{14}C-methylene permethrin, EC formulated permethrin and analytical standards of degradation products were kindly supplied by ICI Plant Products Division, Jealott's Hill Research Station, Bracknell RG12 6EY England.

RESULTS AND DISCUSSION

1. PHOTODEGRADATION

(a) Trans-permethrin

Trans-permethrin degraded rapidly in pond water held in Pyrex glass flasks during the first 24 h and generated an approximate first order plot over the first 6 days (Figure 1). Isomerization of the trans-isomer to the cis-isomer occurred rapidly during the first 6 h in the illuminated flask; no isomerization was observed in the darkened flask. Degradation of the cis-isomer followed an approximate first order plot from day 1 to day 6. The trans-isomer degraded somewhat more rapidly than the cis-isomer, and at the end of 6 days (144 h), 0.04 µg/L (ppb) trans-isomer and 0.08 µg/L cis-isomer remained in the illuminated flask; 2.7 µg/L trans-isomer only remained in the darkened flask.

(b) Cis-permethrin

Cis-permethrin also degraded rapidly in pond water and behaved in much the same way as the trans-isomer had done (Figure 2). Isomerization of the cis-isomer to the trans-isomer occurred rapidly during the first 6 h in the exposed flask; no isomerization was observed in the dark flask. Again, the trans-isomer degraded more rapidly than the cis-isomer, and at the end of 6 days (144 h) 0.15 µg/L cis-isomer and 0.04 µg/L trans-isomer remained; 4.2 µg/L cis-isomer only remained in the dark flask.

The loss of 55-70% of permethrin in the darkened flask indicates that photodegradation is probably not the most important loss mechanism. However, since the cis-isomer has been reported to be more toxic than the trans-isomer (Bigley and Plapp 1978), photodegradation may affect permethrin bioactivity in water due to photo-isomerization.

2. BIOAVAILABILITY

Bioassay results showed 100% mortality of mosquito larvae immediately and for 12 h post-treatment. The bioavailable toxic residue decreased very quickly yielding 4% mortality at 48 h and 0% at 72 h (Figure 3).

3. RESIDUE LEVELS

(a) Water

Residue levels in the pond water were determined in two ways, viz., TLC-autoradiography-LSC, and total ^{14}C residues in the water. Results from ponds 3 and 7 derived from TLC separation, autoradiography, and LSC quantitation corroborated the bioavailability results. The initial concentration of 15.5 µg/L decreased very quickly to 1.5 µg/L by 18 h and 0.04 µg/L at 72 h post-treatment (Figure 4).

Total ^{14}C residues in the water provided further information. Rapid loss of permethrin during the first day paralleled the TLC-LSC results. At approximately 2 days, however, the results show a marked variation: the pool

treated with cyclopropyl labelled permethrin showed a levelling off of detectable ^{14}C residues, and was still above 1 µg/L at 28 days post-treatment, whereas, the pool treated with methylene labelled permethrin showed a steady decline in residual ^{14}C over the same 28 days (Figure 5). The expected degradation pathway was the hydrolysis of the permethrin molecule to yield "cyclopropyl" products and "methylene" products (Figure 7). Since the ^{14}C is associated with these two distinct parts of the molecule, TLC-autoradiography analysis was able to follow each half of the molecule. Results in each case indicated the generation of several degradation products which are as yet not fully identified. Qualitative observation of the radiograms showed the concentration of the cyclopropyl degradation products in the water to be considerably higher than the methylene degradation products. These results indicate a different fate for the two halves of the permethrin molecule.

(b) Hydrosoil

During the first 2 days, the ^{14}C residues in hydrosoil were similar for both ponds. As in the case of the ^{14}C residues in pond water, there is a marked difference in ^{14}C residue levels in the hydrosoil in the two ponds beyond the first 2 days (Figure 6). The pond treated with cyclopropyl labelled permethrin (pool 3) shows a peaking of ^{14}C residue at 3-4 days with a concentration equivalent to 160 µg permethrin/kg dry weight soil, whereas, pond 7, treated with methylene labelled permethrin, peaks at 4 days at a concentration equivalent to 330 µg permethrin/kg dry weight soil. These results are thus complementary to those gathered for pond water and show that the more polar acid metabolites tend to remain in the water whereas the less polar alcohol metabolites tend to associate with the hydrosoil. Anomalous points in Figure 6 are thought to be due to excessive quantities of detritus which collected on top of the soil substrate in these sample jars. The 0.5 cm sample taken for analysis from these jars would have been less dense, thus inflating the concentration values for the residues reported as a µg residue/kg dry weight soil basis.

(c) Fish

Within 24 h of the introduction of the fathead minnows to ponds 3 and 7, considerable uptake of ^{14}C labelled compound had occurred (Table 1). In close agreement with the hydrosoil/water results, the cyclopropyl labelled material was taken up by the minnows to a lesser extent than the methylene labelled material. In general, this appears to be consistent with the polarity of the respective metabolites; i.e., the cyclopropyl products, being more polar than the methylene labelled products are not taken up as efficiently by the fish.

Table 1. Uptake of ^{14}C label (ppb ≡ µg permethrin equivalent/kg wet weight fish) by fathead minnows in ponds treated with ^{14}C-cyclopropyl or ^{14}C-methylene labelled permethrin.

Time (days)	^{14}C-cyclopropyl		^{14}C-methylene	
	ppb	concentration factor	ppb	concentration factor
3	.....Fish added.....			
4	326	151	97	174
11	57	24	107	486
14	93	35	125	499
21	176	73	158	1433
29	231	125		

CONCLUSION

Permethrin is an effective larvicide for Aedes aegypti (mosquitoes) for a period of 12 h post-treatment when pond water is treated at 15 ppb (µg/L). Permethrin is quickly degraded in an aquatic system and is reduced to less than 1% of applied concentration by 5-6 days post-treatment. Photochemical processes increase the degradation rate of permethrin. Other loss mechanisms also take place, but do not lead to cis-trans-isomerization of permethrin.

Degradation of permethrin in the aquatic system was rapid and led to residues of the cyclopropyl metabolites largely in the pond water and residues of the methylene labelled degradation products largely in the hydrosoil and fathead minnows. TLC-autoradiography revealed the presence of several degradation products which have not yet been identified.

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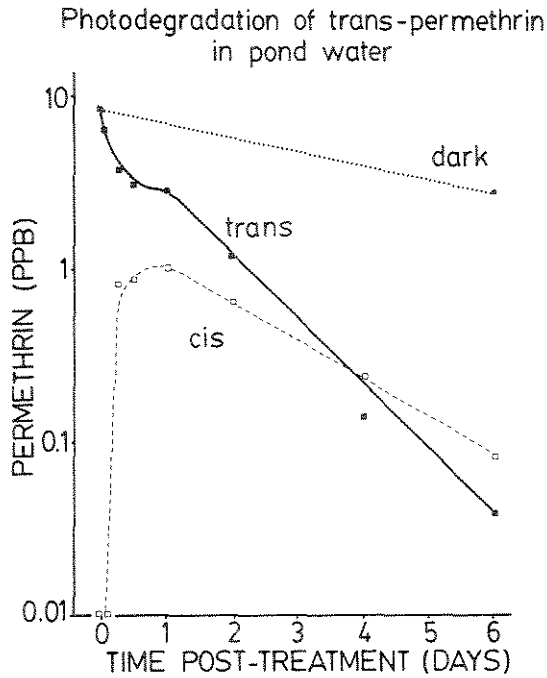
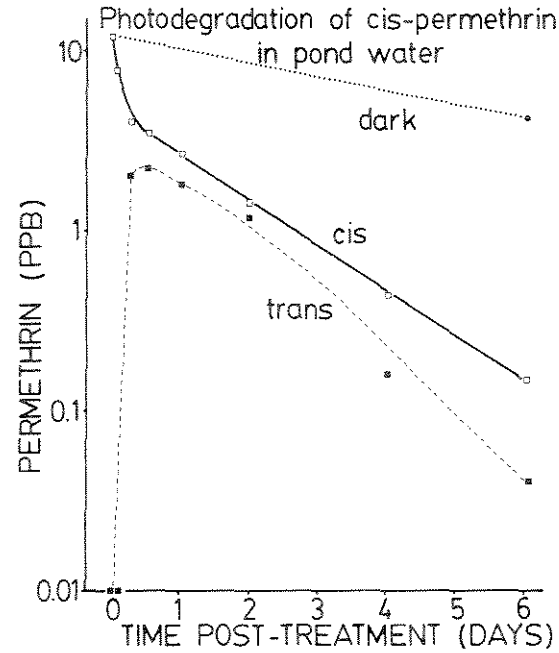
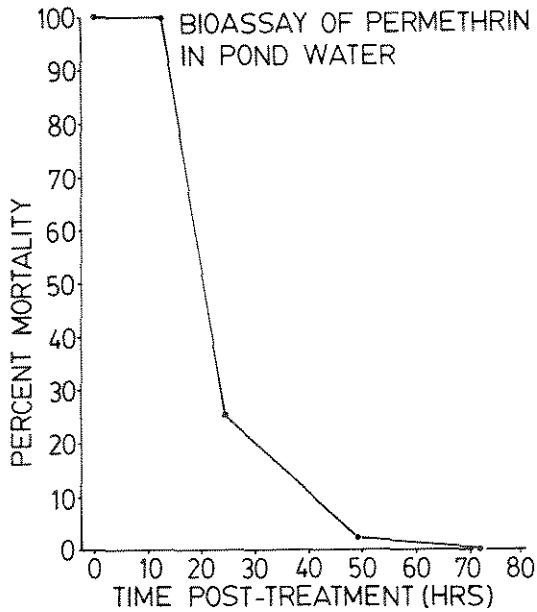
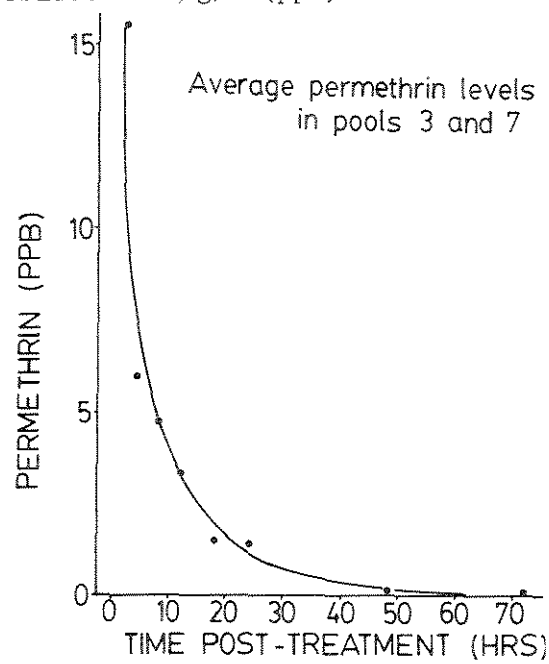




Figures 1 and 2. Photodegradation of trans- and cis-permethrin in pond water. Permethrin residues in µg/L (ppb).





Figures 3 and 4. Bioassay (4th instar larvae Aedes aegypti) and chemical determination of permethrin in pond water. Permethrin in µg/L (ppb).

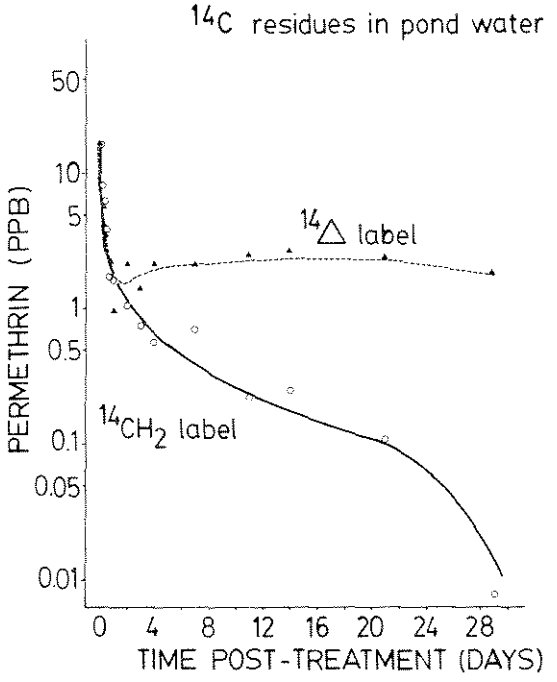


Figure 5. ^{14}C labelled residues in pond water expressed as µg permethrin equivalents/L (ppb).

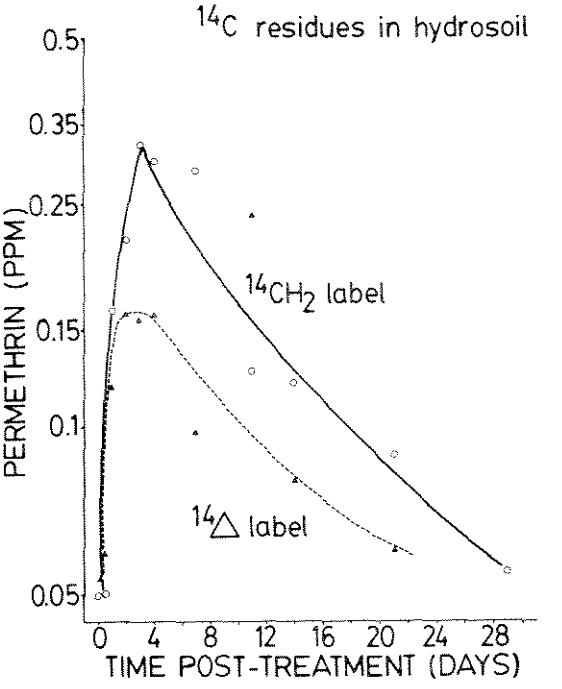


Figure 6. ^{14}C labelled residues in hydrosoil expressed as mg permethrin equivalents/kg dry weight soil (ppm).

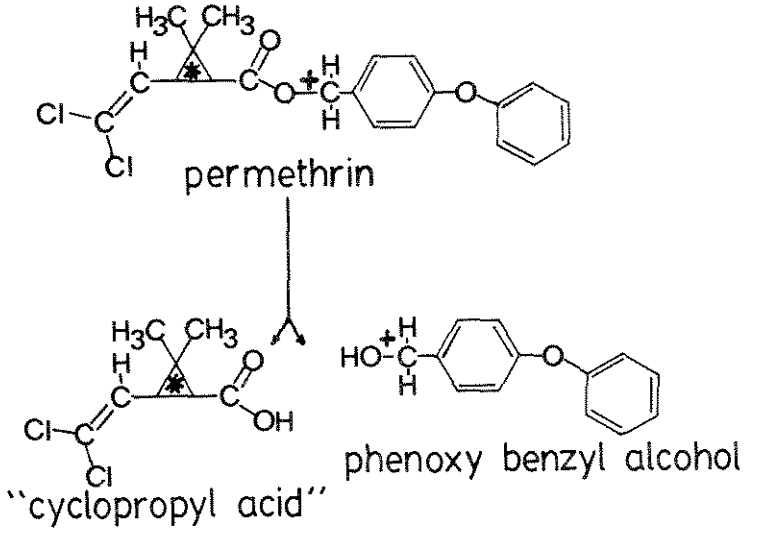


Figure 7. ^{14}C-permethrin labelling: cyclopropyl ring (*)(pool 3) or methylene bridge (+)(pool 7). Hydrolytic cleavage would yield products of the sort indicated with ^{14}C labelling in products derived from one side of the permethrin molecule only.

INFLUENCE OF DIET ON FISH TOXICOLOGY STUDIES

J.W. Hilton

Great Lakes Biolimnology Laboratory, Canada Centre
for Inland Waters, Burlington, Ontario, L7R 4A6

HILTON, J.W. 1980 Influence of diet on fish toxicology studies. Can. Tech. Rep. Fish. Aquat. Sci. 975: 150-151.

Although fish toxicology tests have been standardized, little attention is given to the possible interaction between diet and experimental results. The principal diet characteristics which could cause this interaction are: (1) undesirable levels of environmental contaminants such as heavy metals and pesticides, and (2) poor diet quality, i.e. levels of protein, lipid, vitamins, minerals and carbohydrate. These problems originate with the use of low quality feedstuffs or poor processing and storage. Such problems may affect experimental results by:

(1) altering the toxicity of the test material
(2) causing an unexpectedly high mortality which could invalidate the experiment
(3) causing a high experimental variability that masks treatment effects (false negatives)
(4) inducing spurious responses not characteristic of the test material (false positives).

A standardized test diet would therefore seem appropriate but may be impractical at this time due to lack of availability, difficulty in processing and storage, expense, less than optimum growth response and some potential environmental contamination. Commercial diets are more practical and can be used if their composition, nutrient content and contaminants levels are verified. The same batch of fish feed should be used for the entire experiment and should also be stored in a freezer until required for feeding.

Key words: Diets; fish; toxicology

HILTON, J.W. 1980 Influence of diet on fish toxicology studies. Can. Tech. Rep. Fish. Aquat. Sci. 975: 150-151.

Bien que les essais toxicologiques pour les poissons aient été normalisés, on prête peu d'attention à l'interaction possible entre le régime alimentaire et les résultats expérimentaux. Les principales caractéristiques du régime pouvant causer une interaction sont: (1) des niveaux indésirables de contaminants environnementaux, tels que les métaux lourds et les pesticides, et (2) les régimes alimentaires de qualité inférieure, i.e. sous le rapport de la teneur en protéines, lipides, vitamines, minéraux et hydrates de carbone. Des aliments de mauvaise qualité ou mal transformés et entreposés sont à la source de ces problèmes. Ces derniers peuvent influer sur les résultats par:

(1) altération de la toxicité du matériel d'essai
(2) mortalité exceptionnellement élevée de nature à fausser l'expérience
(3) forte variabilité expérimentale masquant les effets du traitement (fausses valeurs négatives)

(4) induction de réactions anormales qui ne sont pas caractéristiques du matériel d'essai (fausses valeurs positives).

Il serait donc bon d'adopter un régime normalisé pour les essais. Il se peut toutefois que cela soit impossible pour le moment, pour les raisons suivantes : non-disponibilité, difficulté de transformation et d'entreposage, coût, réaction de croissance inférieure à l'optimum et, enfin, contamination possible de l'environnement. Les nourritures commerciales sont plus pratiques et peuvent être utilisées si leur composition, teneur en substances nutritives et niveaux de contaminants sont vérifiés. On devrait utiliser le même lot de nourriture pour toute la durée d'une expérience et cette nourriture devrait être conservée dans un réfrigérateur jusqu'au moment de s'en servir.

THE USE OF *IN SITU* PREFERENCE/AVOIDANCE STUDIES WITH FISH IN MONITORING SULFITE MILL EFFLUENT

E.R. McGreer and G.A. Vigers

E.V.S. Consultants Ltd., 195 Pemberton Avenue,
North Vancouver, B.C., V7P 2R4

McGREER, E.R. and G.A. VIGERS 1980 The use of *in situ* preference/avoidance studies with fish in monitoring sulfite mill effluent. Can. Tech. Rep. Fish. Aquat. Sci. 975: 152-161.

In situ preference/avoidance tests with fish were used to determine the zone of influence of sulfite mill effluent (SME) in Neroutsos Inlet, B.C. Fish avoidance behavior and mortalities were greatest at the mill site and decreased with distance from the outfall. Both avoidance effects and elevated mortality were found at sites up to 10 km seawards of the mill, but the zone of influence varied and was dependent upon the state of the tide. Response times for fish were less than 0.5 h, and juvenile chum salmon (*Oncorhynchus keta*) were found to be more suitable than stickleback (*Gasterosteus aculeatus*) as a test species. Water quality parameters of pH and dissolved oxygen were found to be the most significant ($p < 0.05$) in explaining the vertical distribution of fish.

Key words: Fish behaviour; sublethal effects; pulp mill effluent; impact assessment

McGREER, E.R. and G.A. VIGERS 1980 The use of *in situ* preference/avoidance studies with fish in monitoring sulfite mill effluent. Can. Tech. Rep. Fish. Aquat. Sci. 975: 152-161.

Des essais de préférence/évitement *in situ* avec des poissons ont servi à délimiter la zone d'influence de l'effluent d'un moulin de bisulfite (SME) dans le Neroutsos Inlet (C.-B.). L'évitement par les poissons et leur mortalité sont au maximum au site du moulin et diminuent en fonction de la distance de l'exutoire. On a constaté que l'évitement et la mortalité accrus se faisaient sentir jusqu'à 10 km en aval du moulin. Par contre, la zone d'influence varie et dépend de l'état de la marée. Les temps de réaction des poissons sont inférieurs à 0.5 h, et l'on constate que les jeunes saumons keta (*Oncorhynchus keta*) conviennent mieux que les épinoches à trois épines (*Gasterosteus aculeatus*) comme espèces d'essai. La distribution verticale des poissons semble dépendre davantage des paramètres de qualité de l'eau, pH et oxygène dissous ($p < 0.05$).

INTRODUCTION

Studies on the effects of sulfite mill effluent on fish avoidance behavior in Neroutsos Inlet (Fig. 1) were initiated in 1978 by Rayonier Canada (B. C.) Ltd., Port Alice, B. C. The studies were designed to assess the improvements in environmental conditions that occurred with the installation and start-up of a spent-sulfite liquor recovery system at the Port Alice mill. For 1979, research efforts were directed primarily at assessing the zones of influence of the sulfite mill effluent with respect to fish preference/avoidance behavior. Emphasis was placed on testing reproducibility of the results, and statistical analysis to relate avoidance behavior to water quality parameters. Variables related to test procedure (optimum time of test duration and use of different fish species) were also examined. The avoidance studies were carried out concurrently with investigations by other agencies and included physical and chemical oceanographic data collection, salmonid downstream migration and recruitments studies, and shoreline surveys of vegetation and epifauna.

METHODOLOGY

ACQUISITION, MAINTENANCE AND TRANSPORT OF FISH STOCKS

Juvenile chum salmon (*Oncorhynchus keta*) were obtained from the Qualicum River fish hatchery, Parksville, B. C., and transported to the marine laboratory of E.V.S. Consultants Ltd., North Vancouver. Fish were acclimated to salt water ($25 \pm 2^{\circ}/_{oo}$) over a one-week period. Oregon Moist Pellets and frozen euphasids, in equal proportion, were fed to the fish daily. Average wet weight of juveniles was 0.6 g. Stickleback (*Gasterosteus aculeatus*) were collected from False Creek Inlet, Vancouver, B. C., and transported to the marine laboratory of E.V.S. Consultants Ltd. Fish maintenance was as described for juvenile chum.

The required numbers of chum and stickleback for *in situ* experiments were air shipped to field personnel via Port Hardy, B. C. Approximately 250 fish were placed in 5 L of seawater in double polyethylene bags placed inside styrofoam coolers. The bags were inflated with pure oxygen and sealed. A temperature of approximately 12°C was maintained by freezer packs placed in the containers. Fish were held at the study site in a 1400 L holding/transport tank supplied by the Environmental Protection Service of Environment Canada. Water in the tank was filtered continuously through a Biotech II glasswool filter. Fish were fed daily and mortalities were less than 1% per day.

EXPERIMENTAL PROCEDURES

The preference/avoidance traps and test methods used were those described by Birtwell (1977). The traps consist of six 1 m compartments within an aluminum framework which is suspended in the water column from a surface flotation unit. Doors between each unit may be opened allowing fish access to all compartments. Closing of the doors at test completion traps the fish in each compartment, allowing assessment of their vertical distribution within the top 6 m of the water column. Initial time series tests were carried out to assess the response time and preference/avoidance behavior of chum and

stickleback near the point of mill discharge and at a control site, Site 7 (Fig. 1). Doors in separate traps were closed at intervals of 0.5 h up to 4.0 h. Four replicate traps were used at each of 7 sites (Fig. 1) to determine the zone of influence of sulfite mill effluent. Water quality parameters (temperature, conductivity, dissolved oxygen and pH) were measured at 0.5 m and successive 1.0 m depth intervals using a Hydrolab Surveyor water quality analyzer (Model 6D).

RESULTS AND DISCUSSION

TIME SERIES TESTS

Results of the time series tests are summarized in Figures 2 and 3, and Table 1. Chum fry at the control site (Fig. 2) showed a distinct preference for the surface (0-1 m) waters at all time intervals. Preference for surface waters was not shown as consistently by the stickleback. Relatively large numbers (25-70%) of stickleback were found at depths of 2 to 4 m, particularly at times longer than 2.0 h. The distribution of fish for the time series experiments carried out at the mill site (Site 3; Fig. 1) was very different from that of the control (Fig. 3). The test on May 9, with chum and stickleback, showed a more equal distribution with depth for both species at all time intervals (Fig. 3). Preference for the surface was observed (e.g. 2.0 and 4.0 h) but the largest numbers of fish avoided the 0-1 m depth. In contrast, a second test on May 31 at the mill site showed chum had a subsurface distribution (Table 1) with the majority (65-85%) of fish at the 2.0-3.0 m depth at all times sampled. The different conditions at the mill site on the two occasions was also apparent in the mortalities observed. Up to 53.3% of the fish died during the test on May 31 (Table 20), whereas no mortalities were recorded on May 9.

The above observations on fish distribution were confirmed statistically using Duncan's Multiple Range Test (Table 1). The population mean for subset 1 (depth 0-1 m) was shown to be significantly different from other subsets at the control site and for chum at the mill site on May 9. At the mill site on May 31 subset 3 (depth 2-3 m) was shown to contain significantly more fish than the other depths.

The time series tests were carried out to assess the response times of fish with respect to their avoidance behavior. No significant changes in fish distribution were observed for the time intervals tested. It was apparent from all tests conducted that the response time was 0.5 h or less. This conclusion is consistent with observations from similar experiments using the same apparatus (Birtwell, pers. comm.).

The control test demonstrated the strong preference of chum salmon fry for surface (0-1 m) waters in unpolluted conditions. This response is the working hypothesis upon which these *in situ* preference/avoidance tests have been based. A strong surface preference has been shown for juvenile chinook (Birtwell 1977) and coho salmon (Vigers et al. 1978), but the response has not been as consistent in tests using juvenile Pacific herring (Birtwell 1977; Vigers et al. 1978) or stickleback (present study). Thus, juvenile salmonids (*Oncorhynchus* sp.) seem to be the best species tested to date for use in the *in situ* preference/avoidance experiments.

The marked difference in avoidance response and mortality observed on the two occasions at the mill site suggests that fish were exposed to increasing concentrations of sulfite mill effluent. Effluent from Port Alice has been shown in previous field studies to vary considerably in toxicity and the degree of mixing after discharge (Vigers et al. 1978).

PREFERENCE/AVOIDANCE TESTS TO DETERMINE ZONE OF INFLUENCE OF SULFITE MILL EFFLUENT (SME)

A summary of fish distribution data is shown in Figures 4 and 5. Statistical analyses of fish and water quality data are given in Tables 1 and 2. Two test runs are distinguished; Run #1 (June 1-6) and Run #2 (June 14-18, 1979).

In Run #1, avoidance behaviour was shown in the vertical distribution of chum fry at Sites 1 to 6 (Fig. 4). Fish were completely absent from the surface waters at Sites 2 and 3. Chum at Site 7 (the control) showed the normal preference for surface waters. A very different pattern was apparent in Run #2 (Fig. 5) as avoidance behavior was limited to Sites 1 to 4. Fish were present at the surface (0-1 m) at all sites in Run #2. These differences observed between the results of the two test runs were confirmed statistically (Table 1). Distinctly different subsets for depth were identified between the two runs using Duncan's Multiple Range Test. The largest numbers of fish were found at depths of 1, 3, and 4 m [identified as subset (1,3,4)] followed by depths 2, 5 m in Run #1. In Run #2, the surface 1 m depth had significantly greater numbers of fish than all other depths combined - a more normal distribution pattern. Analysis of variance between the two runs by site and depth for chum (not shown) found a significant ($p < 0.05$) difference in the number of fish in the surface (0-1 m) water at all sites except the control.

The relationship between water quality and avoidance behavior is shown in Table 2. Only those independent variables which made a significant ($p < 0.05$) contribution to the regression equation between numbers of chum and water quality parameters are listed. The relative importance of each independent variable in the regression equation is given by the value for the normal coefficient. In Run #1, at 1 m depth, pH was the only significant variable in the equation where it accounted for 59% of the total variance (r^2). The pH was also identified to be an important variable in explaining the distribution of chum at the 2, 4, 5 and 6 m depths. Distance (from the outfall) was the most significant variable at the 3 m depth. A different relationship between water quality and fish distribution was exhibited in Run #2. Dissolved oxygen, percent saturation of dissolved oxygen, distance and salinity were identified as significant variables. The lack of any significant variable for 3 m suggests that this is a "threshold" with fish being distributed above or below this depth.

The use of *in situ* preference/avoidance bioassays with juvenile salmonids has been shown to be a practical and sensitive methodology for monitoring the effects of sulfite mill effluent in the marine environment. Reproducibility of the test was good as evident from the 95% confidence limits of the data (Figs. 4, 5).

The differences in avoidance and water quality relationships observed between Runs #1 and #2 can be largely explained by tidal conditions at the

time of testing. In Run #1, testing was carried out during ebbing and low slack tides and avoidance behavior was observed up to 10 km from the mill (Site 6). Testing for Run #2 was carried out on a flooding tide and no avoidance was observed at this site. Effluent is dispersed down Neroutsos Inlet on the ebbing tide and subsequently will affect sites seawards of the mill. Conversely, on a flooding tide, effluent will be "concentrated" around the outfall area.

The relationship between water quality and avoidance behavior was shown to be complex with different variables being important on different occasions. During Run #1, when the strongest avoidance of surface water was observed (Fig. 4), pH was identified as the single most significant ($p < 0.05$) variable explaining 59% of the variance (Table 2). These data indicate that some component of the effluent was causing avoidance of surface waters by the fish. Generally, in Run #1, pH was identified as the most common variable in explaining fish distribution. However, stronger relationships between fish avoidance and water quality have been found when an effluent tracer is used (Birtwell, pers. comm.). Color works well with kraft mill effluents, but until recently there have been problems in finding a suitable tracer for sulfite waste liquor in seawater. A new fluorimetric method has been developed in Sweden (Almgren et al. 1975; Josefsson and Nyquist 1976) which may be useful in this regard.

A weaker avoidance response was found in Run #2 (Fig. 5), when the level of dissolved oxygen and the percent saturation of oxygen were identified as the most significant ($p < 0.05$) variables related to the numbers of fish in the surface waters (Table 2). Generally, avoidance of waters with air saturation values below a range of 18-35% was found. These levels for avoidance are very close to the 25-40% air saturation values avoided by juvenile chinook in the Somass River estuary, Alberni Inlet, B. C. (Birtwell, pers. comm.). The behavioral responses of fish to low oxygen conditions have been reviewed by Davis (1975). Most often, fish become more active in hypoxic water and attempt to move away from regions of low oxygen concentrations (Randall 1970).

As both effluent toxicity and low levels of dissolved oxygen were shown to be important variables related to fish distribution, the question of additive and/or synergistic (i.e. more than additive) effects arises. Marier (1973) found that a reduction of dissolved oxygen from 6.5 to 4.0 mg/L increased the toxicity of kraft mill effluent by two to three fold. Similar data for sulfite mill effluent are rare. Davis et al. (1978) reported elevated hematocrit levels in juvenile coho and sockeye salmon at a distance of 10 km seaward from the Port Alice mill during studies in 1973. This observation suggests the possibility of other biochemical changes occurring in the salmonids exposed to SME in the test chambers. If the fish also show greater activity when exposed to low oxygen conditions (Randall 1970), then increased stress may be a factor in producing the mortalities observed. Clearly, more research on the factors responsible for the avoidance responses and mortalities of fish exposed to pulp mill effluents *in situ* is needed.

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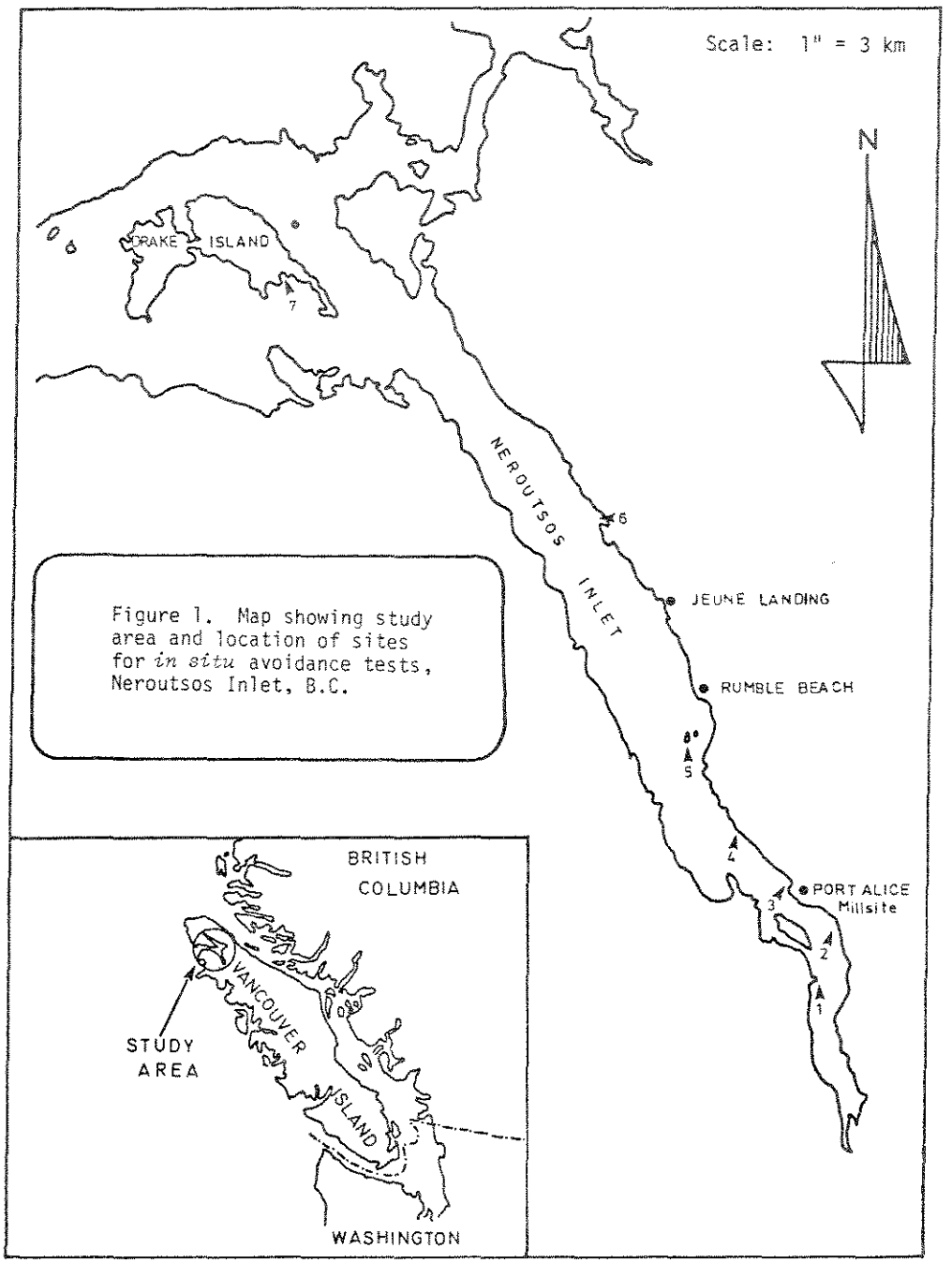

Figure 1. Map showing study area and location of sites for *in situ* avoidance tests, Neroutsos Inlet, B.C.

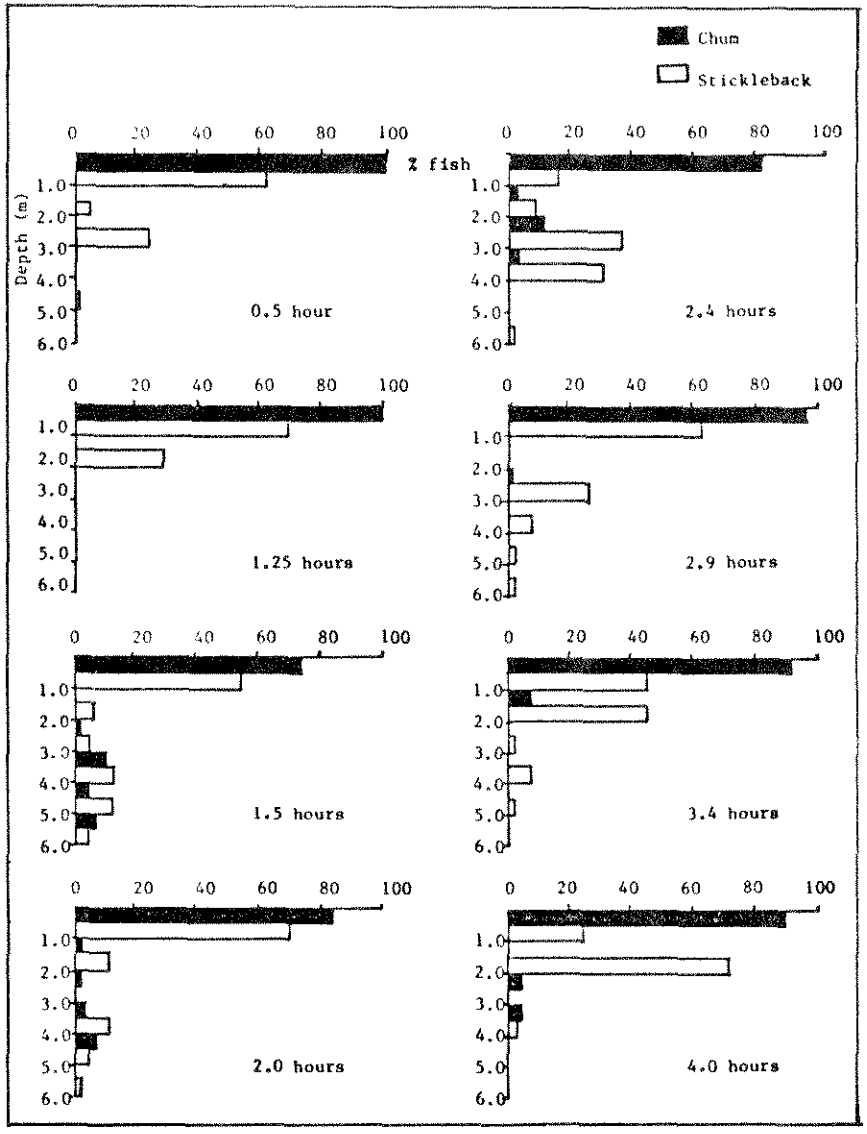

Figure 2. Vertical distribution of fish in time series test at control site - May 8, 1979.

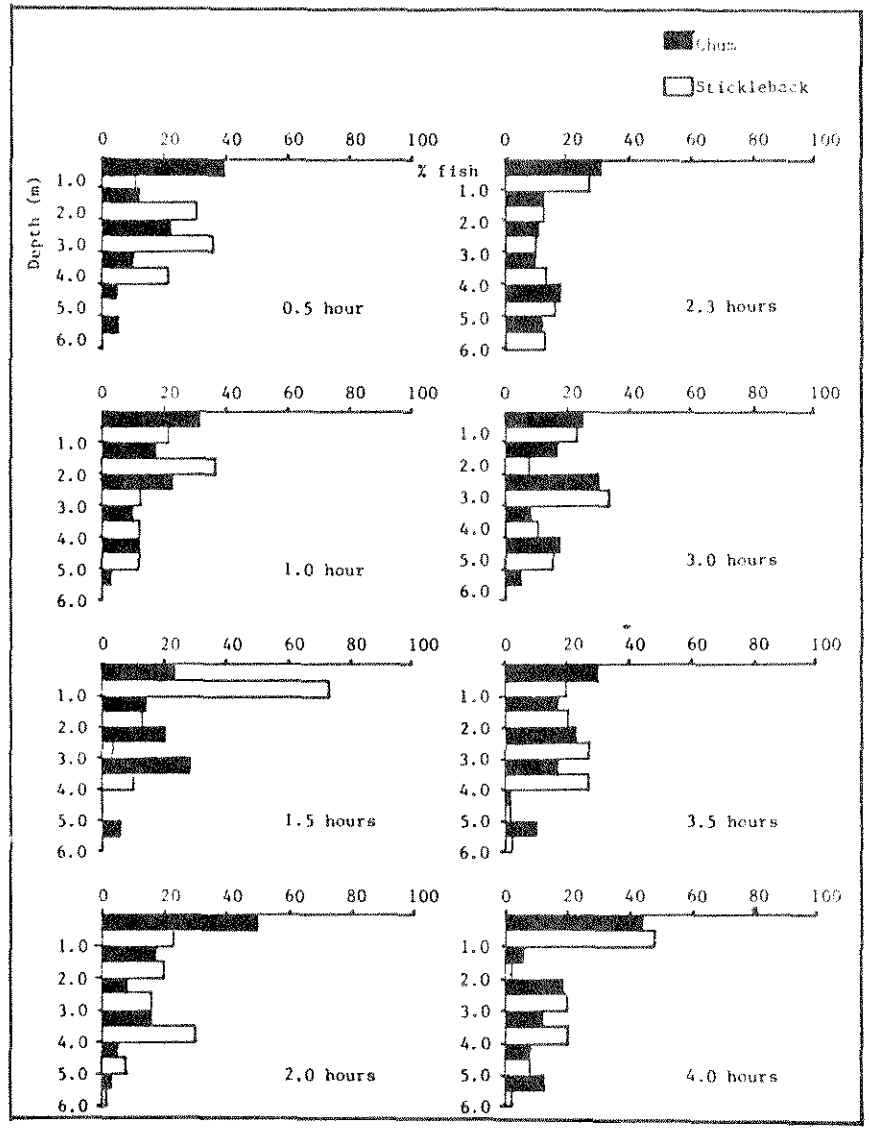

Figure 3. Vertical distribution of fish in time series test at mill site - May 9, 1979.

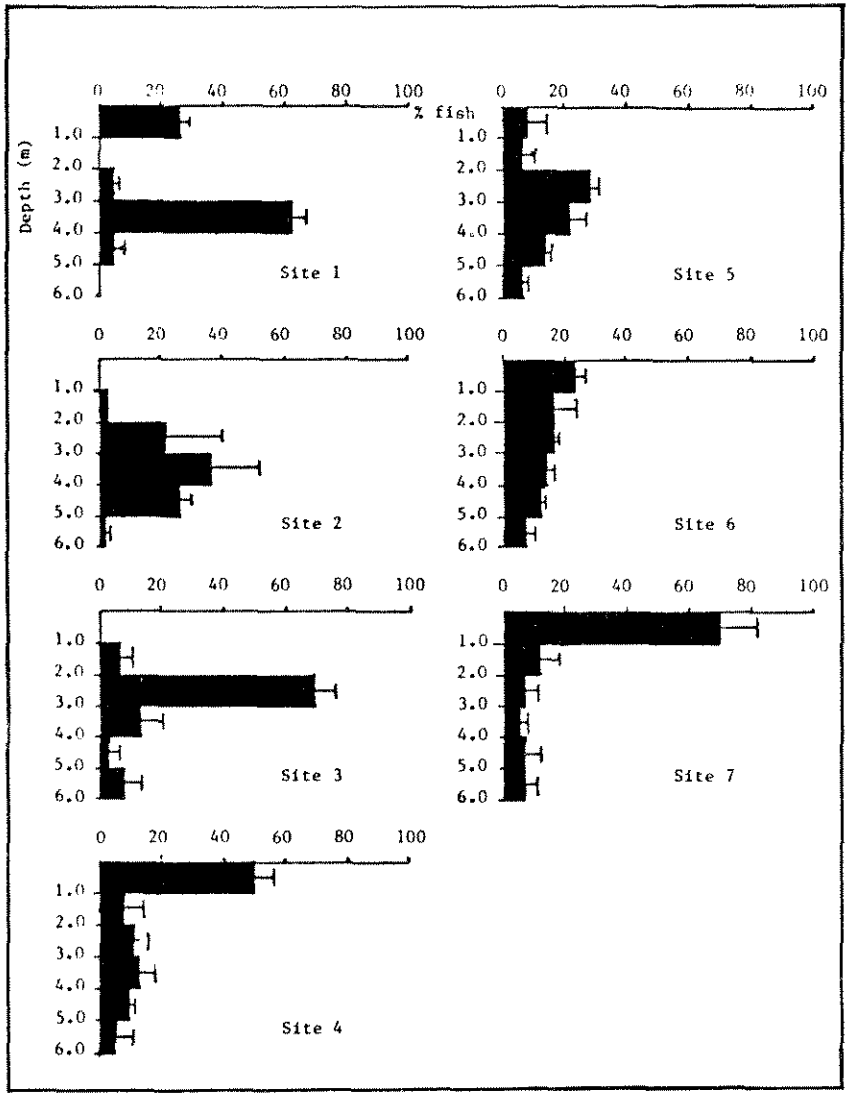

Figure 4. Vertical distribution of chum fry at seven sites in Neroutsos Inlet, for test run #1 - June 1-6, 1979. (Mean and upper 95% confidence limits shown for each depth.)

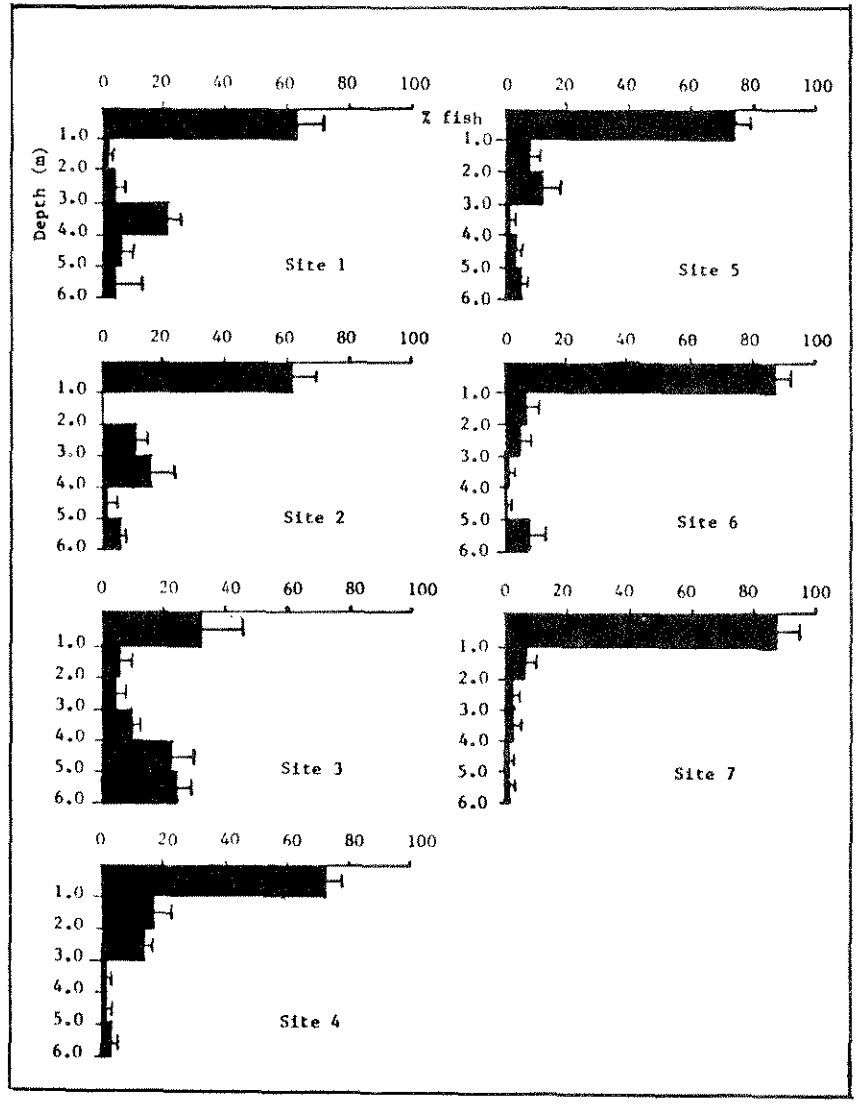

Figure 5. Vertical distribution of chum fry at seven sites in Neroutsos Inlet, for test run #2 - June 14-18, 1979. (Mean and upper 95% confidence limits shown for each depth.)

TABLE 1. RESULTS OF DUNCAN'S MULTIPLE RANGE TESTS FROM ANOVA* BY DEPTH FOR CHUM** AND STICKLEBACK**

Test		No. of Observations	Distinct Homogeneous Subsets (i.e. *Depths*) Identified by Duncan's Multiple Range Test ($p < 0.05$)
Time series - control site	chum	8	(1) (2, 3, 4, 5, 6)
	stickleback	8	(1) (2, 3, 4, 5) (2, 4, 5, 6)
Time series - mill site (May 9)	chum	15	(1) (2, 3) (2, 4) (4, 5) (5, 6)
	stickleback	15	(5) (6) (1, 2, 3) (2, 3, 4)
Time series - mill site (May 31)	chum	8	(3) (2, 4) (1, 5, 6)
Preference/avoidance - June 1-6 (Run #1)	chum	26	(1, 3, 4) (2, 5) (2, 6)
Preference/avoidance - June 14-18 (Run #2)	chum	26	(1) (2, 3, 4, 5, 6)

*All results were significant at $p < 0.05$ level.

**Arc transformed data.

TABLE 2 RESULTS OF STEPWISE MULTIPLE REGRESSION ANALYSIS FOR CHUM WITH WATER QUALITY VARIABLES AND DISTANCE

Test	Date	Depth	Significant* Independent Variables in Stepwise Regression Equation	Normal Coefficient**	Total r^2 for all Independent Variables
Preference/ avoidance (Run #1)	June 1-6	1	pH	0.76	0.59
		2	% sat'n	0.55	0.51
			pH^2	0.39	
		3	distance	0.40	0.16
		4	pH	0.66	0.44
		5	salinity^2	0.40	0.33
			pH^2	0.45	
		6	pH	0.48	0.40
			% sat'n^2	0.35	
Preference/ avoidance (Run #2)	June 14-18	1	dissolved oxygen	0.68	0.46
		2	% sat'n	0.46	0.55
			temperature^2	0.30	
			% sat'n^2	0.41	
		3	none	-	-
		4	distance	2.15	0.81
			% sat'n	2.28	
			temperature^2	0.51	
			% sat'n^2	1.03	
		5	salinity	0.67	0.45
		6	salinity	0.59	0.53
			salinity^2	0.43	

*At $p < 0.05$.

**Expressed as absolute values.

USE OF TELEMETRY IN MONITORING INTENSITY AND ENERGETICS OF ACTIVITY IN FREE-SWIMMING FISH WITH REFERENCE TO ZINC POLLUTION

A.H. Weatherley and S.C. Rogers

Life Sciences Division, Scarborough College,
University of Toronto, West Hill, Ontario, M1C 1A4

D.G. Pincock

Electrical Engineering Department, Nova Scotia
Technical College, Halifax, Nova Scotia

WEATHERLEY, A.H., S.C. ROGERS and D.G. PINCOCK 1980 Use of telemetry in monitoring intensity and energetics of activity in free-swimming fish with reference to zinc pollution. Can. Tech. Rep. Fish. Aquat. Sci. 975: 162-170.

The system overview and design schematics of a radiotelemetry apparatus for sensing/recording electromyograms* from free-swimming fish are given, with preliminary results of its use. The *e.m.g. signals are used to calibrate oxygen consumption rate determined simultaneously during swims of known speed and duration in active swimming, or in spontaneous (= routine) activity, chambers. The purpose is to infer oxycaloric cost of activity in the field from recorded e.m.g. signals so that changes in activity referable to laboratory-determined effects of sublethal zinc concentrations may be examined also in zinc-polluted field situations.

Key words: Radiotelemetry; electromyograms; sublethal effects; oxycaloric costs; trout, rainbow; freshwater pollution

WEATHERLEY, A.H., S.C. ROGERS and D.G. PINCOCK 1980 Use of telemetry in monitoring intensity and energetics of activity in free-swimming fish with reference to zinc pollution. Can. Tech. Rep. Fish. Aquat. Sci. 975: 162-170.

Le présent article décrit un appareil de radiotélémétrie permettant de détecter et d'enregistrer les électromyogrammes (é.m.g.) de poissons nageant librement, ainsi que les résultats préliminaires obtenus avec cet appareil. Les signaux é.m.g. servent à calibrer le taux de consommation d'oxygène mesuré simultanément au cours de nages à vitesse et durée connues dans des chambres de nage active ou au cours d'une activité spontanée (= routinière). L'objectif est de déduire le coût oxycalorique de l'activité sur le terrain à partir de signaux é.m.g. enregistrés. On pourra de cette manière examiner également, dans des situations naturelles de pollution par zinc, les changements d'activité attribuables aux effets de concentrations sublétales de ce métal déterminés en laboratoire.

INTRODUCTION

Many polluted aquatic environments that are not immediately lethal to fish can nevertheless sufficiently damage them so that range, movement, activity and growth are more or less modified. We are developing a new method based on telemetry to assess the effects of sublethal levels of such toxic pollutants as zinc on the activity of free-swimming fish. The reason we need such methods is because, though there is considerable knowledge of the damage to gill epithelium caused by zinc, its accumulation in various tissues and in some cases the functional impairment it may cause (reviewed by Weatherley et al. 1979), it is very difficult to translate the various categories of data on impairment into predictions of behavior of fish in sublethally zinc-polluted environments.

Various investigators have already examined gross movements of fish by means of attached ultrasonic and radiotelemetry packages that transmit simple signals from which fish positions can be instantaneously determined from signal strength and direction (Henderson et al. 1966; Lonsdale 1967; Lonsdale and Baxter 1968; Young et al. 1972; Hawkins et al. 1974; Young et al. 1976; Stasko and Pincock 1977). When many such positions, obtained in succession, are joined in sequence by straight lines, the approximate path of a free-swimming fish may be constructed. This will tend to be a minimal estimate of the true path swum, because it fails to account for curvatures and velocity changes occurring between determined positions. Thus, mean swimming speed based on time to traverse path thus estimated will also be a minimal estimate; and energy consumption of swimming, calculated from oxycaloric equivalents of swimming in laboratory respirometers at fixed speeds that are the same as the underestimated "average" speed mentioned above, will be underestimated too (Weatherley 1976).

Past attempts to overcome such problems have involved the use of variables correlated with swimming activity, such as heart rate, using both ultrasonics (Kanwisher et al. 1974; Wardle and Kanwisher 1974; Priede and Young 1977; Oswald 1978) and radiotelemetry (Frank 1968; Nomura and Ibaraki 1969; Nomura et al. 1972; Weintraub and Mackay 1975). Heart rate is, however, probably a rather inaccurate index because, in addition to responding to circulatory demand, it is also very sensitive to external environmental factors (Randall 1970). Heart rate is, of course, only one of the two factors that determine cardiac output - the other being stroke volume. Since the total bioelectrical changes in the heart probably can be correlated (via the configuration of the electrocardiogram) with strength of heart beat, and therefore with stroke volume, it is quite possible that the electrocardiogram might be usable as an index of cardiac activity in the field that reflects fish metabolic activity, though this is not the way cardiac records have so far been used even when such an approach might have yielded interesting data (e.g., Priede and Young 1977).

TELEMETRY AND ELECTROMYOGRAMS

A description has already been published of the schematics of a radiotelemetry system which depends on sensing and recording of electromyograms (e.m.g.'s) derived from the activity of the main epaxial musculature (Luke et al. 1979). In the epaxial e.m.g., the signal is not necessarily merely a product of directed swimming by the fish, but will result from any contraction of the epaxial muscles. This is obviously an advantage, because it means that what is essentially the biochemical activity (respiration) of the epaxial muscle can probably be related to the e.m.g. signal, whether or not such activity reflects a change in the location of the fish.

In alternative use, the electrodes of this apparatus can be located in small muscles associated with the fish respiratory cycle. The advantage of this location is that it provides the possibility that the configuration of the e.m.g. will be correlated with volume of water crossing the gills with each respiratory movement (Ballintijn and Hughes 1965; Shelton 1970; Oswald 1978), which may in turn lead to better correlation with oxygen consumption, since it is assumed that respiration will be related to the whole of the muscular activity of the fish at the biochemical level, whatever may be its precise relation to gross body movement or positional change.

During periods of extended swims in a Blazka-Fry active swimming chamber (Fry 1971), all e.m.g.'s, from rainbow trout fitted with telemetry packages to transmit either from the epaxial or respiratory muscle sites, are tape-recorded. Evaluation of records consists of computer analysis of statistics and spectra of signals. During the same periods of e.m.g.-recording, oxygen consumption rates are determined, which may be calibrated in terms of corresponding μV averages. Thus oxygen consumption of fish in free-swimming field situations may, in principle, be determined from e.m.g. data - though we will not belittle the difficulties presently opposing the reliable field use of these techniques.

This analysis has two secondary purposes: (i) determination of the most suitable location for electrodes, (ii) evaluation of the relative effectiveness of different kinds of data-processing for assessment of activity and energetics of fish in the field.

The sensor/transmitter is epoxy-insulated and is sutured to a dorsolateral position in the vicinity of the dorsal fin. The e.m.g. is detected by two flexible wire electrodes (kynar-insulated, silver-coated copper) twisted together, tips bared and separated by about 1 mm. When suitably implanted in muscle the electrodes are sutured in place, and pass directly to the attached telemetry package which they enter without an external join (to aid waterproofing). The package is made operational by twisting together two external wires that constitute a switch.

Fig. 1 shows the system overview, and Fig. 2 the schematics of the radiotelemetry packages (Sayre 1979).

PRELIMINARY RESULTS

Fig. 3 is a typical e.m.g. record from the epaxial muscles of a rainbow trout 31 cm long swimming at 1 body length per sec. in a Blazka-Fry chamber. The electrodes were implanted in the "mosaic" muscle which, despite its general designation as a mass of white, fast-contracting, glycolytic fibres (Johnston et al. 1975; Greer-Walker and Emerson 1978) has been shown to be involved in all swimming movements that result in forward movement at speeds greater than 1 body length per sec (Ballintijn and Hughes 1965), and probably at slower speeds.

Fig. 4 (a-upper curve) shows mean μV of e.m.g.'s from the respiratory muscle (levator hyomandibulae et arcus palatini) of a rainbow trout swimming at various velocities in the active swimming chamber. Fig. 4 (b) gives corresponding oxygen consumption rates at the same constant swimming speeds. Both curves are simple exponentials. There is approximately a straight line relationship between oxygen consumption and e.m.g. values for the range of swimming speeds of this experiment (Fig. 4 (c)). Since the fastest swimming speed used approaches the maximum sustainable for rainbow trout of the size used, we can assume that this linear relationship will hold for almost all normal levels of active swimming for the species at the temperature used (12°C). The results therefore provide a laboratory demonstration that, given improved telemetry apparatus and data-processing, it will eventually prove feasible to determine the energy (oxycaloric) cost of free-swimming in a fish from its e.m.g. records by remote sensing. We of course recognize the limitations of the present calibration. Records from taped e.m.g.'s of trout in a spontaneous (= routine) activity chamber are presently being processed for comparison with those in the swimming chamber. It is hoped these will furnish data, on what happens to energy metabolism in situations of unforced activity, collected in a more "naturalistic" and empirical fashion than has usually been the case in such attempts, and providing recourse to other than extrapolations from swimming chamber data.

Fig. 4 also gives data from the epaxial e.m.g.'s of a trout swimming in a Brett (1963) chamber. This also gives a smooth exponential which gives promise of good correlations with oxygen consumption.

THE FUTURE

By further development and application of these techniques we expect to utilize telemetry with fish active up to approx. 1 km removed from the signal reception point, and hence to be able to obtain data on field activity of fish in (zinc) polluted environments at various times of the year, from which energy expenditure can be

inferred.

On completion of the present laboratory calibrations we will measure energetics of fish exposed in laboratory trials to sublethal but damaging concentrations of zinc. As the transmission range and battery life (at present less than 25 m and 2 weeks, respectively) are improved we will move to zinc-polluted experimental ponds and larger water bodies for testing.

The present transmitters are not suitable for field use because of the antenna included in the package, which is the main cause of the limited range, and because of the continuous transmission, which is the cause of short battery life. The range limitation can be overcome with a different antenna. To increase transmitting life of the package to at least several months, pulsed transmission will be needed, which requires that sufficient signal processing, probably in the form of rectification and averaging, be included in the transmitter, so that electrode information can be coded in low duty-cycle transmissions. We envisage the eventual use of temperature- and depth-sensitive telemetry sensors along with the present apparatus, which will greatly increase its potential usefulness in the field.

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FIGURES

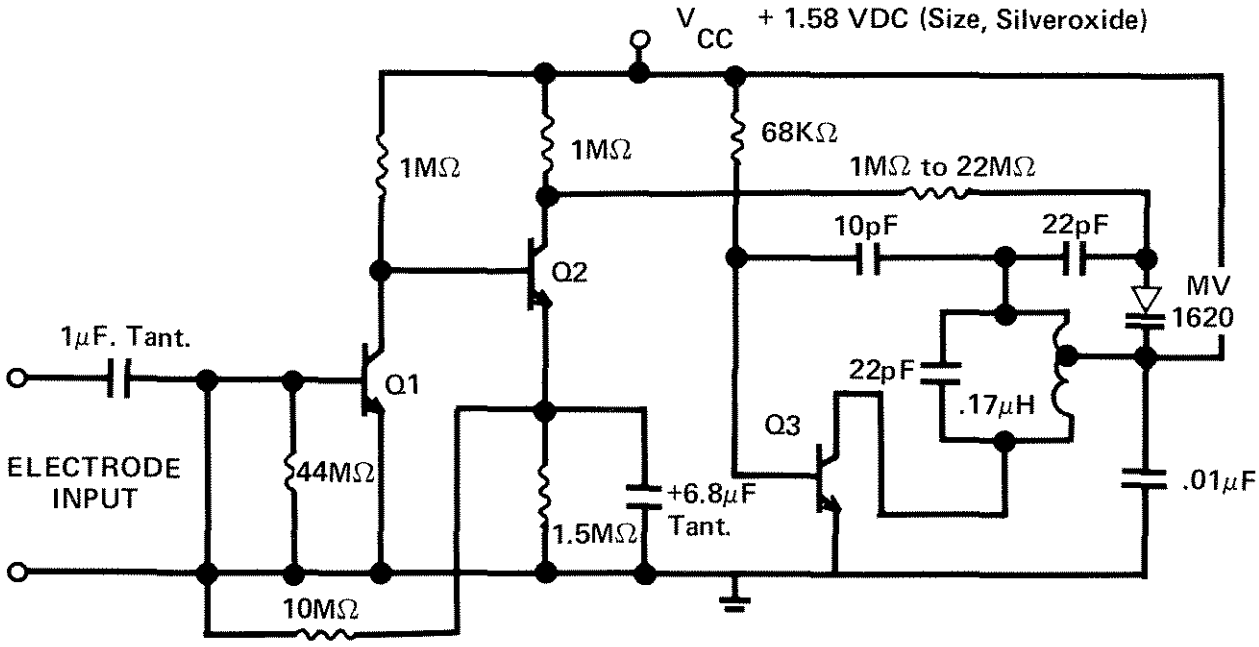

Fig. 1 Schematic of e.m.g. transmitter. The oscillator, Q3, is of the Hartley type, with modulation achieved by means of a varactor diode. The coil is made up by winding 5.3 turns of No. 22 AWG tinned copper tapped 3.5 turns from collector end; space wound on 1/4 in. mandrel; length 0.6 cm. The component values shown determine oscillation at about 90 MHz and the modulation sensitivity is typically 500 Hz/mV. Q1 and Q2 amplify the electrode signal with a gain of about 300 in the bandwidth 0.2 to 300 Hz. Thus the standard 75 kHz deviation, compatible with a commercial FM receiver, is achieved with a 500 μV peak signal at the electrodes.

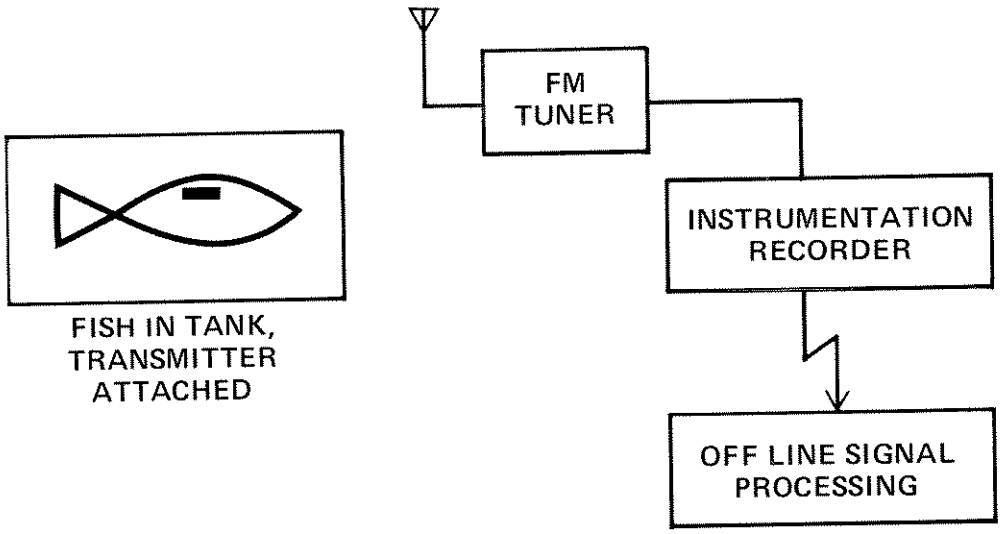

Fig. 2 Overview of telemetry system used in laboratory work. Transmitter uses FM to broadcast electrode voltages on standard FM broadcast band (88 to 1 MHz) detectable by a standard FM tuner. Analysis of recorded tapes is carried out on a PDP-11 minicomputer.

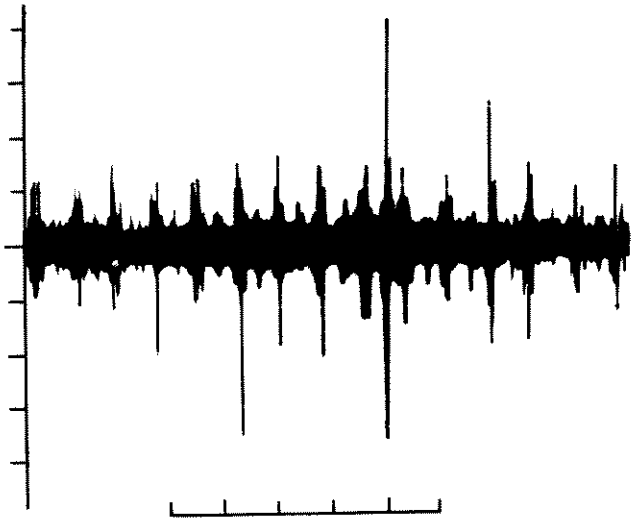

Fig. 3 Representative axial muscle e.m.g. (electromyogram) from 30 cm rainbow trout swimming at 1 body length/sec. Vertical scale, 1 div. = 25 μV; horizontal scale, 1 div. = 0.5 sec. The signal transmitted is taped, demodulated, and filtered (high pass = 20 Hz; low pass = 150 Hz).

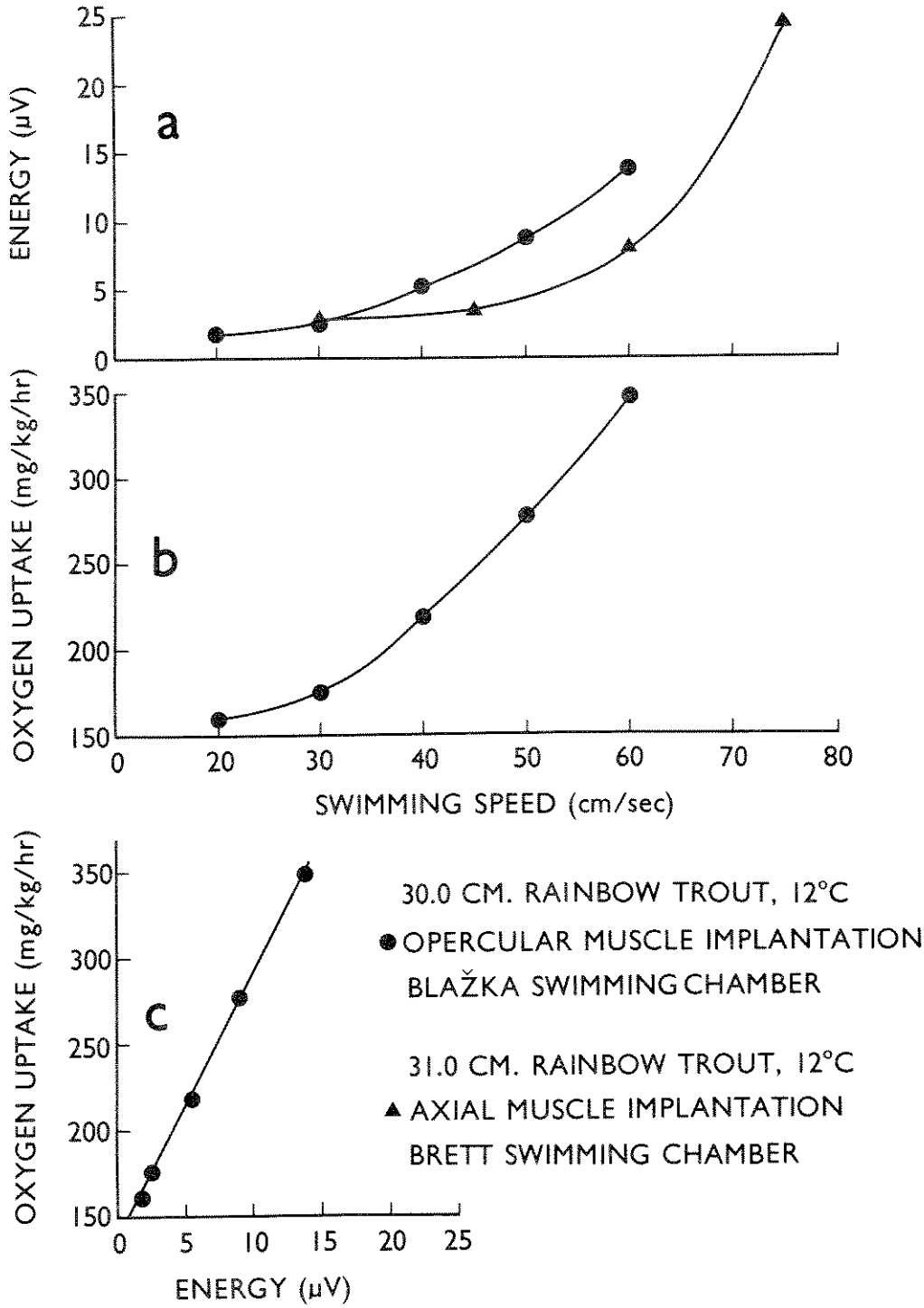

Fig. 4 (a) Mean μV values of e.m.g.'s from respiratory muscle (levator hyomandibulae et arcus palatini) and from epaxial muscles of separate rainbow trout in active swimming chambers. (b) Oxygen consumption rates versus swimming speed of fish with respiratory muscle e.m.g. records. (c) Oxygen consumption versus corresponding mean μV values of e.m.g.'s of fish with respiratory muscle e.m.g.'s.

MAXIMIZING INFORMATION RETURN FROM PREFERENCE AND AVOIDANCE RESPONSE DATA: EXAMPLES AND RECOMMENDATIONS

B.G.E. de March and E. Scherer

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

DE MARCH, B.G.E. and E. SCHERER 1980 Maximizing information return from preference and avoidance response data: examples and recommendations. Can. Tech. Rep. Fish. Aquat. Sci. 975: 171-181.

Data obtained from preference and avoidance experiments in a counter current trough are discussed and disadvantages of the most common method of data presentation, the statistic "percent time spent in one end" are discussed. Several shortcomings of this statistic can be eliminated. Suggested improvements are the use of 1) appropriate data transformations; 2) control data for each animal; 3) time statistics other than % time; 4) replicate data available in each animal's response, and 5) specific response elements instead of time statistics. Several examples of data analyses applying these considerations are presented.

Key words: Experimental design; data analysis; regression analysis; statistics; behavioral reaction; *Gammarus*; whitefish

DE MARCH, B.G.E. and E. SCHERER 1980 Maximizing information return from preference and avoidance response data: examples and recommendations. Can. Tech. Rep. Fish. Aquat. Sci. 975: 171-181.

Les auteurs analysent les données d'expériences de préférence et d'évitement dans une auge à contre-courant, ainsi que les inconvénients de la méthode la plus commune de présentation des données, le "pourcentage de temps passé à une extrémité". On peut éliminer plusieurs défauts de cette statistique et améliorer la méthode en utilisant : 1) des transformations appropriées des données; 2) des données témoins pour chaque animal; 3) le temps exprimé autrement qu'en pourcentage; 4) des données en double de la réaction de chaque animal, et 5) des éléments de réaction spécifique au lieu de statistiques de temps. On donne enfin plusieurs exemples d'analyses de données où l'on met en pratique ces considérations.

INTRODUCTION

Preference and avoidance studies to assess potential effects of toxicants on aquatic fauna have become an accepted part of toxicological testing methodology in recent years (Sprague 1976; Scherer 1977, 1979). We have been analyzing preference and avoidance data from various sources, and it was apparent that potentially meaningful data often were not recorded or were overlooked. In many cases, different experimental design and analysis might have provided a better estimate of the ability of the test organism to detect and respond to the toxicant, and also, would have saved time, effort, and money. Some suggestions for more meaningful results are presented.

In this paper, we will discuss only data obtained from counter current troughs similar to those described by Jones (1947), Sprague (1964), Scherer and Nowak (1973) and Maciorowski et al. (1977), with water entering both ends and an outflow in the middle at the interface. The toxicant is introduced at the desired concentration at one end of the tank. Usually, animals are monitored for a fixed length of time as they swim back and forth in the trough. Often only one measurement, the cumulative time spent in either end, is made during the test. The data are then typically presented in a graphic display of % time spent by the animal in one end *vs* concentration or the logarithm of concentration of the toxicant being tested (e.g. Sprague 1964; Scherer 1975; Fig. 1a). A response different from 50%, either by an individual, or as a mean for a group of individuals, demonstrated by one of a number of statistical tests, is interpreted as either preference or avoidance. These methods have been sufficient to demonstrate general trends in various test organisms' response to toxicants (e.g. Sprague 1964; Kleerekoper 1973; Scherer 1975; Tatsukawa and Hidaka 1978), and such results have often been supported by physiological data (e.g. McLeese 1975; Kamchen and Hara 1980) and field observations (e.g. Sprague et al. 1965).

In our experiments with the freshwater amphipod *Gammarus lacustris* and lake whitefish *Coregonus clupeaformis*, we found that the % time statistics often did not concur with our impression of the type or extent of the behavioral response in individuals. The following are examples of particular problems:

1) Animals often showed a bias for one end of the trough during the control run, which persisted into the toxicant run even though the animal appeared to react to the toxicant. For example, if an animal spent 80% of its time at an end during the control run, and then 60%, after the toxicant was introduced at that end, we may suspect that the animal avoided the toxicant. The final % time statistic suggests no response, or perhaps even preference.

2) The % time statistic did not distinguish between preference of one end of the trough and avoidance of the other. For example, the effects of two concentrations of toxicant are quite different if, at one concentration, animals quickly swim out of the non-toxicant end, and at the other concentration, animals only slow down at the toxicant end. Both results may yield the same % time statistic.

3) Similar changes in time related events in both ends of the chamber were often masked or gave misleading measurements. In some cases, animals were observed to swim faster, slower, or erratically, and this change was not not revealed by the % time statistic.

4) Changes in specific elements of the overall swimming pattern were often

masked. For fish in particular, swimming speeds in and out of an end, the distance of penetration into an end, and the time spent immobile at each end, were all parameters subject to change with toxicant levels, but which were not necessarily revealed by the % time statistic (e.g. Lawrence and Scherer 1974).

5) Information available in each animal's repetition (or non-repetition) of a behavior pattern during the control or test run was lost. A small change in the % time statistic appeared meaningful if the animal moved very regularly, while a large change did not appear meaningful if the swimming pattern was erratic. Also, animals often turned away from the toxicant at the toxicant interface a number of times, and then swam into it, and lay immobile in it for a time, suggesting avoidance but also stupefaction. In all three of these cases, the final % time statistic could be very deceptive.

Because of the above problems, we often found that the mean % time statistic for several animals tested at one toxicant concentration had very large confidence limits, even though each animal appeared to have the same response to the toxicant. It was apparent that data analysis and collection had to be reconsidered.

THE USE OF CONTROL DATA FOR EACH INDIVIDUAL

Preliminary testing with the test species and toxicant will determine whether or not control measurements for each individual are necessary. Individual control runs are not necessary if 1) animals do not show a bias for one end of the trough during control runs, 2) the swimming pattern in the non-toxicant end of the trough does not change after the toxicant is added at the

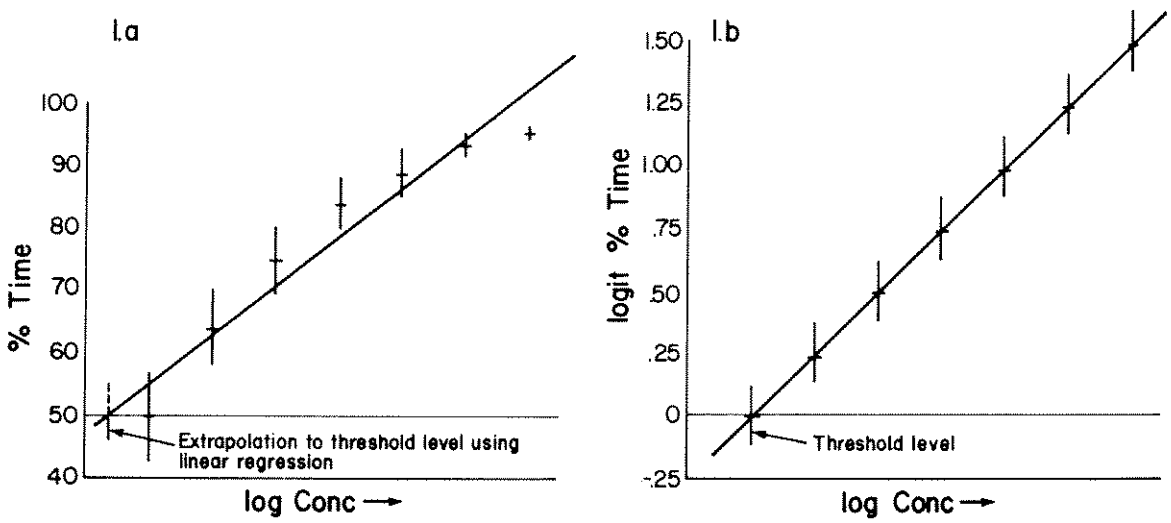

Figure 1. Typical response curves of % time *vs* concentration. Normally distributed simulated data shown in Fig. 1b are used in the example.

other end, and 3) the control activity levels of individuals (e.g. swimming speeds or the number of interface crossings per unit time) do not relate to the degree of preference or avoidance of the toxicant. If individauls do show some of the above tendencies, and control measurements are not taken, it is highly probable that many true responses will be masked.

Control data can be incorporated into final analyses in various ways. The simplest, and least effective would be to subtract (or divide) the control value of any dependent variable from (or by) the final value. A superior method would be to use the control value as a covariate in any statistical analysis, or as an additional independent variable in multiple linear regression analyses. Specific recommendations about the choice of dependent variables will be made in the following sections.

THE USE OF TRANSFORMATIONS FOR % DATA

Most % time curves in the literature resemble the generalized one shown in Fig. 1a. The curve is one-half of a sigmoid curve in the positive response range, and has large variances near the 50% time response and smaller variances near the asymptote. Data distributed in this manner must be transformed with either the probit, the angular (arcsin $\sqrt{(\%-100)}$) or logit (log(%/100-%)) transformation before use in statistical analysis (Bartlett 1947; Ashton 1972). These transformations may make the variances more homogeneous over a range of test values, a necessary prerequisite to further statistical treatments (Cochran 1947). Figure 1b shows the curve obtained with a logit transformation of data in Fig. 1a. The variances are now more homogeneous, therefore, the comparison of responses at different concentrations will yield fewer erroneous results caused by comparing samples with different variances (Cochran 1947). With these transformations specifically, comparisons will show more significant differences near the 50% response, and fewer among the higher and lower % responses.

The response curve may approximate a straight line after the use of one of the suggested transformations, and interpolations using simple regression equations may be valid. One obvious difference between the two curves is their extrapolation to the threshold level (i.e. the highest concentration at which no response is expected) obtained from simple regression equations (Figs. 1a, 1b). In the first curve, (Fig. 1a) the extrapolated threshold level is lower than the concentration at which no response was measured, and its standard deviation is smaller than the actual deviations observed at lower concentrations. The second extrapolation (Fig. 1b) provides a realistic estimate of the threshold level.

We used the logit rather than other transformations for *Gammarus lacustris* data because it relates to a possible model of preference and avoidance behavior. If a given level of toxicant causes the mean trip times into an end (trip time = the elapsed time between crossing the midpoint entering an end, and crossing it leaving that end) to change by a fixed percentage, but we have only % time data, then the logit % time is the most efficient estimator of the response (i.e. the estimator with the smallest variance). The logit % can be factored in the following manner:

$$\log \frac{\%}{100-\%} = \log \frac{\text{Total time in one end}}{\text{Total time in other end}} ;$$

if the number of trips in each end are equal,

$$= \log \frac{\text{Mean trip time in one end}}{\text{Mean trip time in other end}}$$

$$= \log [\text{Mean trip time one end}] - \log [\text{Mean trip time other end}].$$

The logit transformation is therefore a difference of the logarithms of mean trip times in both ends. A difference between logits is the difference between log mean trip times in an end if the mean trip time in the other end does not change. If a given level of toxicant consistently changes a particular mean trip time to n% of its original value, then the difference between logits of % time is always log (n/100). This value would represent the change in response for test organisms with different initial biases and different activity levels. The change in the % time statistic, on the other hand, would be variable.

ALTERNATIVE TIME STATISTICS: TRIP TIMES

The use of ratios such as % time or its transformations forces a particular description to our data. For example, assume that a response can be described by the equation:

$$\text{logit } \% = \log \frac{R}{L} = aC + bC^2 + c$$

where R = total time (or mean trip time) spent in the toxicant end, L = total time (or mean trip time) spent in the non-toxicant end, C = toxicant concentration or a transformation of it, and a, b, c . . . k are regression coefficients determined in multiple linear regression analysis. An equation derived from the above is:

$$\log R = aC + bC^2 + c + \log L.$$

It is highly probable that the addition of any of the terms shown below would describe the response better:

$$\log R = (\text{terms shown above}) + e \log L + f \log L^2 + g \log L \times C.$$

The best possible equation describing the response can be found with proper multiple linear regression techniques. The final equation obtained can then be reconverted to a % time expression for data presentation purposes if desired. For example one could obtain the reconverted equation:

$$\log \frac{R}{L} = a \log L + b \log L \times C + cC + dC^2 + K.$$

This equation is in fact very similar to ones we obtain by multiple linear regression techniques using trip times at the toxicant end as dependent variables, and trip times in the non-toxicant end or during the control run as independent variables (example to follow). The control activity levels

of individuals, measured as either one of the described independent variables, usually relate to the extent of preference or avoidance.

In this type of experiment, one is dealing with two potentially independent responses, i.e. what happens in the toxicant end, and what happens in the pure end. For this reason, trip times in both ends can be considered to be responses, and corresponding trip times in the control run are treated as independent variables or covariates.

In all *Gammarus lacustris* data examined, mean trip time and its standard deviation were directly proportional (S.D.≃ 2/3 Mean). This fact supports the model of preference and avoidance described in the last section: animals are more likely to vary their trip time by a certain percentage than by a fixed amount of time. This fact complicates the analysis. Data distributed in this manner should be log-transformed before statistical analysis (Cochran 1947; Snedecor and Cochran 1964). The statistic "mean log trip time" is in fact not related to its standard deviation, and is therefore a valid one for use in further statistical analysis. Also, the standard deviation of this statistic can be treated as another dependent variable independent of mean trip time. If a toxicant causes erratic behavior, this variable may be the best measure of the response.

The % time statistic is necessarily associated with many statistical problems because of its structure. Among these are: 1) excessive variability compared to numbers used in the statistic, and 2) biased estimation of the true mean value (Sokal and Rohlf 1973; Atchley et al. 1976). These complications do not occur with % data obtained from binomial or multinomial populations, nor with true integrative measures such as means or summations. Green (1979, p. 105) writes, "There is no need to use ratios . . . because other analysis approaches will accomplish what ratios are supposed to and usually do not."

Because of all these considerations, we now treat mean log trip times and their standard deviations as possible dependent variables, and the same parameters measured in the control run as independent variables in multiple linear regression analysis.

THE USE OF REPLICATE DATA FOR INDIVIDUALS

The comparison of mean trip times measured for one individual in one test is a method of testing the significance of its behavioral modification. For *Gammarus lacustris*, we found the t-test to be the most sensitive indicator of differences in mean trip times. The Kolmogorov-Smirnov test used for trip time comparison by Sprague (1964) appears to be less sensitive. It showed significant differences ($\alpha = 0.05$) for only 76% of comparisons shown to be significantly different with the t-test. These results are supported by theoretical results described by Capon (1965) who calculated that the K-S test is 63.7% as efficient as the t-test for normally distributed data. t-test comparisons of mean log trip times are similarly more sensitive than the Kolmogorov-Smirnov test.

Slower individuals of *Coregonus clupeaformis* tested for a fixed lenth of time deviate from the mean responses (measured as % time or mean trip

time) more often, and to a larger extent, than faster individuals. The logit and logarithmic transformations do not remove these deviations satisfactorily. These deviations occur because the response measurement is less precise for slower animals for two reasons: 1) fewer trips are measured in each end in a fixed length of time, and 2) the number of trips measured in each end are less likely to be equal. Such deviations do not exist for *Gammarus lacustris* tested for a fixed number of trips, rather than for a fixed time. Perhaps the two species should not be compared; however, it seems reasonable that all individuals of any test species should be measured with the same precision. We suggest that all individuals be tested for the same number of trips into each end. Of course, if there is a complete breakdown of the behavior pattern one is measuring (i.e. an animal stops moving back and forth), one is observing an independent response with 100% significance, and statistics are not needed to show the significance of this response.

AN EXAMPLE WITH SEVERAL IMPROVEMENTS

Table 1 shows the progressive improvement in the predictive capacity of regression equations describing a response after implementation of suggested changes. All equations were obtained by stepwise multiple linear regression analysis as described by Draper and Smith (1966). All squares

Table 1. r^2 values from multiple linear regression equations using *Gammarus lacustris* exposed to copper at various pH values. All chemical parameters except pH are log-transformed. The control variables are the same measurement as the dependent variable, but obtained during a control run for each individual. Omitted values mean that not all variables have a significant ($\alpha \leq 0.05$) F-value for inclusion (n = 106).

Independent Variables	Dependent Variables	
	logit % time	log mean trip time in toxicant
Cu^{++}	0.046 (Cu^{++}) 0.132 (Cu^{++} and $(Cu^{++})^2$)	0.167
Cu^{++}, Control		0.414
Cu^{++}, $CuOH_2$, pH, control	0.332 (8 terms in equation)	0.429 (3 terms; $CuOH_2$ not included)
Cu^{++}, $CuOH_2$, pH, control control activity level (i.e. mean control trip time)	0.500 (15 terms)	0.581 (3 terms; $CuOH_2$ not included)

and cross-products of the variables of interest were considered. All equations except those for the most complex model were obtained by incorporating variables needed for the comparison if they had a significant F-value for inclusion into the equations. The most complex equations were obtained by backward elimination. Only functional relationships, rather than final equations, are presented in Table 1.

Gammarus lacustris was tested in water of different pH values, and with copper entering one end of the trough during the test run. The speciation of copper and other compounds in our water was estimated in a computer programme designed by R. Wagemann of the Freshwater Institute. This experiment will be presented in detail in another publication.

The better predictive capacity of equations using log mean trip time rather than logit % time in the toxicant end as the dependent variable is evident (Table 1). All r^2 values in the "logit % time" column are smaller than analogous ones in the "log mean trip time" column. Even the simplest relationship of log mean trip time *vs* Cu^{++} (r^2 = 0.167 and 0.046, respectively). The equation of logit % time *vs* all significantly incorporated forms of Cu^{++} is curvilinear (r^2 = 0.132), while that of log mean trip time is a straight line with a higher degree of correlation (r^2 = 0.167). The incorporation of the suitable control statistic improves only the equation with log mean trip time as the dependent variable (r^2 = 0.414), but not that with logit % time. The control logit % time measure, however, is incorporated into the analysis after the chemical variables are entered (r^2 = 0.332). The analogous equation with log mean trip times has a higher predictive capacity (r^2 = 0.429). As expected, the variable "mean control trip time" improves the predictive capacity of the logit % time model (r^2 = 0.500), demonstrating the importance of initial activity levels to the response to the toxicant. The fact that the control response at the toxicant end is important to both models demonstrates that the initial bias for the end later containing the toxicant persists to some extent.

One compound ($CuOH_2$) is not significantly incorporated into the analyses with log mean trip times as the dependent variable, thus producing simpler equations for interactions than with the use of logit % time. The correlation between logit % time and $CuOH_2$ may be a spurious one typically produced by using ratios as dependent variables (Atchley et al. 1976).

The simplest and more traditional analysis of logit % time *vs* log concentration yields only a marginally significant correlation (r^2 = 0.046, $\alpha \simeq 0.05$). The most complex analysis produces a model with a high predictive capacity (r^2 = 0.581, F = 17.7, $\alpha < 0.05$), and a plausible explanation for the varying preference and avoidance of copper.

THE ANALYSIS OF UNDERLYING RESPONSE ELEMENTS

Specific response elements such as swimming speeds, depth of penetration into an end, or time spent immobile in an end, not necessarily related to overall temporal changes, may change in response to a toxicant. The change in these elements is particularly obvious in fish (e.g. Lawrence and Scherer 1974).

The combined results of two experiments testing *Coregonus clupeaformis*' response to methoxychlor are presented in Table 2. In one experiment, each animal was exposed to increasing concentrations of methoxychlor, and in the other, to the same concentrations, but in decreasing order of concentration. Control measurements were made for each animal before being exposed to all concentrations of toxicant. The data are in a preliminary state of analysis and are presented only to demonstrate several points. The statistical methods are as described in the previous section except that squares and cross-products were not considered for incorporating into equations.

The transformed value of % time is most highly correlated with methoxychlor concentration ($r^2 = 0.407$), while the two components which comprise this measure, the trip times into both ends, are significantly, but marginally ($\alpha \leq 0.05$) correlated with concentration ($r^2 = 0.078$, toxicant end; $r^2 = 0.162$, non-toxicant end). Simple correlations of trip times with toxicant levels have approximately the same r^2 values as those in the *Gammarus lacustris* data (Table 1), suggesting that trip times could be used for these data as effectively as for *G. lacustris* data.

Four response elements significantly correlated with toxicant concentrations (exit speed, $r^2 = 0.358$; entrance speed, $r^2 = 0.284$; distance into toxicant, $r^2 = 0.211$; distance into toxicant-free water, $r^2 = 0.070$)(Table 2). These elements are often not highly correlated with each other, suggesting that perhaps individuals,may use only one of several possible avoidance or preference strategies, or use different strategies in different toxicant ranges. It would also be of interest to determine whether true control measurements for individuals could improve the described responses.

The time elapsed between the control and toxicant runs is important to several responses (Table 2); animals apparently slow down with time in both experiments. These are changes which, in one experiment only, cannot be separated from changes occurring in response to the toxicant. Because of this, we suggest that each animal be tested only at one toxicant concentration.

Table 2. r^2 values from various multiple linear regression equations obtained from two experiments using whitefish exposed to methoxychlor. "time" is the time elapsed between the beginning of the control run and the beginning of the toxicant run. Omitted values mean that not all variables have a significant F-value for inclusion (n = 93) (tt = mean trip time.

Independent Variables	Dependent Variables						
	Transformed % Time (angular)	Components					
		tt toxicant	tt non-toxicant	exit speed from toxicant	entrance speed into toxicant	distance into toxicant	distance into toxicant-free water
log Conc.	0.407	0.078	0.162			0.211	0.070
Control				0.358	0.284	0.047	
log Conc. & Control	0.426					0.256	
log Conc. & time		0.092	0.192		0.329		
log Conc. & Control & time			0.207		0.340		

SUMMARY

The improvements suggested here can be implemented in their simplest forms for the analysis of existing data with limited measurements, or in combination to analyze data from well-planned experiments to yield models with maximum predictive capacity. No doubt, most situations will require an intermediate analysis, the details of which will depend on the objectives of the experiment and the type of results obtained.

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BEHAVIORAL REACTIONS OF WHITEFISH (*Coregonus clupeaformis*) TO FOOD EXTRACT: AN APPLICATION TO SUBLETHAL TOXICITY BIOASSAY

R. Kamchen and T.J. Hara

University of Manitoba and Department of Fisheries and Oceans,
Freshwater Institute, Winnipeg, Manitoba, R3T 2N6

KAMCHEN, R. and T.J. HARA 1980 Behavioral reactions of whitefish (*Coregonus clupeaformis*) to food extract: an application to sublethal toxicity bioassay. Can. Tech. Rep. Fish. Aquat. Sci. 975: 182-191.

The behavioral reactions of lake whitefish (*Coregonus clupeaformis*) to food extract prepared from dried pellets were studied using an avoidance-preference trough. Whitefish showed significant preference (attraction) for food extract at the concentration of 0.2 g/L. The preference reaction was eliminated after the nares of fish were cauterized, indicating that the reaction was mediated through olfaction. A significant reduction in the preference was demonstrated in fish exposed to $HgCl_2$ at 0.05 mg/L (1.8×10^{-7}M) for 1 to 2 weeks. Whitefish did not avoid sublethal concentrations of $HgCl_2$ when their direct response to this chemical was tested. Based on these findings, methods of applying the observed preference behavior to sublethal bioassays for aquatic toxicants are discussed.

Key words: Avoidance-preference; mercury; olfaction; sublethal exposure

KAMCHEN, R. and T.J. HARA 1980 Behavioral reactions of whitefish (*Coregonus clupeaformis*) to food extract: an application to sublethal toxicity bioassay. Can. Tech. Rep. Fish. Aquat. Sci. 975: 182-191.

Nous avons étudié le comportement de grands corégones (*Coregonus clupeaformis*) devant un concentré de nourriture préparé à partir de boulettes séchées, utilisant une auge d'évitement-préférence. Les poissons montrent une préférence marquée (attraction) pour l'extrait à la concentration de 0.2 g/L. Cette réaction dépend du sens de l'odorat puisqu'elle est éliminée après cautérisation des narines des poissons. Chez des sujets exposés à $HgCl_2$ à une concentration de 0.05 mg/L (1.8×10^{-7}M) durant 1 à 2 semaines, il y a diminution significative de la préférence. Dans des expériences conçues pour tester leur réaction directe, les grands corégones n'évitèrent pas des concentrations sublétales de $HgCl_2$. Nous examinons en fonction de ces résultats diverses méthodes d'application du comportement de préférence observé à des analyses biologiques sublétales de toxiques aquatiques.

INTRODUCTION

Avoidance-preference tests have attracted some interest in investigators engaged in the development of sublethal bioassays for aquatic toxicants. However, little or no investigation has been attempted to demonstrate whether these tests are in fact expedient in toxicity testing.

Avoidance-preference tests *per se* monitor the ability of the test organism to detect the presence of a chemical, whether toxic or non-toxic, and to respond to the chemical by moving into or away from it. The degree of toxicity and the avoidance level of toxicants are not always correlated, and many toxicants are not avoided at concentrations exceeding the lethal level. In fact some instances of attraction at lethal concentrations are reported (Sprague and Drury 1969; Hara and Thompson 1978; Hara 1980). The relevance of these tests to toxicity testing is thus restricted to determine (1) whether the organism can detect a toxicant, and (2) if so, whether preference for the toxicant will render it more hazardous, or whether avoidance may provide a chance for escape (Scherer 1977).

The alteration of a known behavioral reaction by a toxicant to a non-toxic stimulus has been suggested to be used as a measure for sublethal effects (Scherer 1977; Maciorowski et al. 1977). Feeding behavior of fish is an example. In many species, feeding behavior may be stimulated by various chemical components in foods and is a complicated process of successive individual behavioral components such as detection and attraction (Hara 1971). Olfaction is the major sensory channel for the detection of chemical signals (Hara 1975). Since the olfactory receptors are directly exposed to the environment, man-made alterations in the water quality could easily interfere with their functioning, and a breakdown in communications between fish and environment may consequently result.

In the present paper, methods to monitor and standardize behavioral reactions of fish to food extract with the use of a conventional avoidance-preference trough are described. Based on the toxicity data obtained using $HgCl_2$, possible application of the method to sublethal toxicity bioassay is further discussed.

EXPERIMENTAL

PROCEDURES OF BEHAVIORAL TESTS

Avoidance-Preference Reactions

The behavioral reactions of lake whitefish (*Coregonus clupeaformis*), 12-17 cm in body length, were tested in a avoidance-preference trough similar to that described by Scherer and Nowak (1973). The testing procedures used were described by Hara (1980) and are essentially as follows. Water flowed into each end of the trough and out at the center. By adjusting the flow rate, a distinct separation at the center between the two bodies of water could be attained. The testing procedure included a 5 min acclimation period, a 5 min control run with clean water in both halves, and a 5 min test run with a chemical introduced on one end. The avoidance-preference reactions were measured by calculating the ratio of the amount of time a fish spent in treated water to that in clean water.

Time responses over 50% were considered to be preference while time responses less than 50% were considered to be avoidance. Test solutions were introduced into either side at random.

Movement of fish from end to end of the trough under normal conditions is a prerequisite of this type of test. More than 80% of the total fish placed in the trough swam consistently, though variable in speed, and only those fish were used in the present study. Because the first experience of the chemical by fish affected their subsequent responses, each fish was tested only once at one concentration. Rainbow trout (*Salmo gairdneri*) were also tested for behavioral responses to chemicals in the trough. They swam actively but inconsistently and therefore were less suitable for the test.

Locomotor Activity

The locomotor or swimming activity of whitefish in the avoidance-preference trough was uniform and showed individual consistent rates for end-to-end motions during the control run. Upon encountering an introduced chemical, however, their activities tended to change by showing altered swimming rates, and sudden stoppages accompanied by sharp backward movements or a stationary posture. The change in activity was measured by registering the crossing-frequency of the fish over the boundary between the two halves of the trough. The difference in frequency between the control and experimental runs was expressed as either percent increase or decrease in activity.

Preparation of Food Extract

Food extracts were prepared daily from commercial food pellets (Silver Cup Trout Feed, Murray Elevators, Utah). Weighed amounts of pellets were suspended in distilled water, shaken for 3 min, and left in a refrigerator overnight. The supernatant was used for the behavioral tests after being filtered through a filter paper.

BEHAVIORAL REACTIONS TO FOOD EXTRACT

Control recordings with clean water in both halves of the trough indicated no bias; the mean value of time-responses for all tests was 52.4% ± 8.8% (open square in Fig. 1). The reactions of the fish to food extract in the trough were characterized by a nosing and exploratory behavior followed by a stationary posture. Figure 1 illustrates the results of all tests with food extract at various concentrations, with the mean responses joined by straight lines. At 2×10^{-2} g/L food extract, fish showed slight preference reactions (61.5% ± 11.9%). Although the degree of preference increased gradually with an increase in the food extract concentration, no significant preference was obtained until the concentration reached 2×10^{-1} g/L, where the mean response was 81.1% ± 8.3%.

As the fish became more attracted to the food extract and spent more time in the side containing food extract, fewer crossings over the boundary occurred. This is shown as a decrease in the activity in Fig. 1.

When the nares of whitefish were cauterized using concentrated nitric acid solutions, the behavioral reactions to food extract previously shown in normal fish were eliminated. No preference or avoidance behavior nor a significant

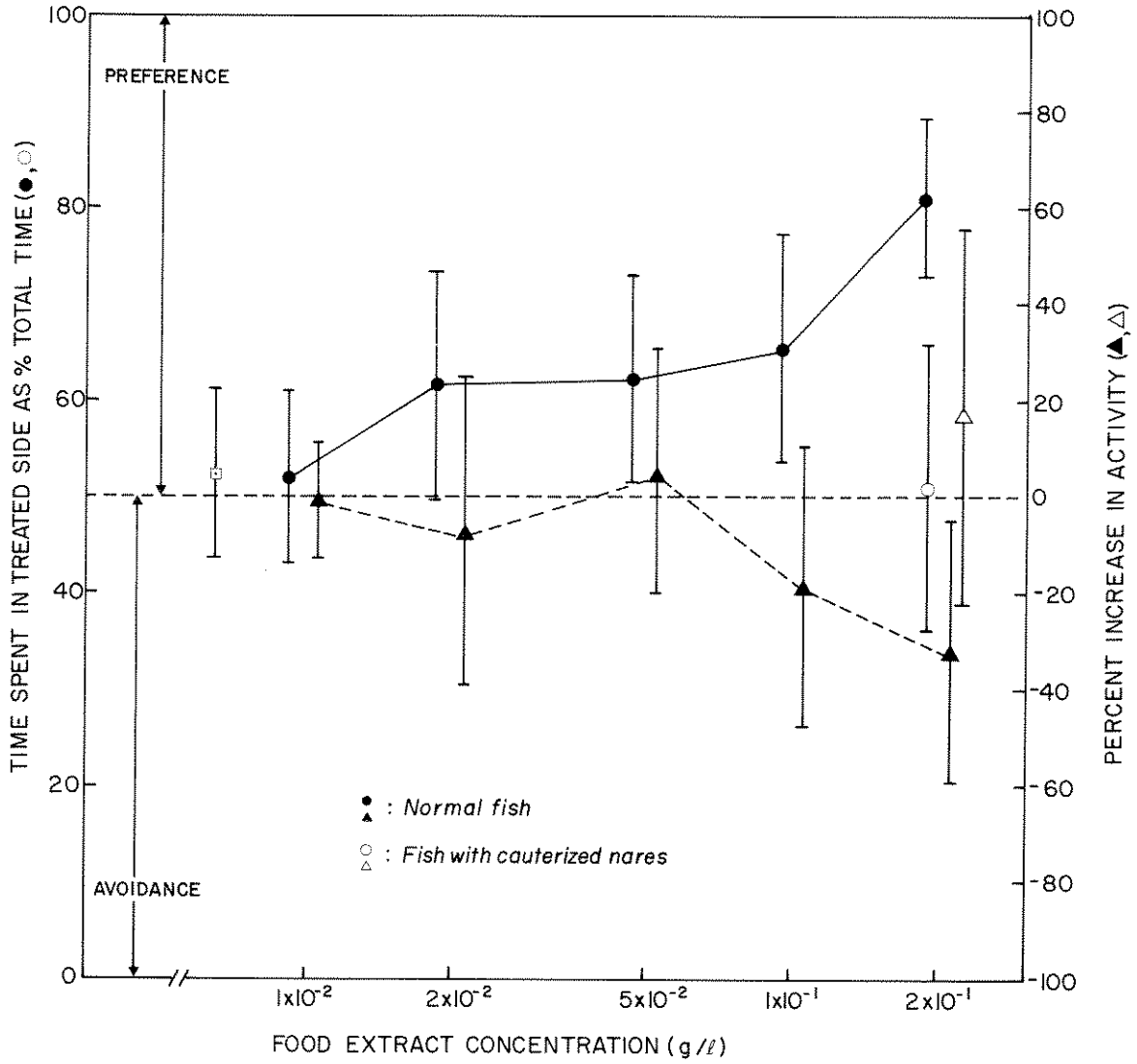


Figure 1. Behavioral reactions (avoidance-preference and activity) of whitefish to food extract. An open square shows the control response. Each point represents the mean of 10-12 tests. Vertical bars represent standard deviations.

change in activity was measured (Fig. 1). The results indicate the reactions to food extract were mediated through the olfactory system.

EFFECTS OF MERCURY ON THE BEHAVIORAL REACTIONS

Reactions to Mercury ($HgCl_2$)

The direct behavioral reactions of whitefish to mercury were biphasic, avoidance at low (1.8×10^{-7} and 1.0×10^{-6}M) and high (1.0 and 2.5×10^{-4})

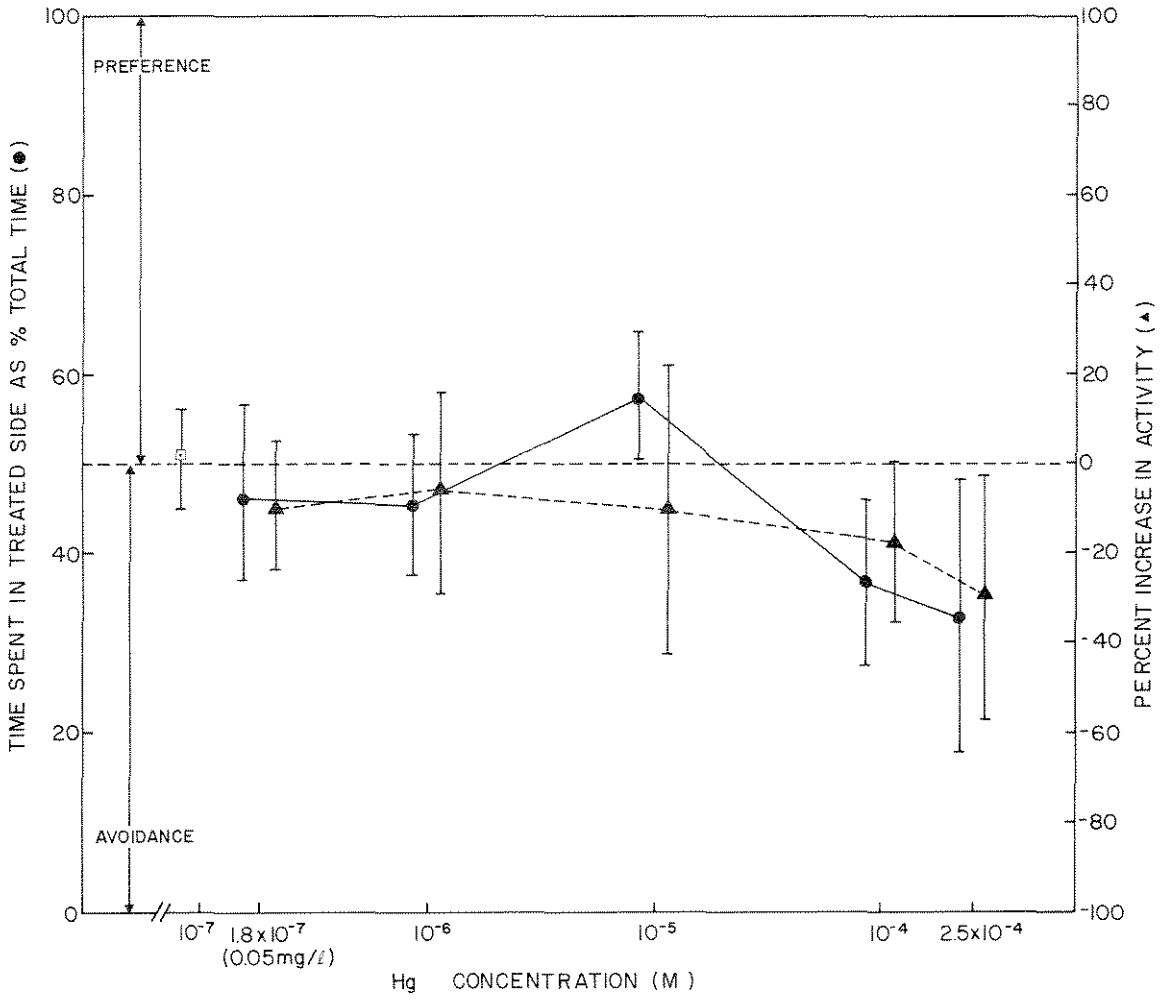


Figure 2. Behavioral reactions of whitefish to $HgCl_2$ solutions. Open square represents the control response.

concentrations and preference at the intermediate (1×10^{-5}M). However, at all except extremely high concentrations, reactions were not significant under the present experimental conditions (Fig. 2). Thus, using the avoidance-preference test method, no evidence was found which indicated that whitefish could detect mercury at sublethal levels.

Effects of Mercury on the Behavioral Reactions

It has been reported that the olfactory responses of fish to chemical stimuli are inhibited by perfusing water containing sublethal levels of mercury through the olfactory organ (Hara et al. 1976; Thompson and Hara 1977). Furthermore, chemical analyses have revealed that when whitefish were exposed to water containing low concentrations (0.01 and 0.05 mg/L) of mercury, the olfactory rosettes accumulated this metal rapidly. Accumulation factors of 800 and 4000, respectively, were estimated after a 2 week exposure (Fig. 3).

In order to investigate a possible effect of mercury on the behavioral reactions, a group of whitefish were exposed to 0.05 mg/L (1.8×10^{-7}M) $HgCl_2$

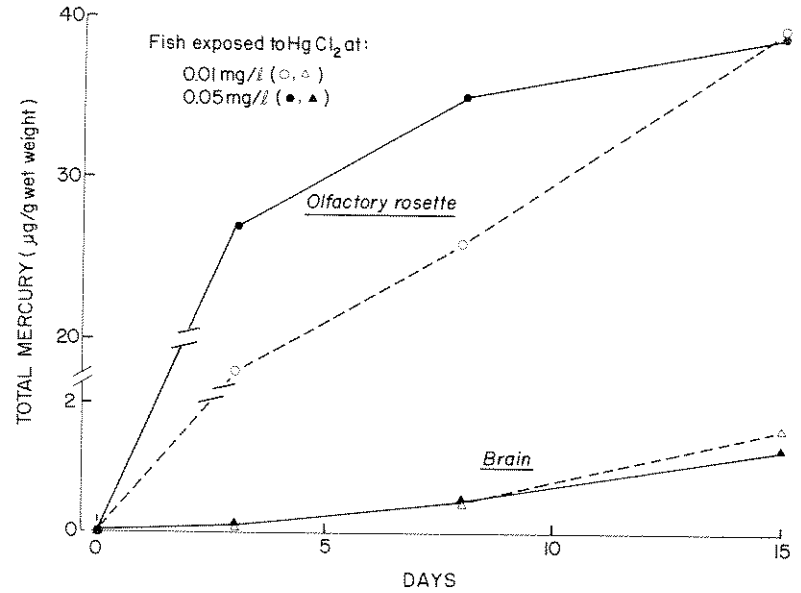


Figure 3. Accumulation of mercury by olfactory rosette and brain tissues of whitefish exposed to $HgCl_2$ at 0.01 0.05 mg/L. Each point represents the mean of determination from five fish.

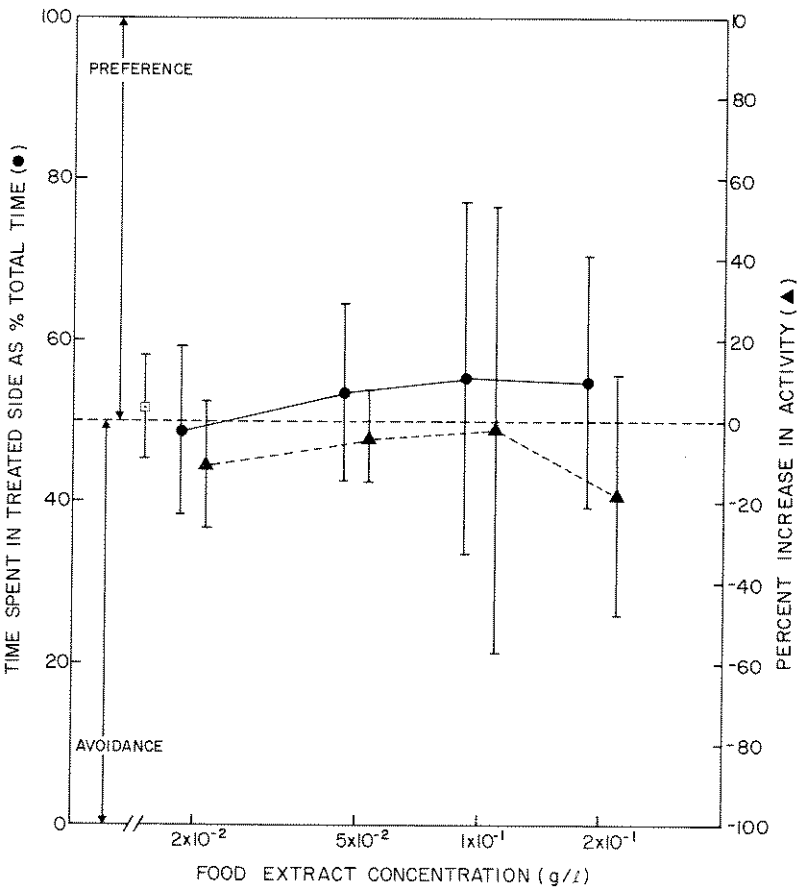
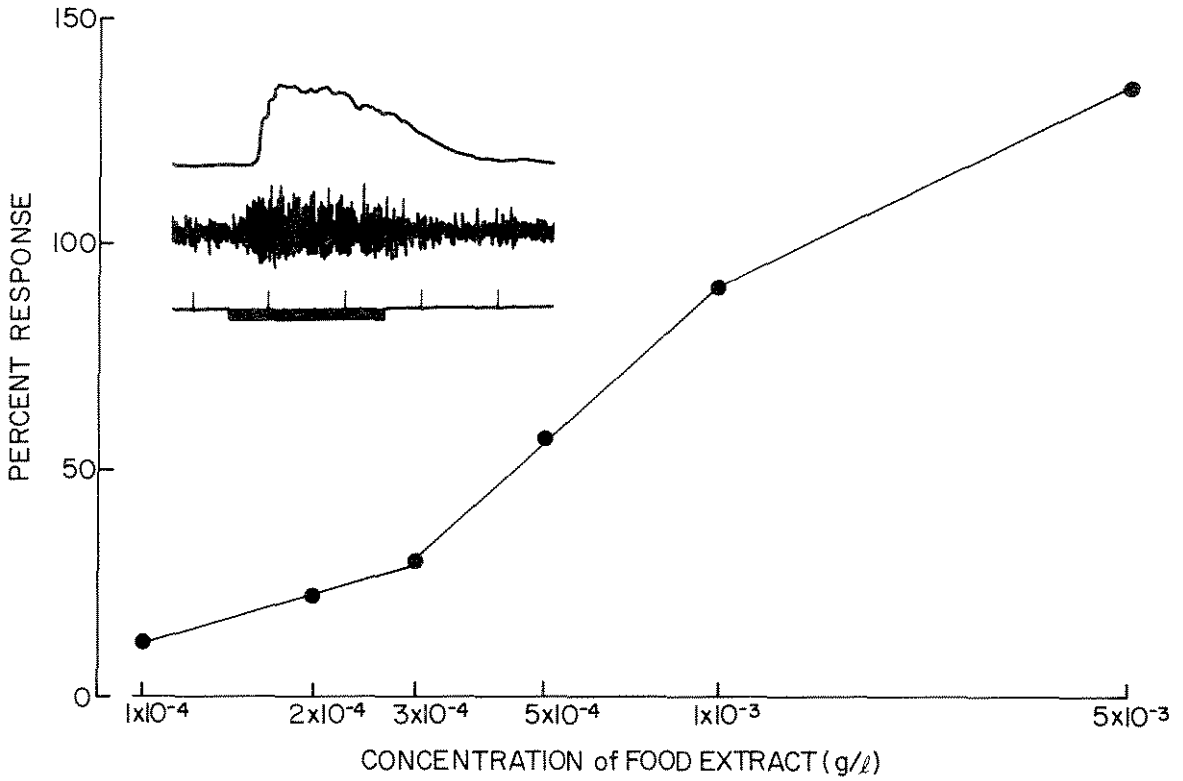


Figure 4. Behavioral reactions to food extract in whitefish pre-exposed to 0.05 mg/L $HgCl_2$ for 1 to 2 weeks.



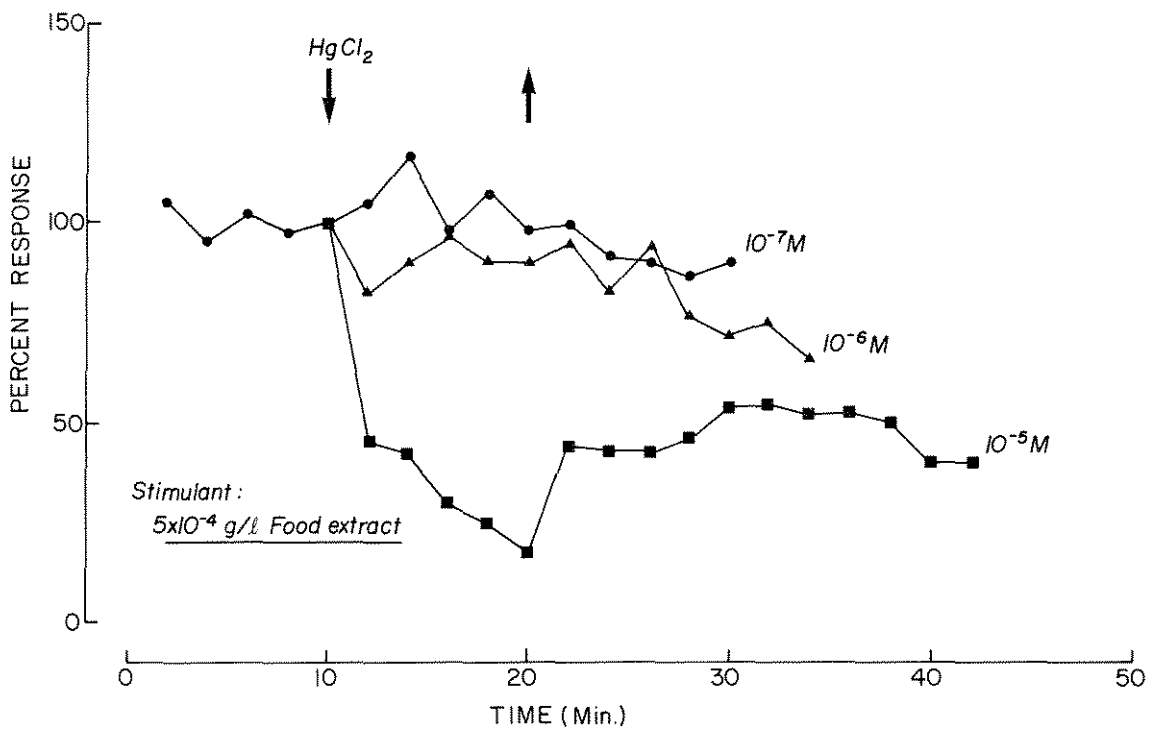


Figure 5. Concentration-magnitude relationships of the olfactory bulbar response to food extract in whitefish. The insert shows a typical recording of the response at 5 x 10^{-4} g/L food extract.

Figure 6. Bulbar responses to food extract over time when the nares were treated with $HgCl_2$ (10^{-7}-10^{-5}M). The arrows delineate the duration of the treatment.

for 1 to 2 weeks, and tested for their responses to food extract using the method described above. The tests showed greatly reduced behavioral reactions to food extract; no significant preference was observed at any of the concentrations tested (Fig. 4). Also, no significant change in the activity was recorded.

ELECTROPHYSIOLOGICAL CORRELATES

Olfactory Bulbar Electrical Responses to Food Extracts

The behavioral studies above have shown that the reactions to food extract are mediated through the olfactory system. The involvement of olfaction in the reaction was further investigated electrophysiologically by recording the electrical activities in the olfactory bulb when the nares were infused with food extract (for details, see Hara 1977). The olfactory bulbar response has been widely adopted as one of the most sensitive monitors of olfactory response of the brain in vertebrates (Hara 1975).

Figure 5 shows a relationship between the magnitude of the bulbar response and the concentration of food extract. The threshold concentration for the electrophysiological response, 1×10^{-4} g/L, was found to be approximately 100 times lower than that for the behavioral response (see Fig. 1).

Inhibition of the Bulbar Response by Mercury

Using essentially the same method described by Hara et al. (1976), the effects of mercury on the olfactory bulbar responses to a standard stimulant, 1×10^{-5}M L-serine or 5×10^{-4} g/L food extract, were examined. The bulbar responses were inhibited by a 10 min perfusion of the nares with 10^{-7} to 10^{-5}M $HgCl_2$ solution; the higher the concentration, the greater the inhibition (Fig. 6). Essentially similar results were obtained using L-serine as the standard stimulant. An accumulative effect of longer exposure to mercury is likely, since the inhibition due to the chemical appears to be irreversible (Fig. 6).

DISCUSSION

The present study has established that by using a conventional avoidance-preference trough, statistically significant preference reactions to proper concentrations of food extract can be monitored and standardized. These reactions may be significantly altered in whitefish by a one to two week exposure to a sublethal concentration of mercury. However, the same and higher concentrations of the toxicant produced no significantly altered response on the fish using the avoidance-preference test. The role of olfaction in the reactions has been investigated, and it appears that the olfactory organs may be the mediators of the reactions to food extract and therefore, the organs may have been affected by the toxicant. These results indicate that this particular series of tests may be appropriate for application to a sublethal toxicity test.

Using the behavioral tests with food-extract, two types of toxicity bioassay can be designed: (1) as in the case of the $HgCl_2$ exposure experiment in the present study, behavioral responses can be compared between two groups

of fish, one control and the other pretreated with test chemicals of varying concentrations and time periods, and (2) food extract and test chemicals can be simultaneously introduced into one end of the avoidance-preference trough. The latter type of test proposed is essentially the same as those reported by McLeese (1973, 1974, 1975), who investigated the effects of copper, fenitrothion, and bleached kraft mill effluent on olfactory responses to pheromone and food extract in American lobster (*Homarus americanus*). This method may be only applicable to those chemicals, such as copper and mercury, whose affinity to biological membranes is extremely high, and should therefore produce satisfactory results (McLeese 1975; Hara et al. 1976; Fig. 6). For most chemicals including insecticides such as fenitrothion whose target organ is assumed to be within the central nervous system, the former method is more applicable. In both types of tests the threshold concentration for the toxicity of a test chemical could be extrapolated from the percent inhibition data obtained from testings at more than one concentration of the toxicant.

The test method presented in this study may be applied to other fish species and toxiants as a measure of toxicant-altered responses to sub-lethal toxicity bioassay. The threshold concentration obtained for food extract, though reasonably low, could be improved in future tests if, for example, natural food materials for food species were used instead of commercial food pellets. Also, other biologically-relevant agents can be used as stimuli to induce behavioral reactions in an organism. It is important to note though, that not all chemical information perceived are integrated in the central nervous system to result in observable behavioral changes.

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A STATISTICAL CORRELATION BETWEEN AN INFILTRATIVE HYALINOCYTIC CONDITION IN A MARINE BIVALVE MOLLUSK AND SEDIMENT LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS

W.J. Cretney, C.S. Wong

Department of Fisheries and Oceans, Institute of Ocean Sciences, Patricia Bay, P.O. Box 6000, Sidney, B.C., V8L 4B2

D.A. Brown, W.A. Heath, K.A. Thompson and B.R. Fowler

Seakem Oceanography Ltd
9817 West Saanich Road, Sidney, B.C., V8L 3S1

CRETNEY, W.J., C.S. WONG, D.A. BROWN, W.A. HEATH, K.A. THOMPSON and B.R. FOWLER 1980 A statistical correlation between an infiltrative hyalinocytic condition in a marine bivalve mollusk and sediment levels of polycyclic aromatic hydrocarbons. Can. Tech. Rep. Fish. Aquat. Sci. 975: 192-203.

Histological examination of bivalve mollusks from Kitimat harbour and its approaches in northern B.C. reveals the presence of a condition in many animals involving extensive infiltration of tissue by hyalinocytes. The incidence of the condition in the mussel, *Mytilus edulis*, correlates positively with the benthic sediment levels of polycyclic aromatic hydrocarbons (PAHs), although there is no evidence as yet to infer whether the correlation is causal or coincidental. Cytological examination of the hyalinocytes indicates that they are normal.

Key words: Histopathology; mussel health; carcinogenesis

CRETNEY, W.J., C.S. WONG, D.A. BROWN, W.A. HEATH, K.A. THOMPSON and B.R. FOWLER 1980 A statistical correlation between an infiltrative hyalinocytic condition in a marine bivalve mollusk and sediment levels of polycyclic aromatic hydrocarbons. Can. Tech. Rep. Fish. Aquat. Sci. 975: 192-203.

Un examen histologique de mollusques bivalves du port de Kitimat et de ses approches en Colombie-Britannique septentrionale indique la présence, chez plusieurs sujets, d'une condition caractérisée par une importante infiltration du tissu par des hyalinocytes. Il y a corrélation positive entre l'occurrence de cette condition dans la moule, *Mytilus edulis*, et les niveaux d'hydrates de carbone aromatiques polycycliques (PAH) du sédiment benthique. On ne peut toutefois, pour le moment, décider si cette corrélation est causale ou coïncidente. L'examen cytologique des hyalinocytes indique qu'ils sont normaux.

INTRODUCTION

The Kitimat area of B.C. has only recently entered the modern industrial age. Prior to the spring of 1951, the only permanent settlement in the area was the Haisla Indian village, Kitimaat, on the east side of Kitimat Harbour. That spring marked a beginning of construction in the area resulting in a few years in the Alcan aluminum smelter and the town of Kitimat. In August 1954 the smelter commenced production. Today, the plant produces just under 300,000 metric tons of aluminum per year and the town has grown to a population of about 14,000. The Eurocan pulp and paper mill established in 1970 became the second major industry of Kitimat and today there is serious talk of other major industrial concerns locating there. This winter, moreover, Alcan Smelters and Chemicals Ltd. announced plans to treble the firms smelting capacity by the construction of three new smelters.

A recent study of aluminum plants on Norwegian fjords (Palmock 1974) produced evidence that the plants may be a major source of PAHs in the local marine environment. Indeed, our study of marine sedimentary hydrocarbons in the Kitimat area implicates the aluminum plant there as a major chronic source of environmental PAHs (Cretney et al. in prep.). The hydrocarbon distribution in dated cores indicates that there has been a more than two orders of magnitude increase in the concentration of PAHs in sediments of Kitimat Harbour since the middle of this century, paralleling a similar increase of PAH concentration in benthic surficial sediments between remote and harbour sites.

Because of the large PAH gradient, the Kitimat area is a potentially good natural laboratory in which to study the biological effects of PAH pollution, provided of course that such effects can eventually be distinguished from the effects of natural stresses and other pollutants. The last consideration prompted us to initiate a study of Alberni Inlet and will lead us to study a suitably pristine inlet as well. Alberni Inlet was chosen for comparison because Port Alberni at its head has a similar population as Kitimat, a large pulp and paper mill, but no aluminum smelter. The blue mussel, *Mytilus edulis*, was chosen as the principal study organism because of its role in the International Mussel Watch program (Goldberg in press) and also because of reports of putative neoplastic lesions in marine bivalve mollusks (Farley 1976; Mix et al. 1977), particularly those reports of lesions in bivalves from petroleum oil (a source of PAHs) spill sites (Barry and Yevich 1975; Brown et al. 1977; Yevich and Barszcz 1977).

METHODS

BIVALVE COLLECTION

In the Kitimat study area samples of mussels (*Mytilus edulis*) and/or clams (*Macoma inconspicua*) were collected from shore sites near regular oceanographic stations in Kitimat Arm, Douglas Channel, Kitkiata Inlet and Wright Sound. In the Port Alberni study area *Mytilus edulis* samples were obtained from Christie Bay and the mouth of Uchucklesit Inlet at the entrance to Alberni Inlet and at intervals along the inlet shore up to Port Alberni. In both areas, mussels were obtained from the mid-tide level during periods of low tide from rock and natural wood surfaces. At Alberni, however, some mussels were also collected by diving. Mussels having about a 50 mm shell length were collected at each station for histopathological examination and handled as described below. At the same time about 500 g of bivalves were collected, wrapped in aluminum foil and frozen for chemical analysis.

SEDIMENT COLLECTION

A Smith-McIntyre grab sampler (Kahlsico International Corp.) was used. This sampler provides 0.1 M^2 cross sectional area of relatively undisturbed benthic surficial sediment. An aluminum ring (90 mm diam. x 50 mm deep) cut from a can was pressed fully into the sediment surface with the lid in place to minimize its deformation. A broad bladed spatula was used to separate the core at the base of the ring and lift the core and ring from the bulk of the sediment. The core was transferred to an intact aluminum can (90 mm x 53 mm deep) and the can's lid was sealed in place with Teflon tape covered with masking tape. The sealed aluminum cans were immediately transferred to a freezer. Sediment samples were kept frozen until the time of analysis, when they were thawed and subsampled.

PREPARATION OF TISSUE SAMPLES

The animals were maintained for 24 hours in unfiltered seawater to allow self-cleaning of sand, mud, and other abrasives. This procedure was a precaution taken to reduce problems from grit during microsectioning of the tissue. Following flushing, the bivalves were shucked into Helly's fixative (Barszcz and Yevich 1975), sagitally sectioned if necessary, and fixed for 8 to 24 hours depending on size; less time for smaller animals more for larger ones. The tissues were then rinsed continuously in seawater or freshwater for 16 hours to remove excess fixative before being stored in 70% ethanol until further processing. Sections (6 µm) were prepared according to Bayne et al. (1979) and examined using a Zeiss Jena Ergaval microscope. Photomicrographs were prepared using a Carl Zeiss Universal R microscope with camera attachment.

HISTOPATHOLOGICAL RATING OF INFILTRATIVE HYALINOCYTIC CONDITION (IHC)

Ratings for *Mytilus edulis* collected during February, 1979 in the Kitimat study area were done "single blind" with the raters knowing the sampling sites, but not the PAH levels in the tissues or nearby benthic sediments. Ratings for all subsequently collected samples were done "double blind". Indices for the condition were assigned for given tissue sections (3 per animal) using a set of integer-rated "benchmarks" (Table 1).

Fractional indices were given for tissue sections showing characteristics intermediate to the benchmarks.

Table 1. Integer-rated benchmark characteristics for IHC[a].

Rating	Characteristics
0	no detectable[b] hyalinocytes[c]
1	high level of hyalinocytes relative to granulocytes[d]; indication of tissue degeneration
2	as for a 1 rating, but widespread degeneration of muscle tissue, digestive diverticula and reproduction follicles, the last in pre-spawn animals and distinct from natural resorption in post-spawn animals
3	as for a 2 rating, but massive degeneration and infiltration

[a] animals considered to exhibit the condition were those for which the IHC index was ≥ 1. [b] in only one animal were no hyalinocytes observed in any of the 3 sections. [c] hyalinocytes (4.5-6 µm diam.) have a small amount of basophilic cytoplasm, enlarged ovoid nucleus, pale granulated or non-granulated nucleoplasm and visible nucleoli. [d] granulocytes (8-11 µm diam.) have a small intensely coloured blue (basophilic) nucleus and orange-red (eosinophilic) granular cytoplasm.

PAH ANALYSIS

PAHs in tissues and sediments were analysed by GC/MS/MIM according to procedures described in detail elsewhere (Wong et al. 1976; Cretney et al. 1980). In the case of the sediment analyses, a modification of the method was made in which each isooctane wash of the sediment sample was carried out at reflux temperature with stirring. Also, a 3.05 m x 2.0 mm i.d. silanized glass column packed with 2.3% Dexsil 300 on Chromosorb W(AW), and, more recently, with 3.1% Dexsil 300 on Chromosorb 750 was used in the GC/MS/MIM system. Silyl 8 was used as necessary to eliminate chemisorptive broadening of peaks from individual PAHs in amounts of about 1 ng. The moisture content of sediments was determined as the weight loss on drying to constant weight in a forced air oven at 80° C.

RESULTS

Of the bivalve populations studied (Table 2), those from inside Kitimat Harbour, the limit of which is about 4 km south of sampling site L on Markland Point, show the highest incidence of the infiltrative hyalinocytic condition. This condition is characterized by the presence of high numbers of hyalinocytes (Figures 1a and 1c) and tissue degeneration in affected animals compared to unaffected animals. Reduction in the number of muscle bundles is observed in animals having a large number of infiltrative hyalinocytes (cf. Figure 1a and 1b) and is likely reflected in the ease with which many animals from affected populations can be shucked. Infiltration of digestive diverticula by granulocytes occurs in mussels in Alberni Inlet (Figure 1d). This infiltration by granulocytes is concommitant with the degeneration of the

Table 2. Sampling periods and locations, IHC indices of bivalve mollusks, and benz(a)anthracene group concentration in sediments.

Bivalve [nearby[a] sediment] sites	Location	Distance[b] from inlet head (km), bivalve[c]	Mean IHC Index SE [n][d] February[e]	April/July[f]	% with IHC (Feb., Apr./July)	BaA group[g] conc. (µg/kg)
	Kitimat Area:					
A [6]	Gil Isle.N. tip	88,m	0.45±0.04 [25]	0.41±0.07 [25]	0,4	99
F [7]	Gertrude Point	58,m	n.d.[h]	0.67±0.07 [17]	n.d.,12	148
G [CTD 6,7]	Hawkesbury Isle.	48,m	0.51±0.04 [25]	n.d.	0, n.d.	171
L [8,G-16]	Markland Point	20,m	1.29±0.12 [25]	n.d.	56, n.d.	587
M [G-16]	Bish Creek, S.side	14,m	0.99±0.09 [25]	n.d.	52, n.d.	780
N [G-9]	Kitimat Arm, W. side	8,m	0.80±0.06 [25]	0.91±0.05 [25]	24, 36	1037
O [G-1]	Kitimat Arm, W. side	1,m	0.73±0.05 [25]	0.98±0.06 [25]	16, 32	1402
X3 [10-2]	Minette Bay, E. side	0,m	n.d.	0.76±0.07 [24]	n.d., 33	405
P [G-4]	N. of Wathl Creek	2,m	0.74±0.06 [25]	n.d.	16, n.d.	288
X4 [G-4]	At Wathl Creek	2,m	n.d.	0.86±0.06 [24]	n.d., 25	288
F [7]	Gertrude Point	58,c	n.d.	0.33±0.06 [12]	n.d., 0	148
X1 [10-1,G-2]	near Alcan dock	-1,c	n.d.	0.67±0.08 [29]	n.d., 21	1063
X2 [G-2,G-3]	E. of Eurocan dock	0,c	n.d.	1.04±0.12 [29]	n.d., 45	475
X5 [10-1]	S. of Alcan dock	0,c	n.d.	0.87±0.12 [29]	n.d., 28	1250
	Port Alberni Area:					
1 [1]	Christie Bay	44,m	n.d.	0.26±0.03 [25]	n.d., 0	78
2 [2]	Brookby Point	32,m	n.d.	0.16±0.01 [25]	n.d., 0	85
3 [3]	Nahmint Bay, S. side	21,m	n.d.	0.18±0.01 [25]	n.d., 0	75
4 [5]	S. of Underwood Cove	11,m	n.d.	0.27±0.01 [25]	n.d., 0	n.d.
5.5 [6]	Stamp narrows W. side	6,m	n.d.	0.37±0.01 [25]	n.d., 0	78
7 [6,7]	W. Alberni Inlet	4,m	n.d.	0.23±0.01 [25]	n.d., 0	108
8 [7]	Polly Point	2,m	n.d.	0.26±0.01 [25]	n.d., 0	138

[a] Separation of bivalve and sediment sampling stations varies from a few hundred meters in harbour area to several kilometers in some distant stations. [b] Straight line distance. [c] m=mussel, c=clam. [d] S.E.=standard error, n=number of animals examined from station. [e] All bivalves were sampled in 1979. [f] Clams and mussels in Kitimat area were sampled in April; mussels in Port Alberni area, in July. [g] mean conc.(dry wt.) for equidistant sediment samples or for samples taken at same site on different cruises; in the latter case, mean rel.standard dev. = 0.41 for 9 stations sampled June, October, 1978 and also February, 1979; rel.standard dev. = 0.07 for a well homogenized sediment sample. [h] n.d. = not determined.

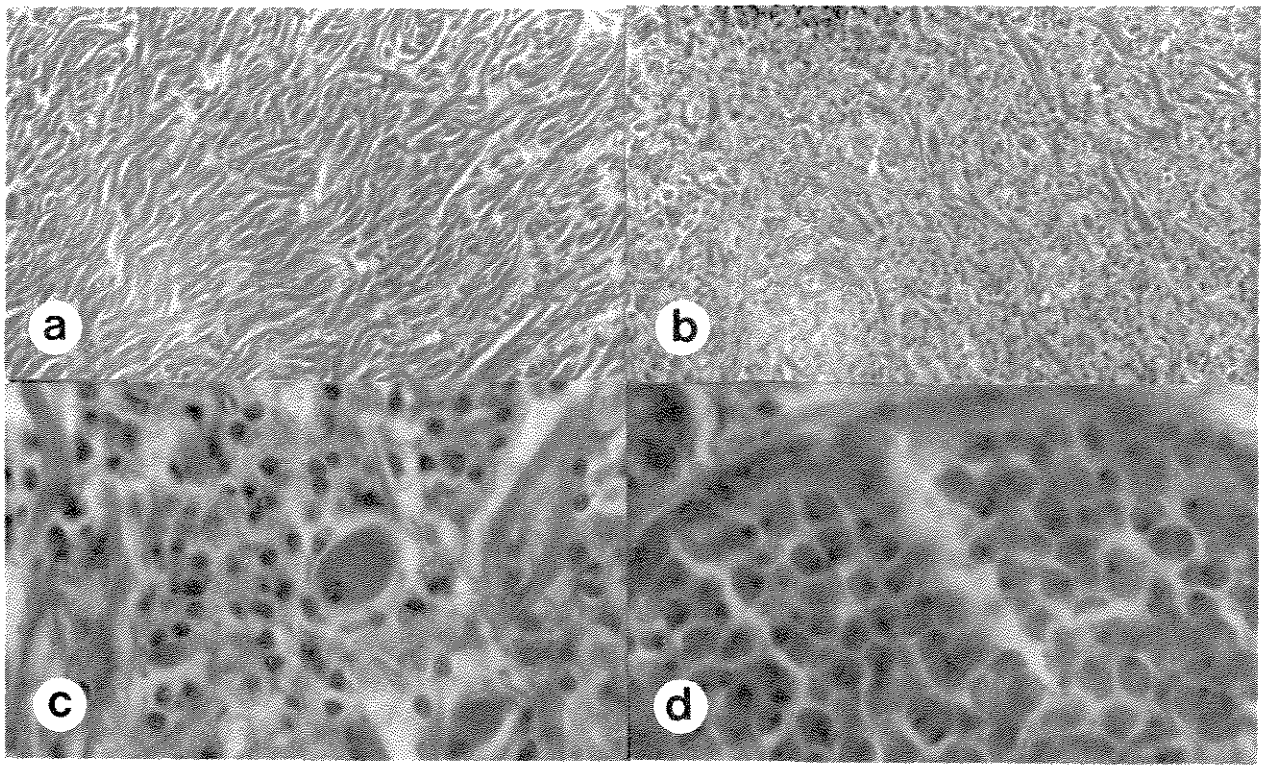

Figure 1. (a) normal muscle tissue from a mussel collected at site A in February, 1979. IHC index 0.2. 193X. (b) hyalinocyte infiltrated muscle tissue from a mussel collected at site L in February, 1979. IHC index 3.0. 193X. (c) another muscle tissue section from same animal as in (b). IHC index 3.0. 772X. (d) granulocytes in a digestive diverticulum of a mussel collected at site 5.5. IHC index 0.7. 772X.

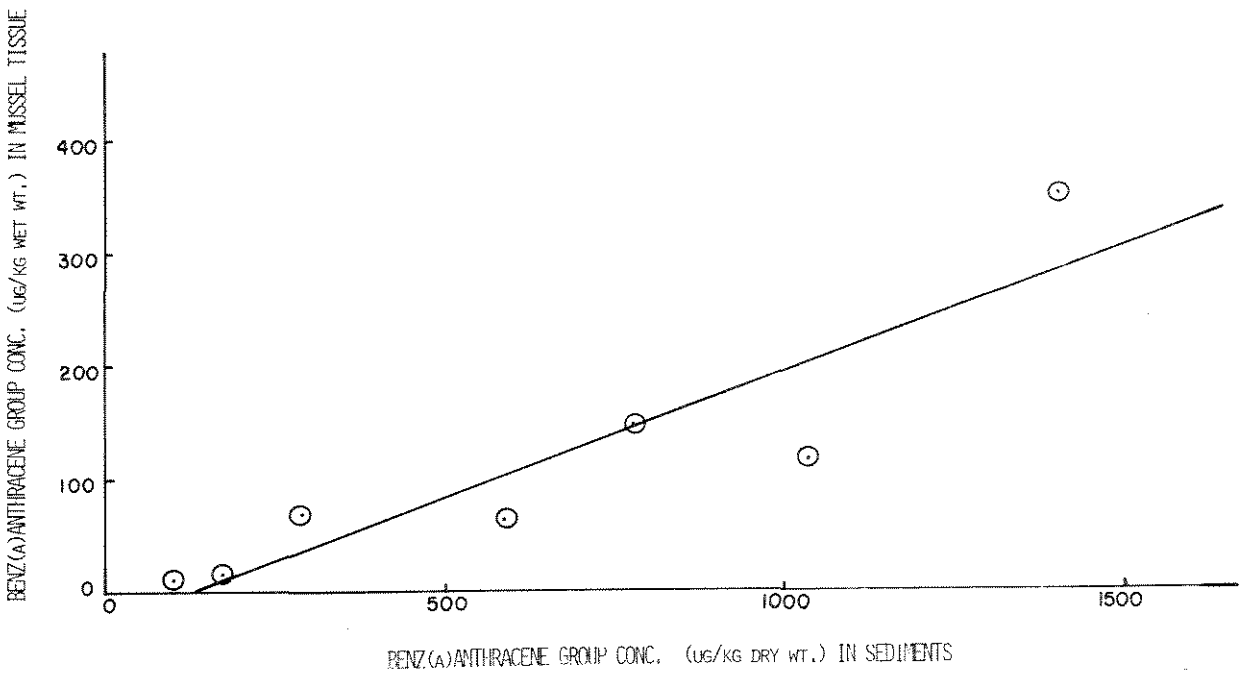

Figure 2. Relationship between benz(a)anthracene group concentrations in mussels (collected in February, 1979) and sediments in the Kitimat study area.

ciliated columnar epithelium of the digestive diverticula. The degeneration of the digestive diverticula in mussels from Kitimat Harbour is histopathologically similar, with the infiltrative hemocytes being hyalinocytes rather than granulocytes. A similar contrast is observed in the nature of reproductive degeneration between post spawn, site A and pre-spawn, harbour populations in the Kitimat study area sampled in April 1979. Degeneration of reproductive follicles and remaining unspawned gametes from unspent follicles in post spawn animals is associated with granulocytes. The reproductive degeneration in unspawned, harbour populations is associated with the presence of hyalinocytes. Lack of space precludes a more detailed histopathological report and a broader analysis of the relationship between mussel condition and natural or anthropogenic stressors in the study areas. Our mussel health survey has included an assessment of a number of factors such as population size and average individual size, reproductive stage and degeneration, digestive transformation and degeneration, myodegeneration, nutritive state, parasitism, infiltration by hyalinocytes and granulocytes, water temperature and salinity, and probable sources and kinds of pollutants (Brown et al. in prep.; Cretney et al. in prep.).

The similarity between the trends in the values of the mean IHC indices for different mussel populations and the concentrations of PAHs in nearby benthic sediments (Table 2) led us to examine the possibility that the two parameters were statistically correlated. In Figure 3 the mean IHC index values are plotted as a function of the sediment concentrations of the benz(a) anthracene group of PAHs, which consists of isomers such as chrysene. The plot indicates that the parameters are correlated. Since the sediment PAH values are heteroscedastic, having approximately constant relative standard deviations, and the values of the IHC indices for individual mussels from a given population appear not to be normally distributed (Cretney et al. in prep.), a non parametric correlation method seems appropriate. The Spearman rank correlation method (Zar 1974) gives a rank correlation coefficient, r_s, of 0.842 ($p < 0.001$) for all the data in Figure 3. If the Alberni Inlet stations are omitted from the correlation, $r_s = 0.648$ ($p = 0.02$) with a correspondingly higher, though still low, probability of error in rejecting the proposition that the IHC indices and benz(a)anthracene group concentrations are not correlated.

The plot in Figure 3 presumably could be fitted using a polynomial or some non-linear (asymptotic regression) model. Nevertheless, because of the heteroscedasticity in the data the plot does not provide a true picture, tending to overemphasize the importance of points having high IHC index and PAH concentration that are known with less precision. In this regard it is noteworthy that the Spearman rank correlation coefficient increases only marginally to 0.856 ($p < 0.001$) on leaving out the data for stations M, N, and O. Nevertheless, a plot of the log transformed data, which is probably statistically more appropriate (Eberhardt 1976), indicates that the levelling off in the IHC index for the west-side harbour mussels may be real. Additional work is planned to broaden the data base, both seasonally and geographically, to corroborate the findings so far, particularly with respect to the levelling off in the IHC index at higher concentrations of PAHs.

Although only a small number of mussels have been analysed up to the present, the possibility of a correlation between tissue and sediment concentrations of PAHs has been examined. The benz(a)anthracene group

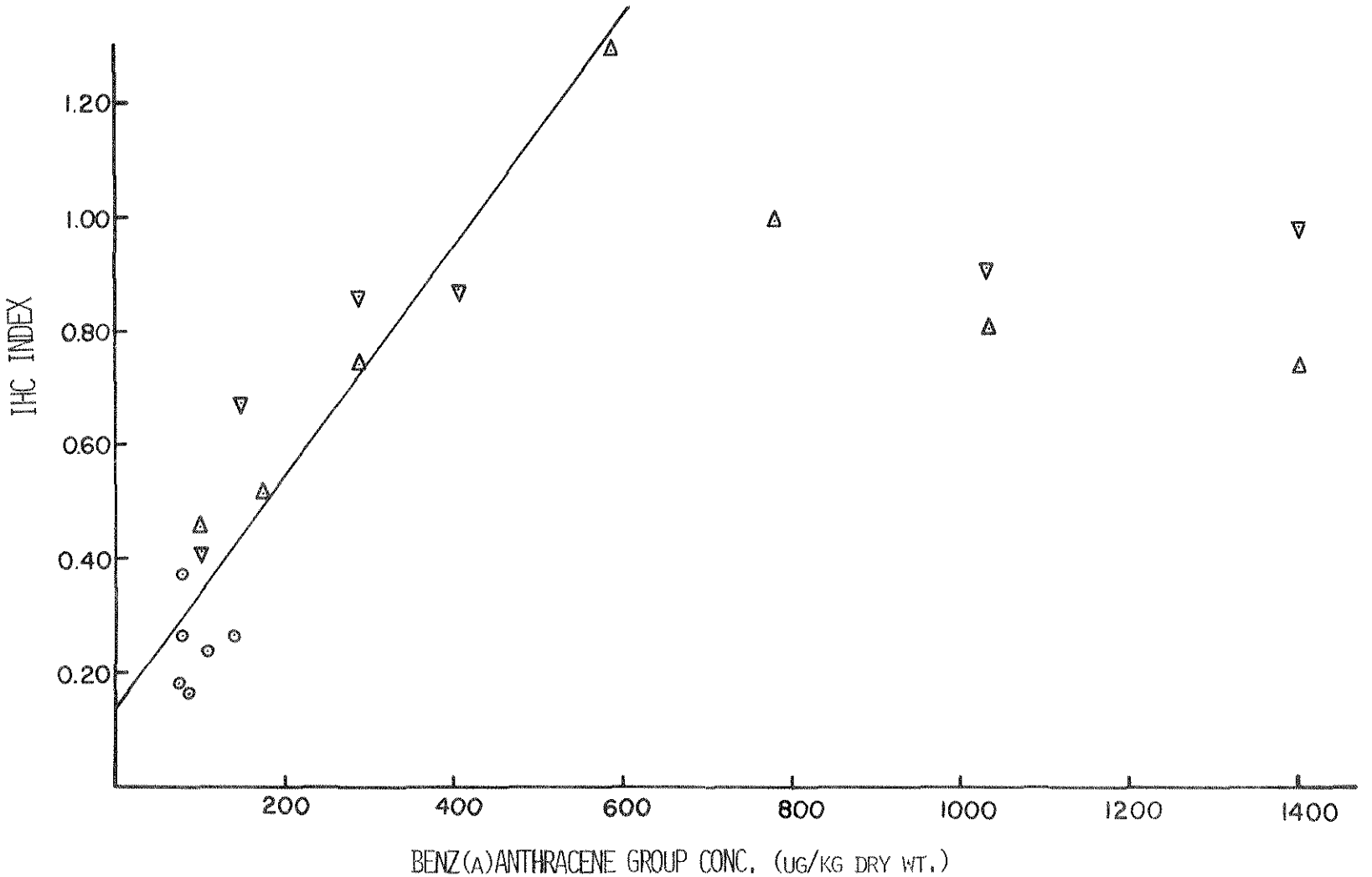

Figure 3. Relationship between IHC index for mussels and the concentration of benz(a)anthracene group PAHs in nearby benthic sediments. Port Alberni area mussels, July, 1979 (○); Kitimat area mussels, April, (▽), February (△), 1979.

concentration in tissue from mussels collected in February 1979 is plotted as a function of the benz(a)anthracene group concentration in nearby benthic sediments in Figure 2. A correlation coefficient of 0.907 ($p < 0.005$) is obtained for the untransformed data and a correlation coefficient of 0.957 ($p < 0.001$), for the log transformed data. Dunn (1979) has reported a similar correlation between mussel tissue and sediment concentration of benzo(a)pyrene.

DISCUSSION

While recognizing the preliminary nature of the evidence gathered so far, some speculation can be made regarding the processes that might be governing the correlations presented above. The approximately linear portion of the plot (indicated by the oblique straight line) suggests a possible direct relationship between the IHC indices and the PAH concentrations up to a limit. With regard to the apparent levelling off in the IHC index with increasing PAH concentration, a number of potential explanations present themselves. The most attractive explanation to us at present would have the mussels with the infiltrative hyalinocytic condition succumbing at a faster rate than those without the condition because of the toxicity of the PAHs where their sedimentary concentrations are highest along the west side of the inner harbour (sites M, N, and O) or because of the toxicity of associated chemicals (eg. fluorides). In this regard, however, any stress affecting the inner harbour that is either absent outside it or not severe enough to have an observable effect could account for the apparent phenomenon through selective removal of animals with the condition. Since the animals that display the condition (IHC index ≥ 1) by definition (Table 1) are in poorer health than those that do not, it does not seem unreasonable to expect them to be less resistant to stress. Alternatively, the west side, inner harbour populations may be genetically more resistant to developing the condition. If an infectious agent is involved, the west side inner harbour sites may be less conducive to its development. If chemical agents are involved, photooxidation or similar processes may increase the potency of the mixture by generating new compounds or removing antitoxins, with an effectiveness that counters the dilution that occurs with distance from the source. Clearly, a number of possible explanations exist and those presented here are admittedly biased by our desire to establish a link, if one exists, between the infiltrative hyalinocytic condition and pollutants, particularly PAHs, in the study area.

The high correlation between the mussel tissue concentrations of PAHs and the sediment concentrations in our view is a consequence of there being a chronic point source of highly concentrated PAHs on suspended particles. As in the case of mussels and benthic sediments, a steep gradient in the PAH concentration of unfiltered seawater is observed between stations inside and outside of the harbour (Cretney et al. in prep.). Both the mussels, which are filter feeders,and the sediments receive PAHs from the common pool of PAH containing suspended particulates. The steep gradient in the seawater concentration of PAHs thus results directly in a steep gradient in the concentration of PAHs in benthic sediments and mussels. The high correlation results from there being a sufficiently large range of concentrations attributable to the gradient to minimize the effect of local differences in such factors as rate of uptake, partitioning, and rate of depuration in mussels collected at the same time of year and dilution of the highly

concentrated PAH containing particulates with less concentrated PAH containing particulates from other sources.

The infiltrative cells involved in the condition described in this paper appear to be normal hyalinocytes (immature hemocytes). Since no mitotic figures have been observed, the hyalinocytes do not appear to be proliferating in the infiltrated tissues where they are seen in elevated numbers. Since the hyalinocytes are neither atypical nor actively mitotic, the condition does not meet the criteria (Pauley 1969) of molluscan neoplasia. In this regard the condition is distinct from the putative neoplastic conditions reported by Yevich and Barszcz (1977), Brown et al. (1977), Mix et al. (1977, 1979) and Lowe and Moore (1978). The condition does resemble however, an epizootic disease found in clams, *Mya arenaria*, collected in Umpqua Bay, Oregon (Farley 1976). Farley characterized that disease as appearing "... to be similar to the leukemoid response seen in mammals ...(and) ... associated with the presence of small, difficult to see, protistan parasites." The Kitimat bivalve condition does not appear to be associated with such parasites, but the possibility that it is simply a normal inflammatory response to an infectious agent (Bayne et al. 1979), or a chemical one for that matter, cannot be ruled out. The presence of an infectious agent, however, would not necessarily preclude the possibility of some involvement of environmental contaminants. Conceivably,for example, the infectious agent could act as a cocarcinogen (Bingham et al. 1976; Stich et al. 1977; Jarret et al. 1978) with certain PAHs.

The correlation reported here between the infiltrative hyalinocytic condition in mussels and PAHs in sediments, it must be emphasized, is purely statistical. No cause and effect relationship is claimed or implied. It is our intent for the near future to characterize the condition more fully by using additional histopathological techniques such as electron microscopy and verify the correlation by gathering more information on the seasonal and geographic variability of the condition and tissue concentrations of PAHs.

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MONITORING ENVIRONMENTAL CONTAMINATION FROM CHLOROPHENOL CONTAMINATED WASTES GENERATED IN THE WOOD PRESERVATION INDUSTRY

G.A. Vigers[1] and A. Maynard[2]

[1] E.V.S. Consultants Ltd., 195 Pemberton Avenue, North Vancouver, B.C., V7P 2R4

[2] Can Test Ltd., 1650 Pandora Street, Vancouver, B.C., V5L 1L6

VIGERS, G.A. and A. MAYNARD 1980 Monitoring environmental contamination from chlorophenol contaminated wastes generated in the wood preservation industry. Can. Tech. Rep. Fish. Aquat. Sci. 975: 204.

An exploratory field and analytical program was undertaken to investigate the presence and bioaccumulation of chlorophenol and chlorobenzene contaminants from the forest industry in fresh water, estuarine, and marine biota at ten sites on the Strait of Georgia, British Columbia. Sediments, surface waters, and biota were sampled and analysed for chlorophenol isomers, pentachloroanisole, and chlorinated benzenes. Commercial PCP formulations were found to be extensively contaminated with related compounds. Livers from prickly sculpins (*Cottus asper*) and staghorn sculpins (*Leptocottus armatus*) contained penta- and tetrachlorophenol at concentrations 1-3 orders of magnitude greater than skeletal muscle. Sculpin liver tissue exhibited preferential uptake of pentachlorophenol with levels averaging 402 and 448 ppb respectively.

Key words: PCP; body burdens; bioaccumulation; forest industry

VIGERS, G.A. and A. MAYNARD 1980 Monitoring environmental contamination from chlorophenol contaminated wastes generated in the wood preservation industry. Can. Tech. Rep. Fish. Aquat. Sci. 975: 204.

Un programme analytique et d'exploration sur le terrain a été entrepris dans le but d'étudier la présence et la bioaccumulation des contaminants chlorophénol et chlorobenzène de l'industrie forestière, dans des biocoenoses d'eau douce, d'estuaire et de mer à dix sites du Strait of Georgia (Colombie-Britannique). Nous avons déterminé les isomères du chlorophénol, le pentachloroanisol, et les benzènes chlorés dans des échantillons de sédiment, d'eau de surface et de biocoenose. Nous constatons que les PCP de commerce sont fortement contaminés par des composés apparentés. Le foie de chabots piquants (*Cottus asper*) et de chabots armés (*Leptocottus armatus*) contient du penta- et du tétrachlorophénol à des concentrations de 1-3 ordres de grandeur plus grandes que celles du muscle du squelette. Le tissu hépatique de ces chabots absorbe le pentachlorophénol plus que tout autre contaminant à des niveaux moyens de 402 et 448 µg/L respectivement.

CONTAMINANTS IN TRACE METAL ANALYSIS

A. Lutz

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

LUTZ, A. 1980 Contaminants in trace metal analysis. Can. Tech. Rep. Fish. Aquat. Sci. 975: 205-213.

In trace metal analysis on environmental samples, great care should be exercised from sampling to final assay to prevent loss or contamination, in order to provide reliable data. A brief summary of contaminated sources which may be encountered during trace metal analyses is presented.

Key words: Pollutants; tissues; water analysis (chemical); mercury; cadmium; water filters; plastics; glassware

LUTZ, A. 1980 Contaminants in trace metal analysis. Can. Tech. Rep. Fish. Aquat. Sci. 975: 205-213.

Si l'on veut obtenir des données fiables dans une analyse de métaux en traces sur des échantillons prélevés dans l'environnement, il faut procéder avec précaution, depuis l'échantillonnage jusqu'à l'essai final, de façon à prévenir toute perte ou contamination. L'auteur résume brièvement les sources de contamination possible dans des analyses de métaux en traces.

CONTAMINANTS IN TRACE METAL ANALYSIS

There is a growing need in recent years for the services of the analytical chemist, when the biologist goes afield to study the effects of pollutants in the environment. From initial identification and quantitation to tracing its pathway within the ecosystem, the chemist is faced with the challenges of analyzing a variety of samples; such as biological tissue, water, and sediment, at concentrations from the ultra-trace level, to the trace level. Trace levels are defined as milligram per kilogram (part per million), while ultra-trace as microgram per kilogram (part per billion). Expressed in layman terms a part per million may be expressed in a number of ways:

- one ounce of salt in 31 tons of potato chips,
- a very dry martini, one drop of vermouth in 80 "bottles" of gin,
- one inch in 15.8 miles.

A part per billion:

- one pinch of salt in 10 tons of potato chips,
- one drop of vermouth in 500 "barrels" of gin.

The concentrations of the metals to be analyzed at environmental levels are very small indeed, consequently, there is a great need for care in sampling, storage, and preservation for valid results to be obtained. Sampling should be designed with the chemist in mind. In the case of water, the biologist should be made aware of the contamination from sampling containers, Moody and Lindstrom (1977), Table 1. Containers should be soaked overnight in acid solution and thoroughly rinsed with distilled water and soaked in quartz distilled water. The sampling device should be free from rubber or other contaminating material, metal components such as springs, etc., should be coated with Teflon, Robertson (1968).

Numerous papers have been written on the preservation techniques of water samples and perhaps the most comprehensive review to date on this subject is by Batley and Gardner (1977).

Walls of containers often act as an ion-exchanger therefore, trace metals are absorbed from solution, conversely, if metals are already present they are desorbed and contaminate the sample. Consequently, the need to have clean containers as well as some method of preventing adsorption is accomplished by acidification to a pH 2.0 for most metals.

Decontamination of pyrex bottles used for mercury analyses is best accomplished by heating in a muffle to 450^{o} C. for two hours. Samples are then preserved by addition of dichromate (0.05%) as oxidant and acidified (1%), Feldman (1974). In our laboratory a 10 liter tap water sample spiked with inorganic mercury to a level of approximately 0.05 micrograms per liter, and used as a daily control, was analyzed repeatedly for two months. The mean value of 39 analyses was 0.058 micrograms mercury per liter with a relative standard deviation of 7.2%.

Contamination may often be introduced into the sample in other ways. Should filtration be necessary contamination of metals from filters is always present, Tables 2 - 5. Filters should be acid washed with high purity acids and rinsed thoroughly with double quartz distilled water.

A source of contamination may be found in glassware during digestion procedures. Sodium ions may leach out and cause positive interference in cadmium analysis when non-flame atomizers are used for atomic absorption analysis, Culver and Surles (1975).

LEACHABLE CADMIUM FOUND IN PYREX STANDARD FLASKS

Flask	Initial Cd found, ng	Initial Cd found*, ng
A	82	1.5
B	38	0.96
C	26	1.3
D	49	0.30
E	50	2.1

* After soaking for 1 week in hot nitric acid, Smith (1978).

In our own laboratory aluminum was leached from glassware during wet digestion, resulting in high and variable blanks; the method of decomposition was changed in favor of dry ashing in platinum crucibles. This alleviated the problem. Similarly high blanks are obtained from glass on analysis of boron, Green et al. (1976).

Contamination may also be introduced through the use of plastic pipette tips, especially when analysis is by non-flame atomization techniques. Copper and cadmium have been known to be present as contaminants and washing with dilute acid prior to injecting the sample has alleviated the problem, Benjamin and Jenne (1976); Salmela and Vuori (1979).

With the advancement in electronics and carbon rod or graphite atomization in atomic absorption, the increase in sensitivity has created more difficulties for the analyst. More than ever before ultra pure acids and water are necessary as well as clean metal free areas in laboratories. Hydrofluoric, hydrochloric and nitric acids may be prepared in a specially designed Teflon® still, Mattinson (1972).

Thus many factors must be taken into consideration to obtain precise and accurate results at trace and ultra-trace levels in environmental samples.

Table 1. Impurities Leached from Plastic Containers in One Week by (1 + 1) HCl (ng/cm^2) Determined by IDMS.

Elements	Teflon FEP	LPE	CPE	PC
Pb	2	0.6	18	10
Tl	< 1	< 0.6	3	0.7
Ba	2	1	0.3	3
Te	2	-	0.7	-
Sn	1	< 1	< 0.8	13
Cd	0.6	0.2	0.2	< 8
Ag	< 6	-	-	-
Sr	< 1	0.2	0.2	0.3
Se	0.8	0.4	< 0.3	< 0.5
Zn	4	9	1.0	-
Cu	6	1	0.7	< 6
Ni	0.8	0.8	0.3	0.3
Fe	16	1	1.0	< 49
Cr	4	0.8	0.3	< 5
Ca	2	60	0.8	< 16
K	1.6	1	0.7	< 5
Mg	1.0	0.4	0.7	0.8
Al	4	4	10	3
Na	2	6	42	8

Reference: Moody and Lindstrom (1977)

Table 2. Impurities in Various Filter Types (ng/cm^2).

	Glass fiber	Silver membrane	Cellulose ester (MF-Millipore)	Polystyrene (Microsorban)	Polycarbonate (Nuclepore)
Al	-	-	10	20	6
Be	40	200	0.1	-	0.03
Cd	-	-	5	-	< 0.06
Ca	-	-	260	300	6
Cr	80	60	14	2	3
Cu	20	20	40	320	3
Fe	4000	300	40	85	30
Pb	800	200	8	-	< 0.06
Mg	-	-	< 200	< 1500	6
Ni	< 80	100	< 50	< 25	6
Si	$7x10^6$	$1.3x10^4$	100	-	30
Na	-	-	330	90	30
Ti	800	200	5	70	3
Zn	$1.6x10^5$	10	20	515	6

Reference: Dulka and Risby (1976)

Table 3. Carbon and nitrogen content in different types of glass fibre filter before and after they were subjected to heat treatment and washing.

Type of glass fibre filter and treatment	Carbon µg/filter	S.D.	Nitrogen µg/filter	S.D.
Whatman GF/C, not heat treated not washed	76(4)*	19	25(4)	6.9
Whatman GF/C, heat treated not washed	24(11)	14	5.4(12)	1.1
Whatman GF/C, heat treated washed	31(6)	5.3	9.5(6)	6.3
Gelman Type A, not heat treated not washed	69(3)		6.4(3)	
Gelman Type A, heat treated not washed	20(13)	5.9	5.4(13)	1.5
Gelman Type A, heat treated washed	34(6)	5.6	6.7(6)	1.2
Sartorius, not heat treated not washed	255000(3)		2000(3)	
Sartorius, heat treated not washed	27(9)	2.9	4.5(8)	2.5
Sartorius, heat treated washed	19(6)	7.6	1.2(6)	1.3

*Numbers in brackets are the number of filters analyzed.

Reference: Wagemann and Graham (1974)

Table 4. Metal content of 37 mm millipore type HA filters.

Metal	µg/filter	Standard Deviation (n=20)
As	0.893	± 0.253
Ca	2.657	± 1.980
Cd	0.145	± 0.155
Cr	1.201	± 0.683
Cu	0.665	± 0.269
Fe	3.695	± 1.536
Mg	1.301	± 0.437
Mn	0.250	± 0.128
Mo	0.335	± 0.260
Ni	0.485	± 0.290
Pb	1.199	± 0.523
Sb	1.621	± 1.539
Sn	0.821	± 1.053
Zn	1.195	± 0.488

Above analysis by inductively coupled Argon Plasma Spectrometry

Reference: Zimmerman et al. (1979)

Table 5. Metal content of unwashed and acid-washed Whatman 41 filters.

Metal	Unwashed ng cm^2	HCl Washed ng cm^2	HF/HNO_3 Washed ng cm^2
Al	8 ± 1	1.2 ± 0.7	<1
Cd	0.05 ± 0.03	0.02 ± 0.003	0.021 ± 0.009
Cr	6 ± 2	3 ± 1	2.2 ± 0.8
Cu	2.1 ± 0.3	0.09 ± 0.03	0.39 ± 0.05
Fe	64 ± 18	5 ± 1	3.2 ± 0.6
Mn	0.7 ± 0.2	0.16 ± 0.03	0.14 ± 0.03
Ni	5 ± 2	0.15 ± 0.02	0.12 ± 0.03
Pb	0.44 ± 0.03	0.06 ± 0.02	0.08 ± 0.03
Zn	1.4 ± 0.7	0.14 ± 0.06	1.7 ± 0.03

Analysis by Atomic Absorption Spectrophotometry

Reference: Wallace et al. (1977)

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SOME PITFALLS IN USING FISH TISSUES OR OTHER BIOLOGICAL SAMPLES AS INDICATORS OF METAL CONTAMINATION IN NATURAL FRESHWATER

W.G. Franzin and G.A. McFarlane

Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba, R3T 2N6

FRANZIN, W.G. and G.A. McFARLANE 1980 Some pitfalls in using fish tissues or other biological samples as indicators of metal contamination in natural freshwater. Can. Tech. Rep. Fish. Aquat. Sci. 975: 214.

Organisms or their tissues have been used as indicators of aquatic metal contamination for many years, often by comparing a putative contaminated site with a known uncontaminated site. These sorts of data often are extrapolated to the more general case to include other situations having similar environmental metal concentrations. Our observations of metal concentrations in biological samples and in the physical environments of six lakes of varying contamination near a metal smelter demonstrate the possibilities for erroneous interpretation of metal concentrations in biological material as indicators of present or past metal pollution.

Key words: Fish; pollution (freshwater); heavy metals

FRANZIN, W.G. and G.A. McFARLANE 1980 Some pitfalls in using fish tissues or other biological samples as indicators of metal contamination in natural freshwater. Can. Tech. Rep. Fish. Aquat. Sci. 975: 214.

Depuis plusieurs années, on utilise des organismes ou leurs tissus comme indicateurs de contamination aquatique par les métaux, souvent en comparant un site qu'on suppose contaminé avec un autre que l'on sait non contaminé. Souvent, des données de ce genre sont appliquées par extrapolation à un cas plus général, de façon à englober d'autres situations à concentrations de métaux semblables. Nous avons déterminé les concentrations de métaux dans des échantillons biologiques et dans le milieu physique de six lacs contaminés à divers degrés près d'une fonderie. Nos observations démontrent la possibilité d'interprétations erronnées des concentrations de métaux dans un matériel biologique utilisé comme indicateur de pollution présente ou passée.

THE UNRELIABILITY OF SOLUBILITY DATA FOR ORGANIC COMPOUNDS OF LOW SOLUBILITY IN WATER

J. Todd, D.E. Orr and G.W. Ozburn

Lakehead University, Thunder Bay, Ontario

TODD, J., D.E. ORR and G.W. OZBURN 1980 The unreliability of solubility data for organic compounds of low solubility in water. Can. Tech. Rep. Fish. Aquat. Sci. 975: 215-219.

In an effort to establish a reliable method of solubility determination for liquid organic chemicals in water the solubility of a trichlorobenzene mixture was measured by five different methods. No reasonable similarity could be found among the results from the various methods and it was concluded that a given solubility value is strongly dependent on the experimental method used to obtain it. A standard method for determining the solubility of trichlorobenzene and other liquid organic compounds is still in question.

Key words: Methodology; solubility; trichlorobenzene; organic compounds

TODD, J., D.E. ORR and G.W. OZBURN 1980 The unreliability of solubility data for organic compounds of low solubility in water. Can. Tech. Rep. Fish. Aquat. Sci. 975: 215-219.

Nous avons mesuré par cinq méthodes différentes la solubilité d'un mélange de trichlorobenzène dans le but d'en trouver une qui soit fiable dans la détermination de la solubilité de substances chimiques organiques liquides dans l'eau. Les résultats de chacune de ces méthodes diffèrent. Nous avons donc conclu qu'une valeur donnée de solubilité dépend fortement de la méthode utilisée. On est encore à découvrir une méthode standard de détermination de la solubilité du trichlorobenzène et d'autres composés organiques liquides.

Solubility in water of compounds with low solubility, such as polychlorinated biphenyls, trichlorobenzenes and diphenyl oxides is important because it might provide useful clues as to the bioaccumulation and movement in aqueous systems for these and other industrially useful compounds.

LITERATURE REFERENCES

The literature contains several methods for solubility determination. The method used by Wallnofer (1973) involved coating a glass vessel with the chemical in question by dissolving a small amount of the chemical in solvent, pouring the solution into a flask and spreading the solution over the surface of the glass until all the solvent had evaporated. Distilled water was then added to the flask and it was shaken at 30°C for 10 days. Samples were taken, extracted and analyzed by gas chromatography (GC). This group worked with 21 chlorobiphenyls and did not claim that their values were absolute physical constants but rather a means of comparison for use in their biological experiments.

Haque et al. (1974) determined the solubility of the PCB, Aroclor 1254 (Monsanto) which is a mixture of PCB isomers with different numbers of chlorine substituents. The method involved equilibration of five grams of Aroclor 1254 with 6 litres of water. The solution was stirred slowly with a magnetic stirrer and separated from the magnetic motor by a one-half inch polystyrene sheet to prevent dispersion and heating, respectively. Aliquots were removed periodically and centrifuged at 1085g for 10 minutes, then extracted and analyzed by GC. This was done at one week intervals. It was found that "true" solubilization was achieved in six months but values close to the solubility were achieved in the first few days. It was observed from relative size of GC peaks that solubility decreased as chlorination increased. Another method by Haque and Schmedding (1975) seemed to take into account most factors that could cause discrepancies in readings. They coated 25 litre carboys with a film of chemical as Wallnofer did. Water was then added and it was stirred slowly. Samples were analyzed over three months, after which time stirring was discontinued as equilibrium was reached. After stirring was discontinued the equilibrium concentration decreased to a new equilibrium probably due to the fact that if there were any aggregates of chemical in the water they would float or sink (depending on the density of the solute) rather than be stirred into the sample. Samples were removed below the surface of the solution. Water used was rendered free of organics by house distillation. When a steady figure of solubility was reached it was assumed to be the true physical constant for solubility.

SOLUBILITY METHODS FOR TRICHLOROBENZENE

The solubility of a sample of a technical mixture of trichlorobenzene (TCB), found by several methods, was used as an example of the unreliability of solubility data. The mixture is composed of 1,2,3 and 1,2,4 TCB in a 1:2 ratio.

METHOD I

A supersaturated solution of TCB in one litre of water was magnetically stirred overnight. A 400 mL sample (sample A) was removed and extracted with 50 mL of hexane. The other 600 mL were centrifuged at 27000g for one hour and another 400 mL sample (sample B) of the supernatant was removed and extracted.

In sample A the solubility of TCB was found to be greater than 100 ppm due to emulsified TCB present. It was thought that this emulsion would be broken by

centrifugation but values remained high at 5.3 ppm. All gas chromatographic analyses were performed on a Hewlett-Packard 5730a gas chromatograph equipped with an ECD detector. Values for TCB content in hexane extracts were determined from TCB standards prepared in hexane.

Some attempts were made, at this point, to determine the solubility of TCB in water containing 100 and 600 ppm acetone (ie. the environment in our fish testing tanks). The same experiment for water with 100 and 600 ppm acetone was carried out as above but all samples were centrifuged. Results in all cases were higher than the result of 5.3 ppm for distilled water but results for both concentrations of acetone varied widely. On this basis, we decided to try another method of determining solubility. Also, we had noticed TCB dropping out of the water and plating out on the tank bottoms at concentrations as low as 2.0 ppm in 250 ppm acetone in water, so we assumed the solubility must be less than that.

METHOD II

In order to determine whether another type of centrifuge would produce different results, the ultracentrifuge was used in the same experiment at 80000 g for 60 minutes at 20°C. Samples were taken and extracted yielding two solubility figures of 1.60 and 1.23 ppm. Again there was a discrepancy of values.

METHOD III

Our next attempt involved the method for determining the solubility of PCB's by Zitko (1970). Technical TCB (1.5 mL) was added to 50 mL of fresh water at room temperature in a blender and homogenized for 10 minutes. It was observed that after 10 minutes the homogenate had become very warm. Two ultracentrifuge tubes of the homogenate were saved and cooled to room temperature by refrigerating for a period of 30 minutes. They were then centrifuged for 30 minutes at 30000g and 5°C after which the concentration of TCB in 5 mL samples of the supernatant was determined. This first trial (trial A) yielded results of 8.0 and 27.4 ppm. A second trial (trial B) was performed and the samples were prepared as in trial A but instead were spun at 5°C at 48200g for three hours. Trial B produced solubilities of 25.45, 25.90, 28.65 and 26.15 ppm. We were skeptical of these high values due to the previous problem in the fish tanks with "plating out" at 2.0 ppm. We felt that the homogenization and heating in the blender must have caused unbreakable micelles of TCB in the water resulting in artificially high values of solubility.

METHOD IV

In order to check the solubilities obtained using Zitko's method of blender emulsification, another experiment was carried out.

We thought it necessary to determine if a less "violent" means of mixing the TCB and water had an effect on the solubilities obtained, testing the theory that an emulsion formed as a result of the blending.

Therefore, as suggested in a paper dealing with the solubility of hydrocarbons in water by McAuliffe (1966), an excess of TCB in water was mixed on a magnetic stirrer for 24 hours at a speed such that the vortex reached only one-

quarter of the way to the bottom of the flask. Samples were taken, being careful not to take any visible TCB, and centrifuged at 20°C and 48200g for 2 hours. Samples of the supernatant were taken and extracted with hexane. Four solubilities were found by GC analysis of the extracts. These were .464, .472, .528 and .464 ppm.

METHOD V

An intermediate method of mixing the TCB and water involving mechanical shaking was next attempted. Four 500 mL separatory funnels were shaken containing 400 mL of water and enough TCB to supersaturate the solution. These were shaken at maximum speed for 15 minutes on a wrist-action mechanical shaker. Four samples were taken and centrifuged at 48200g for two hours at 20°C. Samples of the supernatant were taken and extracted and extracts were analyzed using GC. Results were .712, .868, 1.39 and .887 ppm. All the results were in between the results for magnetic stirring and blending.

SUMMARY

It can be concluded from these results that the solubility of trichlorobenzene is highly dependent on the experimental method used. The method in which the slightly soluble organic liquid is introduced to the water for equilibration is very important. This fact is probably true for many similar compounds. It is evident from this research that work must be done to determine a standard method for the solubility of a liquid in a liquid. Please refer to Table 1 for a summary of the results obtained in this experiment.

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TABLE I

SUMMARY — SOLUBILITY DATA FOR TRICHLOROBENZENE

DESCRIPTION OF METHOD	TRIAL #1	TRIAL #2	TRIAL #3	TRIAL #4
OVERNIGHT MECHANICAL STIRRING	.464 ppm	.472 ppm	.528 ppm	.464 ppm
MECHANICAL SHAKING - 15 MINUTES AT MAXIMUM SPEED	.712 ppm	.868 ppm	1.39 ppm	.887 ppm
BLENDER - 10 MINUTES AT MINIMUM SPEED	25.45 ppm	25.90 ppm	28.65 ppm	26.15 ppm

PETROLEUM INDUSTRY ACTIVITY IN AQUATIC TOXICOLOGY

E.C. Birchard

Imperial Oil Limited, Toronto, Ontario

BIRCHARD, E.C. 1980 Petroleum industry activity in aquatic toxicology. Can. Tech. Rep. Fish. Aquat. Sci. 975: 220.

The Canadian petroleum industry individually and through its environmental associations has been sponsoring and conducting aquatic toxicology research and studies on a regular basis since the early 1970's at the refinery level. Studies have been conducted on developing and assessing novel bioassay techniques for routine and spot monitoring of liquid effluents, as well as running conventional 24-hour static and 96-hour flow-through tests. A 3-year study was undertaken to assess sublethal effects of refinery effluent on growth, survival, reproduction, behaviour and respiratory rate in fish as well as life cycle studies in an invertebrate. Current emphasis is centered on determining presence and levels of priority contaminants in effluents and assessing their degree of hazard based on such criteria as carcinogenicity, mutagenicity, persistence, accumulation and exposure levels.

Key words: Toxicity tests; bioassays; sublethal; growth; survival; reproduction; behaviour; respiratory rate; fish; invertebrates

BIRCHARD, E.C. 1980 Petroleum industry activity in aquatic toxicology. Can. Tech. Rep. Fish. Aquat. Sci. 975: 220.

Depuis le début des années 1970, l'industrie pétrolière canadienne, individuellement et par le biais de ses associations, a encouragé et poursuivi des études de toxicologie aquatique au niveau des raffineries. Des études visant à mettre au point et à tester de nouvelles méthodes d'analyses biologiques routinières ou à des intervalles irréguliers d'effluents liquides, de même que des essais conventionnels statiques de 24 h et de 96 h à flot continu. On a entrepris une étude de 3 ans en vue d'évaluer les effets sublétaux de l'effluent d'une raffinerie sur la croissance, survie, reproduction, comportement et taux respiratoire des poissons, ainsi que des études du cycle biologique d'un invertébré. On met présentement l'accent sur la détermination de la présence et des niveaux de contaminants prioritaires dans les effluents et sur l'évaluation de leurs dangers. Comme critères, nous utilisons leurs propriétés carcinogènes et mutagènes, leur persistance, accumulation et niveau d'exposition.

SOME ASPECTS OF RADIATION TOXICOLOGY*

J.E. Guthrie

Environmental Research Branch, Atomic Energy of Canada Research Company, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, ROE 1LO

GUTHRIE, J.E. 1980 Some aspects of radiation toxicology. Can. Tech. Rep. Fish. Aquat. Sci. 975: 221-230.

Biological effects on organisms resulting from exposure to ionizing radiation are many and varied. In general, the effects on a species are dependent on the type of radiation, the dose rate, the duration of exposure, and whether the radiation source is internal or external to the organism.

When establishing permissible release limits of radionuclides to an aquatic environment, biological accumulation by the organism must be considered. If significant accumulation occurs, the radiation dose to the organism from ingested radioactivity may be much larger than the external dose received from the immediate surroundings. This aspect of radiation toxicology is illustrated by results from laboratory experiments in which mosquito larvae were reared in media containing radioactive strontium or radioactive phosphorus.

Determination of biological accumulation simplifies the task of specifying the amounts of radioactive materials which may be released to aquatic environments with minimal risk. Such coefficients are important input for ecological system models using computer codes such as RAMM, for calculation of the radiation dose commitment to various species including man.

Key words: Radioactive pollution; bioaccumulation; mosquitos; toxicology

GUTHRIE, J.E. 1980 Some aspects of radiation toxicology. Can. Tech. Rep. Fish. Aquat. Sci. 975: 221-230.

Les effets biologiques sur les organismes résultant de l'exposition au rayonnement ionisant sont multiples et divers. En général, les effets sur une espèce dépendent du type de rayonnement, du débit de dose, de la durée de l'exposition, et si la source de rayonnement est interne ou externe à l'organisme.

On doit tenir compte de l'accumulation biologique par les organismes lorsque l'on définit les limites admissibles de libération des radionuclides dans un milieu aquatique. Si une accumulation importante se produit, la dose de rayonnement reçue par l'organisme provenant de la radioactivité ingérée peut être beaucoup plus élevée que la dose externe provenant de l'environnement immédiat. Des expériences en laboratoire au cours desquelles des larves de moustiques ont été élevées dans des milieux contenant du strontium radioactif ou du phosphore radioactif illustrent cet aspect de la radiotoxicologie.

La détermination de l'accumulation biologique rend plus facile la spécification des quantités de matières radioactives qui peuvent être libérées dans un milieu aquatique avec un minimum de risques. Ces coefficients sont des données importantes pour les modèles de systèmes écologiques employant des codes d'ordinateurs tels que RAMM pour le calcul des doses engagées pouvant être reçues par diverses espèces, y compris l'homme.

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INTRODUCTION

In discussing the role of radiation toxicology in assessment of the potential risks associated with the radioactive by-products of the nuclear industry, I must emphasize that, with the exception of uranium, the permissible amounts of radioactive isotopes that may be released to the environment are not governed by their chemical toxicity. Therefore, for purposes of this discussion, we may take it as granted that radiation toxicology is concerned with the quantity of ionizing radiation i.e., the radiation dose, absorbed from a radioactive isotope or mixture of isotopes.

Radiation toxicology is obviously an important aspect of the Health Physics Field. The primary concern of this discipline is development and application of scientific knowledge whereby man and the environment are protected from the harmful effects of radiation. Radiation toxicology is also an integral part of radiation ecology, which is concerned with the fate of radioisotopes in the environment and the effects of ionizing radiation on organisms, populations, communities and ecosystems. This paper discusses some aspects of external and internal exposure to radiation, and thus is mainly in the field of Health Physics. In addition, some attention is given to the importance in internal dosimetry of uptake of isotopes, which aspect is in the preserve of Radiation Ecology.

RADIATION EXPOSURE

Determination of the minimum dose of radiation which has no apparent biological effects on a test animal is beset by the problem of biological variation, as is conventional chemical toxicology. This problem and the test methods which minimize it, have been reviewed by Sprague (1969) who has also discussed the questions of sub-lethal effects and the "safe" concentration of a toxicant (1970, 1971). Within the context of Sprague's use of the term, if we substitute "permissible" for "safe", we have, in principle, the recommendations of the International Commission for Radiological Protection (1959) as they pertain to radiation dose.

FACTORS AFFECTING RADIATION DOSE

Three kinds of ionizing radiation (radiation) are commonly encountered in the environment,viz, alpha and beta particles, and gamma photons or rays. The alpha particle is the nucleus of a helium atom, He^{+2ve}; the energy of most alpha particles ranges from 3 to 6 MeV. The beta particle is an electron emitted from the nucleus of an atom; its energy may be only a few keV to more than 2 MeV. Gamma photons, unlike alpha and beta particles, are electromagnetic radiation; they range in energy from a few keV to several MeV. It is the deposition of the energy of alpha, beta or gamma radiation in the cell's components which may promote chemical reactions that cause biological damage. An example of such damage is the breaking of some of the chemical bonds which link together the strands of the DNA molecule. If the rate of breakage exceeds the capacity of the organism's repair systems, biological damage will result.

Whether or not the capacity of an organism's repair systems will be exceeded, depends on the rate at which the radiation dose, (dose-rate), is delivered and the amount of radiation that is absorbed (the total dose, i.e., dose-rate x exposure time). The amount of radiation absorbed is important.

When speaking of the damage that could result from a dose of radiation, it is important to distinguish between external exposure and internal exposure. An alpha particle causes no damage if it is outside the organism because its penetrating power is quite small. When an alpha particle is emitted from an isotope which has been ingested, i.e., it is inside the organism, the potential for causing damage is considerable because a large amount of energy will be deposited per unit mass of tissue. The penetrating power of beta particles is greater than that of alpha particles but even the most energetic beta particles from fission products are barely able to penetrate the human skin. When beta particles are emitted from an ingested isotope, however, the damage potential will be greater. In contrast to alpha and beta particles, the penetrating power of gamma photons is theoretically infinite and only a small fraction of their energy is deposited per unit mass of tissue. Thus, in the case of gamma photons, it makes little difference if the gamma-emitting isotope is inside or outside the organism.

UPTAKE OF RADIOACTIVE ISOTOPES

A radioactive isotope in a river, lake or ocean is a source of external radiation to organisms living in these habitats. If the isotope is ingested and accumulated by the organism, it becomes, in addition, a source of internal radiation. Consequently, the amount of uptake or biological accumulation has an important bearing on the total dose of radiation absorbed by the organism.

The extent to which an isotope might be accumulated (by an organism) can be anticipated from its chemical properties. Radioactive isotopes of elements which are essential to the organism's growth and metabolism, e.g. radioactive iodine, will be accumulated. Isotopes of elements which are chemical relatives of metabolically essential elements, may also be accumulated, e.g. radioactive strontium is concentrated in the bones of vertebrates because of its chemical similiarity to calcium.

EXTERNAL AND INTERNAL DOSE

The importance of measuring the amount of radiation absorbed from internal and external exposure (dosimetry) in radiation toxicology can be illustrated by using data from a study of the effects of exposure of mosquito larvae to beta radiation (Guthrie and Brust (1971) and Scott and Guthrie (1972)). Newly hatched *Aedes aegypti* larvae were reared in media containing different concentrations of radioactive strontium in equilibrium with its daughter product radioactive yttrium, or radioactive phosphorous. The 'apparent concentration ratio (ACR)' for each larval instar was determined as:

$$\text{ACR} = \frac{\text{radioactivity per unit mass of larvae}}{\text{radioactivity per unit mass of rearing medium}}$$

The ACR's (Tables 1 & 2) show that the larvae accumulated significant amounts of radioactive strontium, yttrium, and phosphorus. Also, the ACR for

TABLE 1

AVERAGE ACTIVITY VALUES OF *AEDES AEGYPTI* LARVAE REARED IN MEDIA CONTAINING 410 Bq/g ^{90}Sr IN EQUILIBRIUM WITH ^{90}Y AND 0.01 mg/g Sr^{+2} CARRIER. THE 95% CONFIDENCE LIMIT FOR ALL AVERAGES IS ± 15%.

INSTAR	NUCLIDE CONC. kBq/g LARVA		APPARENT CONC. RATIO (ACR)*	
	^{90}Sr	^{90}Y	^{90}Sr	^{90}Y
1st	22	122	54	300
2nd	23	46	56	110
3rd	39	115	95	280
4th	77	130	190	320

$$^{*}ACR = \frac{\text{ACTIVITY OF LARVA (Bq/g)}}{\text{ACTIVITY OF REARING MEDIUM (Bq/g)}}$$

TABLE 2

AVERAGE ACTIVITY VALUES FOR *AEDES AEGYPTI* LARVAE REARED IN MEDIA CONTAINING 22 Bq/g ^{32}P (CARRIER FREE). THE 95% CONFIDENCE LIMIT FOR ALL AVERAGES IS ± 15%.

INSTAR	^{32}P CONC. kBq/g LARVA	APPARENT CONC. RATIO
1st	81	3700
2nd	25	1140
3rd	19	840
4th	10	440
ADULTS		
MALE	8	340
FEMALE	13	590

these isotopes differed between instars, especially for radioactive phosphorous (Table 2). The accumulation of yttrium (Table 1) is surprising in that there is no requirement for this and the other elements in Group III B of the periodic table for mosquito larvae growth.

The percentage of adult females with follicular development greater than or equal to Clement's stage III, was one of the indicators used to score biological effects of larval exposure to beta radiation. There was no apparent relationship between this percentage and the concentration of radioactive isotope(s) in the larval rearing medium. The method for determining the internal dose component of the total dose received by the larvae has been published (Scott 1972). The external, internal, and total beta radiation

doses, are given in Tables 3 and 4. The biological effects observed were highly dependent on the stages of larval development exposed, and reflect the different accumulation ratios of individual instars. When, for example, the logarithm of total dose (external + internal dose) is regressed against the percentage of adult females with follicular development greater than stage III, the two parameters are highly correlated (Figure 1).

Another aspect of isotope accumulation which should be pointed out is its effect on the relative contributions to the total dose of the external dose absorbed from the larval rearing medium, and the internal dose from accumulated isotope (Tables 3 & 4). If the ACR $\leq$ 1, the internal dose contribution will never exceed that of the external dose. If significant biological accumulation does occur, i.e., ACR > 1, the internal dose will be the larger contribution to the total dose absorbed by the organism.

TABLE 3

DOSE-RATE (Gy/h) TO *AEDES AEGYPTI* LARVAL INSTARS REARED IN MEDIA CONTAINING 37 kBq/g (1 µCi/g) ^{90}Sr IN EQUILIBRIUM WITH ^{90}Y.

INSTAR	^{90}Sr + ^{90}Y EXTERNAL DOSE-RATE	ACR	^{90}Sr INTERNAL DOSE-RATE	ACR	^{90}Y INTERNAL DOSE-RATE	TOTAL DOSE-RATE
1st	0.019	54	0.05	300	0.19	0.26
2nd	0.019	56	0.07	110	0.09	0.18
3rd	0.019	95	0.16	280	0.35	0.53
4th	0.019	190	0.43	320	0.67	1.12

ACR = APPARENT CONCENTRATION RATIO
1 GREY (Gy) = 100 rad

TABLE 4

DOSE-RATE (Gy/h) TO *AEDES AEGYPTI* LARVAL INSTARS REARED IN MEDIA CONTAINING 37 kBq/g (1 µCi/g) ^{32}P.

INSTAR	EXTERNAL DOSE-RATE	ACR	INTERNAL DOSE-RATE	TOTAL DOSE-RATE
1st	0.013	3700	2.55	2.56
2nd	0.013	1140	1.07	1.08
3rd	0.013	840	1.18	1.19
4th	0.013	440	1.00	1.01

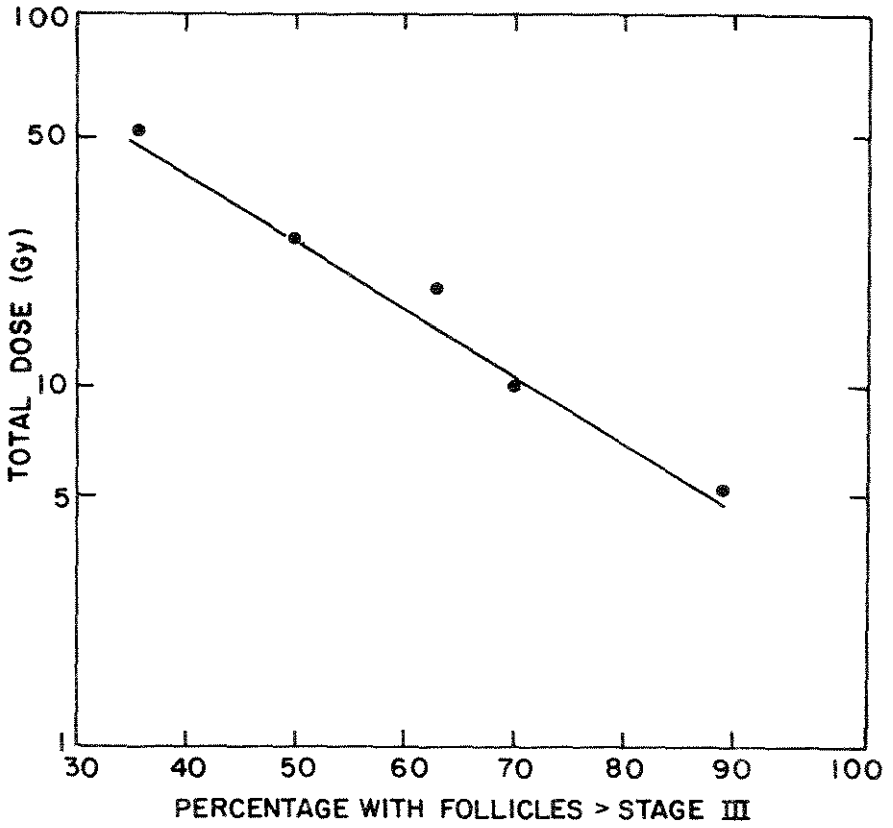

FIGURE 1: Effect of total beta dose on percentage of adult female *Aedes aegypti* with follicle development more advanced than Clement's Stage III. Larvae were irradiated for the duration of 1st and 2nd instar stages only.

As already mentioned, the accumulation of yttrium (Figure 1) was not anticipated. However, the largest contributor to the total dose absorbed by the larvae reared in the media containing radioactive strontium and yttrium, was yttrium.

PREDICTING RADIATION EXPOSURE

Compared to the quantification of biological effects, measurement of isotope accumulation is relatively simple. Transfer coefficients can be derived from accumulation ratios. They lend themselves readily to the prediction of the maximum radiation dose resulting from a release of a known quantity of radioactive isotopes to the environment at any point of interest in an ecosystem. Such predictions, i.e., ecosystem analysis can be made by the computer code RAMM (Radioactive Materials Management) developed for handling such system models. This system of computer programs for radioactive isotope pathway analysis calculations (Lyon 1976) is very useful for inputting time-dependent data into complex ecological system models such as FOOD II (Zach 1978).

RAMM uses a compartmental or nodal approach, i.e., the system to be analysed is divided up into 'nodes'. Nodes are 'compartments' containing isotopes or other potential contaminants. Nodes may be volumes, such as a volume of water, or the biomass of a species, or areas of a surface. Each node is of such size that the distribution of isotopes within it can be considered to be homogeneous. Next, pathways are defined between nodes. The appropriate transfer coefficients are then generated as inputs for each pathway.

The outputs from RAMM include: time-dependent contents of the nodes, dose-rate and intregrated doses, and the dose commitments for selected nodes.

SAMPLE CASE

To illustrate the application of RAMM, consider the following simplification of a "sample case" taken from Lyon. This case involves a section of the Winnipeg River and the lake at Lac du Bonnet, Manitoba. Assume radioactive cesium in groundwater enters the river (Figure 2) at a rate of 1×10^{10} Bq/d. During its passage to the river, it interacts via ion exchange with the ground. On entering the river, the isotope concentration becomes less as a result of mixing with river water. The radioactive cesium eventually enters the lake where it is accumulated by fish which are eaten by man. In the lake, the isotope interacts with sediment and interstitial water. Deposition of sediment causes it to be packed on the bottom of the lake so that some of the isotope is trapped and lost from the system. The object of this sample case is to predict the dose-rates to a man drinking water from the river, and to a man eating fish caught from the lake.

Figure 2 illustrates the physical model for the sample case while Figure 3 gives the combination of nodes and pathways comprising the computer model.

Some of the nodes belong to streams for which a distance and a velocity are defined. At the appropriate time intervals, the contents of each node are transferred down the stream. Eventually, the lake empties into the dump node - OUT.

To maintain sensible computing time, the sections of pathways should be 'well balanced' in terms of time constants. For example, the model includes a pathway through soil which takes hundreds of years, followed by transfer down the river which takes a few hours. It would be impractical (and should be unnecessary) to model the transfer down the river in detail in the total calculation. In this case, the spatial distribution due to dispersion in the river is derived in a separate RAMM calculation, followed by the total calculation in which the river transfer is assumed to reach its predicted spatial distribution instantaneously.

The groundwater is modeled by using a stream (UNSTR(1) to (4)) interchanging with ground nodes GROU1 and GROU2. The river is modeled with two parallel streams STRE1(1) to (5) and STRE2(2) to (5) to take into account the transfer of isotope from one side of the river to the other by diffusion. The lake is modeled with a water node (LAKE), an interstitial water node (INSWA), and sediment nodes INSED, FISED, and BOSED. The man drinking water

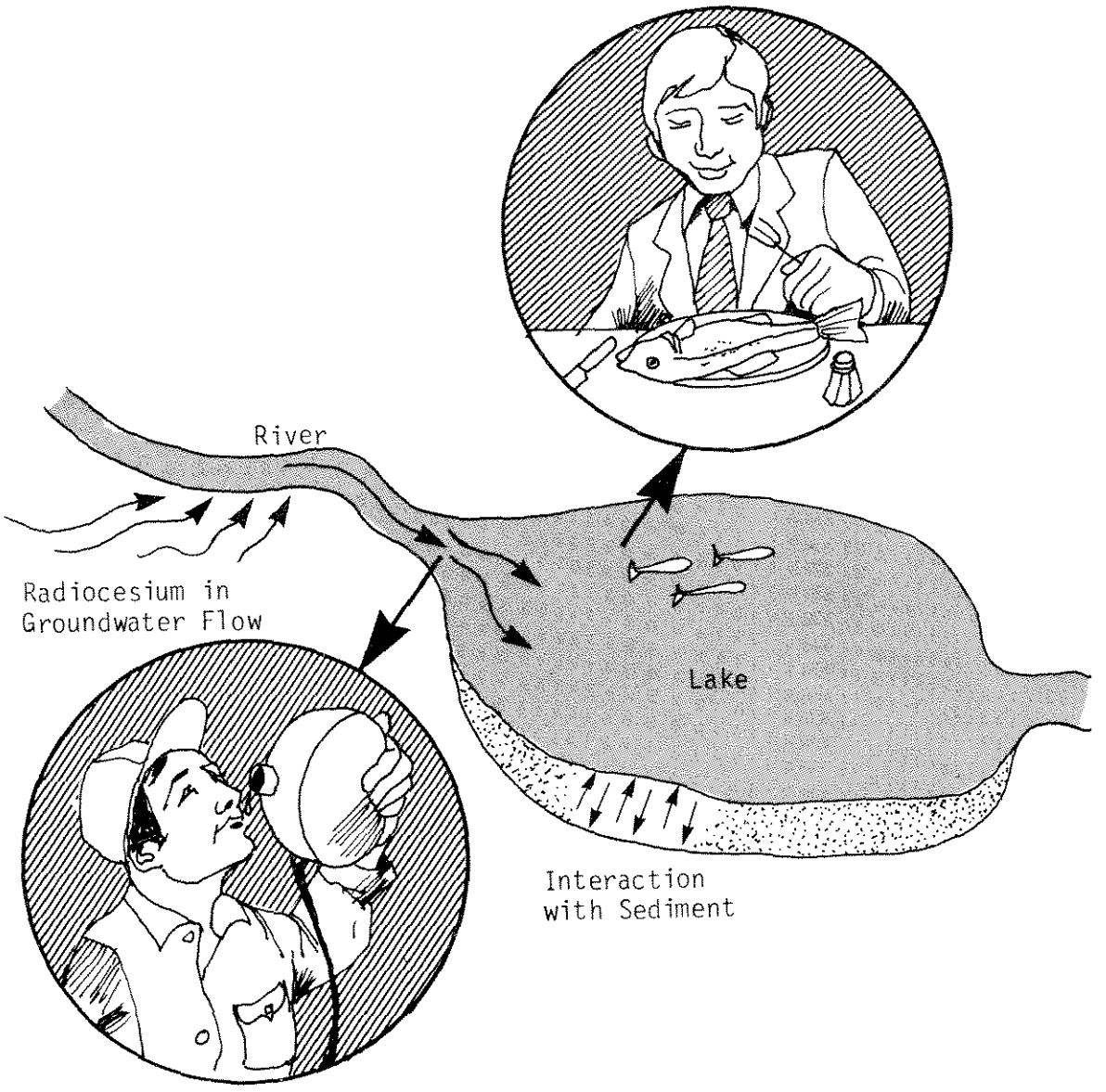

FIGURE 2: Physical model of a sample case illustrating an application of the RAMM computer code for aquatic ecosystem analysis.

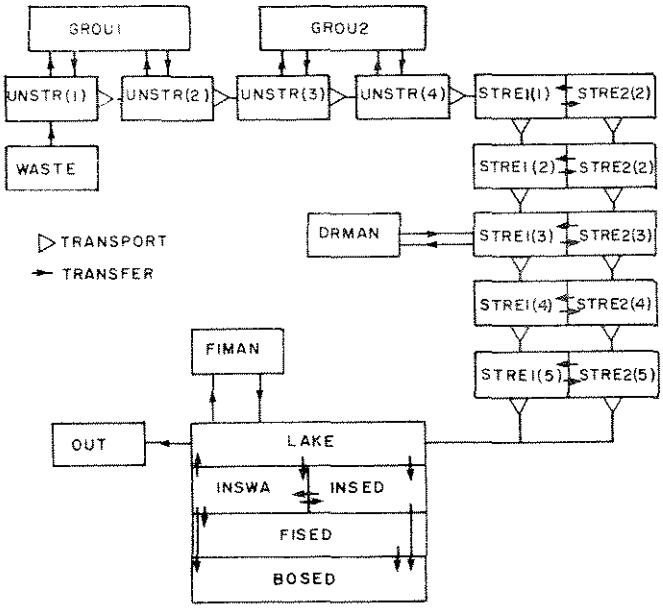

FIGURE 3: Computer model of a sample case illustrating an application of the RAMM computer code for aquatic ecosystem analysis.

and the man eating fish are modeled by nodes DRMAN and FIMAN. The OUT node provides for flushing out of the lake.

One of the several transfer coefficients required by RAMM to run the model, is derived below to illustrate the use of apparent concentration ratios. The ACR of radioactive cesium by commercial freshwater fish species is 2000. The consumption rate of a man eating the maximum amount of fish = 18 kg/a. The volume of the lake is estimated to be 3.24×10^9 m^3. Therefore, the transfer coefficient for radioactive cesium from lake to man via fish is:

$$\frac{18 \times 2000}{3.24 \times 10^9 \times 365} = 3 \times 10^{-8}/d$$

The output from RAMM also provides a periodic node status. It gives the number of atoms in each node at the time of interest, and the radiation dose statistics for those nodes for which dose calculations have been requested. For example, at 20 days, the accumulated dose commitments will have reached 1.1×10^{-4} rem for the man drinking water (DRMAN) and 3.8×10^{-2} rem for the man eating fish (FIMAN). The doses integrated over the period of the calculation are 9.8×10^{-6} and 2.3×10^{-3} for the drinking and fish-eating man respectively (Lyon 1976). Thus, the dose commitment, resulting from the radioactive cesium entering the river via groundwater, is greater for the man eating fish. This prediction, subsequently confirmed by experimental studies, illustrates another benefit of modeling, that direction of experimental effort to the most critical or sensitive parts of the system is possible.

CONCLUSION

While systems analysis cannot replace conventional toxicological assessment, modeling can be used to show how one aspect of toxicology, biological accumulation, can predict the impact of potentially toxic substances on the environment and on man. The toxicological data required for good, reliable, predictive models, and for validation of the models in nature, must still be provided by experimental measurement.

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THE SELECTION OF SUB-LETHAL TESTING PROCEDURES FOR ASSESSMENT OF CHEMICAL TOXINS IN THE AQUATIC ENVIRONMENT AND EXPERIENCES IN THE USE OF STEROID HORMONES METABOLISM

J.F. Uthe[1], H.C. Freeman[1] and A.D. McIntyre[2]

[1] Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada

[2] Department of Agriculture and Fisheries for Scotland, Aberdeen, Scotland

UTHE, J.F., H.C. FREEMAN and A.D. McINTYRE 1980 The selection of sub-lethal testing procedures for assessment of chemical toxins in the aquatic environment and experiences in the use of steroid hormones metabolism. Can. Tech. Rep. Fish. Aquat. Sci. 975: 231-242.

The detection of deleterious effects of current chemical pollutant levels in the marine biosphere has been the topic of a recent meeting held under the auspices of the International Council for the Exploration of the Sea. The first part of this paper reviews the criteria utilized by the Biochemistry Panel to judge and rate the applicability of current biochemical techniques to this question. The second part of the paper reviews our experiences in the use of steroid hormone metabolism for either scanning hormonal effects of a number of chemicals or for a detailed hormonal investigation of one chemical during an animal's reproductive cycle.

Key words: Bioassays; sublethal; reproduction; steroids; methodology

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Le Conseil international pour l'exploration de la mer organisait récemment une réunion consacrée à la détection des effets délétères des niveaux actuels de contaminants chimiques dans la biosphère marine. L'article qui suit, dans une première partie, passe en revue les critères qu'utilise le Comité de biochimie dans l'évaluation des possibilités d'application des techniques courantes à cette question. Dans une seconde partie, nous présentons une revue de nos expériences sur le métabolisme des hormones stéroïdes visant à déterminer les effets de plusieurs substances chimiques ou d'étudier en détails les effets sur le métabolisme hormonal d'une substance chimique au cours du cycle reproducteur d'un animal.

In recent years a number of testing protocols have been developed for studying the sub-lethal effects of contaminants upon aquatic organisms (U.S. National Academy of Sciences 1975; Giam 1977). In general the observed effects are believed to be deleterious but, in actual fact, it is extremely difficult to relate many of these effects to death of either the individual or of the species (eg. through impairment of reproduction). An additional problem arises; since many of these tests are carried out using contaminant levels rarely, if ever, encountered in the environment, assessment of the effects of current ambient levels is extremely difficult.

Early in 1979, under the auspices of the International Council for the Exploration of the Sea a meeting was held in Beaufort, North Carolina to consider the applicability of available testing procedures for determining if current levels of aquatic pollutants are having deleterious effects on aquatic animals. Participants were divided on the basis of their expertise, into seven different panels; biochemistry, physiology, pathobiology (disease), bioassay, genetics, behaviour and ecology. Panels were requested to consider first of all criteria which must be met in determining if contaminants, in particular currently observed levels in the more polluted areas of the ocean, are having effects upon life in the seas and secondly if feasible, comment upon the applicability of currently available testing procedures within their area of expertise to detect sub-lethal or deleterious effects. The final document is currently being published by the International Council for the Exploration of the Sea and will include A: Papers by each participant discussing their individual areas of expertise and the application of these techniques to effects testing, B: A series of plenary papers setting the stage and discussing theories and limitations of effects testing and C: Reports from each panel discussing certain tests, requirements of effects testing procedures and, in some cases, a ranking of available testing procedures. It is hoped that documents such as these, representing the agreed opinion of groups of active environmental scientists, will assist regulatory and other agencies in extending their testing protocols and requirements beyond tests based upon lethality.

Due to the time limitations only the efforts of the biochemistry panel will be described. Prior to this a few thoughts on the nature of effects may be beneficial. Chemical induced effects (responses) may be of four types (Table 1): 1) Negative or no-effect responses. In this case the assay system does not respond to the stress in any demostrable manner and the system may be described as being "refractive". 2) Dose/response or linear responses. In this case increments in stress result in response increments and the relationship can generally be described as linear following appropriate transformation. Probably the majority of effects testing procedures fall into this category. Here, considering the range of responses found another division may be made (A) In the first division one would place all of those effects whose range of contaminant-induced effects is within the range of effects that can be induced by normal environmental stressors. A test such as carbon fixation in the sea (Lassig et al, 1978) can vary between zero and maximum under normal environmental changes. This makes it extremely difficult to determine the significance of changes induced in carbon fixation by pollutants unless other studies indicate the system has been deleteriously affected in some other manner. (B) The second division would obviously include all dose/response effects in which the range of contaminant induced effects exceeds the range of the normal environmentally induced effects. These tests are, of themselves, more meaningful than the first group since they show the system has been stressed beyond normal. A test such as the

adenylate energy charge (ATP + ADP)/(ATP + AMP) (Giesy et al, 1978) could be included in this group. 3) "Quantum" responses. In this case increasing doses of a stressor lead to the appearance at some level of stress of a response that is not observed under normal environmental conditions. The appearance of abnormal steroid metabolites with increasing chemical toxin stress (Freeman et al. 1979) and the appearance of non-metallothionen bound mercury in tissues (Brown and Parsons 1978) illustrate this type of response. Death, as currently used in bioassay, is obviously another "quantum" response.
4) Inflective Responses. These responses are those which over the range of stressor induced effect show an inflection point or change in the direction of the effect. The most prominant effect of this type is undoubtably the generalized stress syndrome described by Selye (1976) in which the initial effect of a stress is an increase in circulating steroid hormones, particularly gluocorticoids. As the stress increased in magnitude or duration the level increased to a certain point after which stressor increases lead to a collapse of the response.

TABLE I. Types of Response Induced by Chemical Stressors.

Response Type		
1.	"Refractive"	No change within dose range.
2.	"Dose/Effect"	Lineal response within dose range
		A. Response stays within normal limits over dose range
		B. Response does not stay within normal limits over dose range.
3.	"Quantum"	Appearance of abnormal metabolites within dose range
4.	"Inflective"	Inflection appears in change in response/change in dose curve within dose range.

The last three types of response are all dose/response relationships but 2B, 3 and 4 and have obvious advantages in that a cleaner delineation between contaminant- or toxin- induced change and changes induced by normal environmental stresses can be demonstrated.

Now, let us turn to the recommendations of the Biochemistry Panel from the Beaufort meeting. The panel, in addition to recognizing the variety in types of response, considered other criteria for judging the usefulness of various biochemical testing procedures. These are shown in Table 2.

TABLE 2. Criteria Selected by the Biochemistry Panel for Evaluating Biochemical Testing Procedures.

1. Consistent relationship of response to pathology
2. Specific vs general response
3. Precision and range of response
4. Time relationship between stress exposure and response
5. Applicability of response to various phyla
6. Technical difficulty
7. Cost
8. Dose/response relationship
9. Applicability to general survey or pollution "hot spot" situation.

Under the first criterion i.e. relationship to pathology and in its discussion the panel emphasized the importance of utilizing a battery of tests, not only biochemical but from any other area of science to enable an overall assessment of the situation to bemade. They further emphasized that as environmental scientists we are assessing health. This means that as researchers into environmental effects we must realize that we are indulging in an environmental "medical practice" and that the final judgement of environmental health and risk includes both an objective (quantitative response relationship) and subjective (judgemental) aspect. Also medical practice is always willing to modify, adapt and use tests specific to the situation being investigated. This flexibility, along with the ability of medical practitioners to come to a consensus with respect to disease detection and treatment, recognizing the intangible benefits of experience, represents an extension of our more usual science practice that would be of benefit to the environment. In the second criterion i.e. whether or not the response is related to a specific toxin or to stress in general, the panel recognized the importance of developing techniques that respond to environmental toxins in general since the nature of all the chemical inputs to an area may not be known. Other tests are specific for a certain type or class of toxicants. The third criterion recognized the realities of measurement, especially the biological response of a heterogeneous species. It is simply easier to see a response which shows a narrow deviation at a constant stress and a wide response range over the stress dose range. Criterion 4 recognized the difficulties in working with delayed response syndromes. Tests which are applicable to many phyla and can be applied to small individuals offer advantages over tests which are applicable to say only large members of one or two similar species (Criterion 5). Criterion 6 and 7 recognized the realities of life with respect to man power and money limitations. Criterion 8 emphasized the necessity of demonstrating some type of a dose-response relationship. Without this it is doubtful that a test will have an adequate degree of predictability associated with it or be of much use in assessing the degree of environmental degredation an area is undergoing. Criterion 9 simply indicated if the panel believed the test is applicable in a general survey situation, eg. to projects such as the "Mussel Watch" program (Goldberg et al. 1978) or of value in a specific hot spot study where known toxicant is being added. The panel used these criteria to rate the tests shown in Table 3. It should be noted that the panel recognized that many other biochemical tests exist that may be of use but have not had adequate testing yet. In addition to a multi-disciplinary investigation of animals the importance of water, food and tissue residue analysis was emphasized.

TABLE 3. Current Biochemical Techniques Evaluated by the Biochemistry Panel.

Mixed Function Oxygenase	Inducible microsomal oxygenase system
Metal Binding Protein	Excess metal to inducible binding capacity
Lysosomal Fragility	Cytochemical assay of membrane stability
Steroid Hormone Metabolism	Quantitative shifts and strange metabolites
Adenylate Energy Charge	Measure of available metabolic energy
Routine Blood Biochemistry	Blood constituent and enzyme assay
Taurine/Glycine Ratio	High ratio indicates stress
Primary Productivity	Commonly as C^{14} fixation

One of the tests in Table 3 is based upon the effects of chemical pollutants upon steroid hormone metabolism. Steroid hormones are responsible

not only for maintenance of salt and water metabolic balance (mineralocorticoids) but are also involved in response to stress (glucocorticoids) and also are involved in reproduction (androgens and estrogens). In the case of the sex hormones we have a tool for determining the effects of a toxin on a process that is anything but homeostatic and obviously of vital importance from a species survival point of view. One should keep in mind that in the case of reproduction in fish and many other lower species it is not only important that both males and females ripen normally, both sexes must also ripen at the same time for successful reproduction to occur.

A number of different tissue/assay combinations can be used for effect studies. In fish three different tissues are utilized: 1) blood to assay blood level changes in hormonal level over the course of exposure of the animal to a toxicant, 2) interrenal tissue (adrenal homologue in fish) to study effects on glucocorticoid and mineralocorticoid metabolism and, 3) gonadal tissue to study effects on sex hormone metabolism. Metabolic effects in interrenal and gonads can be assayed following in vivo exposure to the chemical of interest or following in vitro addition of chemicals to incubates. Assays can be intensive, utilizing DIDA techniques for determining levels and isotopic ratios of each metabolite (the dual pathway of steroid hormone synthesis necessitates the use of both [^{3}H]-pregnenolone [^{14}C]-progesterone as precursors). The [^{3}H]/[^{14}C] ratio changes indicate effects upon the individual pathways of metabolism. In addition to this somewhat lengthly procedure it is possible, using autoradiograms of chromatograms of steroid hormone extracts to assess effects rapidly in a qualitative and roughly quantitative manner. Coupling of autoradiographic assay with in vitro additions of a chemical can be used to rapidly assess the potential of a chemical to induce steroidogenic effects. In Figure 1 the effects of the in vitro addition of a variety of metallic compounds upon adrenal incubates of grey seals (Halichoerus grypus) at a level of 0.45 ppm are shown (Freeman and Sangalang 1977). It is readily apparant that two important effects on steroid hormone metabolism are present. In the first instance alterations of hormone levels are found Secondly, one should note the appearance (from the precursors) of labelled material that is not found in control incubates. The appearance of such abnormal metabolites represents a type 3 or "quantum" response. Changes in hormone levels are noteworthy since they are probably beyond the normal range of hormonal metabolism encountered during the animal's exposure to natural environmental changes but this remains to be assessed in detail. Figure 2 shows a similar assessment of phthalate ester, di-2-ethylhexyl phthalate, effects on hormone metabolism in interrenals (adrenal homologue) from Atlantic cod (Gadus morhua). Even at levels of 1000 ppm (approximately 2000 x levels studied with the metallic compounds mentioned above) no changes in hormone metabolism were found. These observations were confirmed by study of the isotopic ratios ([^{3}H]/[^{14}C]) of the hormones, cortisol and cortisone which remained essentially constant over the range of di-2-ethylhexyl phthalate studies (Freeman and Sangalang 1979).

Changes in blood levels of hormones can be of two types, the first of which is a change in level induced by a toxin as compared to a relatively static level of a hormone. Secondly during the course of a non-homeostatic process in an animal, e.g. reproduction, blood hormone levels may change in a necessary manner (eg. sex hormones) over time. Alterations in these changes may induce serious effects e.g. reproductive changes. Consider Figure 3. Here the levels of 11-ketotestosterone have been monitored over the course of an experiment in which groups of sexually maturing cod were fed levels of

0,1,5,10,25 or 50 µg Arochlor 1254/g diet 3 times a week for 92 days. The usual sexual maturation rsponse (o µg Arochlor 1254/g diet) should be noted. The level of 11-ketotestosterone increases during ripening and then falls as regression occurs. At the lower feeding (1 and 5 µg/g) levels the blood levels of 11-ketotestosterone increased much more rapid than control levels and the normal higher level was reached much earlier than usual. As exposure continued blood levels in these groups increased to much higher levels than control levels. At higher doses of PCB, however, levels of 11-ketotestosterone fell below control values. This shift in response is an example of Type 4 or "inflective" response. Animals from field situations which do not show a elevation of these blood levels upon administration of low levels of a standard toxicant would be judged much less healthy than animals which do respond with elevated levels. In addition to monitoring of blood hormone levels over the course of exposure, at the end of the exposure period animals were sacrificed and the steroid hormone metabolism of the testes and interrenals from each group studied. Figure 4 shows the results obtained from the testicular incubations. The initial enhanced synthesis of testosterone and 11-ketotestosterone is noteworthy and corresponds to the blood level observations. At the highest dose level little hormone was observed and since considerable amounts of the precursor was still present the lack of labelled hormone is due to impaired synthesis rather than accelerated breakdown. Figure 5 shows the resultant autoradiograms from the interrenal incubates. Once again the increased hormonal levels were found at the lower doses. Again at the higher levels only small amounts of labelled metabolites appears and a large amount of unchanged precursor remains.

What was the reproductive status of these animals? Control fish, both male and female ripened but, in the case of the exposed male fish no ripe animals were obtained at the end of the exposure period, showing even in the cases of lower exposure, the enhanced hormonal levels interfere in reproductive maturation (Freeman et al. 1978)

To round off this experiment histological investigation of the testes, s gills and liver determinations of tissue PCB levels and enzymological studies of the gonads were carried out.

Testicular sections from control and exposed cod are shown in Figure 6. Even at 1 µgPCB/g diet lobular derangement and arrest of spermatogenesis at the spermatocyte stage was noted. Hyperplasia of the lobule boundary cells and some necrosis was found at the lower dietary exposure levels while at the highest levels there was total disintegration of the lobular contents and marked hyperplasia of the lobule boundary walls.

The gill sections from exposed fish (10 µgPCB/g diet) showed prominent structural changes. These alterations consisted of hyperplasia of the epithelial layer of the secondary gill lamellae. This resulted in clubbing, fusion and thickening of the epithelial layer of the secondary lamellae (Figure 7).

Fatty degeneration of the liver (Figure 8) was observed in samples from all PCB-fed fish although the correlation between diet and degree of degeneration was not high. Severe degeneration was observed in the fish from the higher dietary PCB levels. The relative absence of nuclei in livers from PCB fed fish as opposed to control livers is noteworthy. The livers of a few of the control fish also showed some degree of degeneration.

PCB levels were determined in selected fish from each group and are shown in Table 4. It should be noted that levels in the lowest exposed group (1 µgPCB/g diet) were only about double the control levels. This doubling of the control levels was apparent in all four tissues studied and demonstrates that the experiment does indeed represent realistic exposure doses and routes. (Freeman et al, 1978).

TABLE 4. Concentrations of PCB in Male Cod Tissue (µg/g Wet Wt.)

PCB in Diet µg/g	No. of fish	Livers Range	Livers Mean	Testes Range	Testes Mean	Body Muscle Range	Body Muscle Mean
0	7	2.0-9.0	5.1	0.20-0.06	0.03	0.01-0.03	0.02
1	3	7.3-3.5	10.1	0.05-0.06	0.06	0.02-0.04	0.03
10	5	43.5-101	81.0	0.40-0.66	0.51	0.29 (1 fish)	
25	1	156		1.3		0.33	
50	1	374		5.3		0.98	

Investigation of intermediary metabolic enzymes (malic, lactic, glutamic and isocitric dehydrogenases and aspartic and alanine aminotransferases) in mitochondrial and supernatant preparation from gonads from these cod showed that by and large the activities of these enzymes are very refractive in spite of the marked histological damage observed (Mounib and Eisan, 1978). The levels of mitochondrial malic dehydrogenase, supernatant and mitochondrial lactic dehydrogenase, supernatant and mitochondrial isocitic dehydrogenase, supernatant and mitochondrial glutamic dehydrogenase, mitochondrial aspartic aminotransferase and supernatant alanine aminotransferase were unaffected. Supernatant malic dehydrogenase and supernatant aspartic aminotransferase levels were elevated in the higher PCB-exposed animals while mitochondrial alanine aminotransferase was depressed. These results show two points: 1. the importance of separating enzymes of different intercellular components prior to study and, 2. the refractivity (Type 1) response of many intermediary metabolic enzymes.

The above is only a short review of some of the progress being made in the field of sub-lethal effects detection in our laboratory. It is obviously impossible to carry out such tests with the ease with which lethality tests can be carried out. However such tests as these are of fundamental importance if we wish to ensure adequate species survival as well as individual survival. Reproduction in animals probably, the most non-homeostatic and stressful time in a normal adult animals life, and the addition of further stress at this time, whether of a chemical, physical, psychological or sociological nature likely has a greater deleterious effect upon the animal than at any other time of its adult life. Testing procedures to document the effects of stress at this period represent, we believe, one of the most important areas of investigation for the environmental toxicologist of today.

ACKNOWLEDGEMENTS

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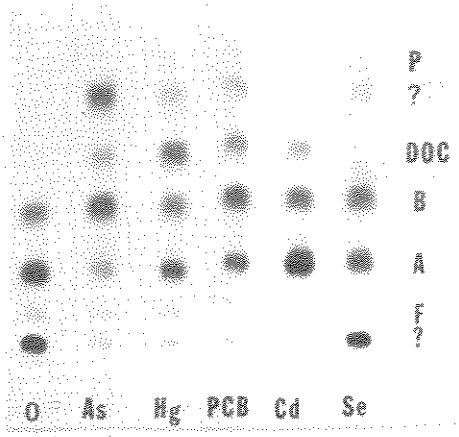


Figure 1. Autoradiogram of grey seal in vitro incubates. Symbols, P-precursors ?-unknown, DOC-11-deoxycorticosterone, P-corticosterone, A-aldosterone, F-cortisol, 0-control, As-As_2O_3, Hg-CH, HgCl, PCB-Arochlor 1254, Cd-$CdCl_2$, Se-SeO_2.

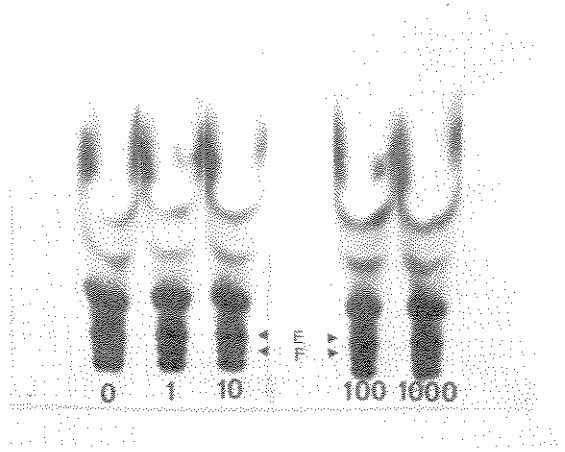


Figure 2. Autoradiogram of cod head kidney (interrenal) incubates. Symbols, E-cortisone, F-cortisol, 0,1,10,100, 1000 µg/ml additins of di-2-ethylhexyl phthalate to incubates.

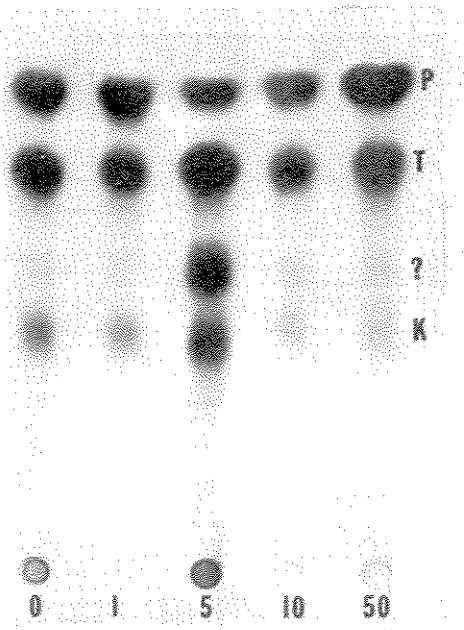


Figure 4. Autoradiograms of cod testes incubates. Symbols, P-precursor, T-testosterone, ?-unknown, K-11-ketotestosterone 0,1,5,10,50-µgPCB/g diet.

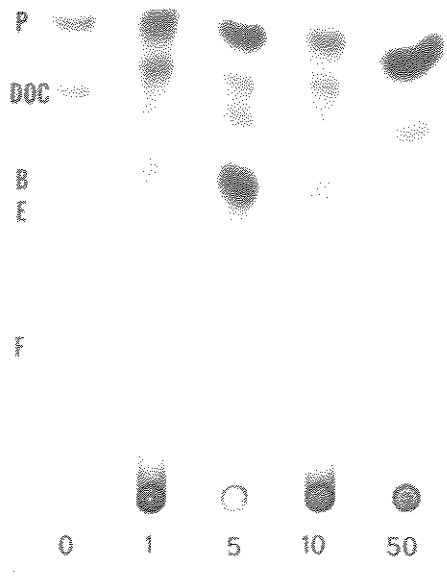


Figure 5. Autoradiogram of cod head kidney (interrenal) incubates. Symbols, P-precursor, DOC-11-deoxycorticosterone, B-corticosterone E-cortisone, F-cortisol, 0,1,5,10,50- µgPCB/g diet.

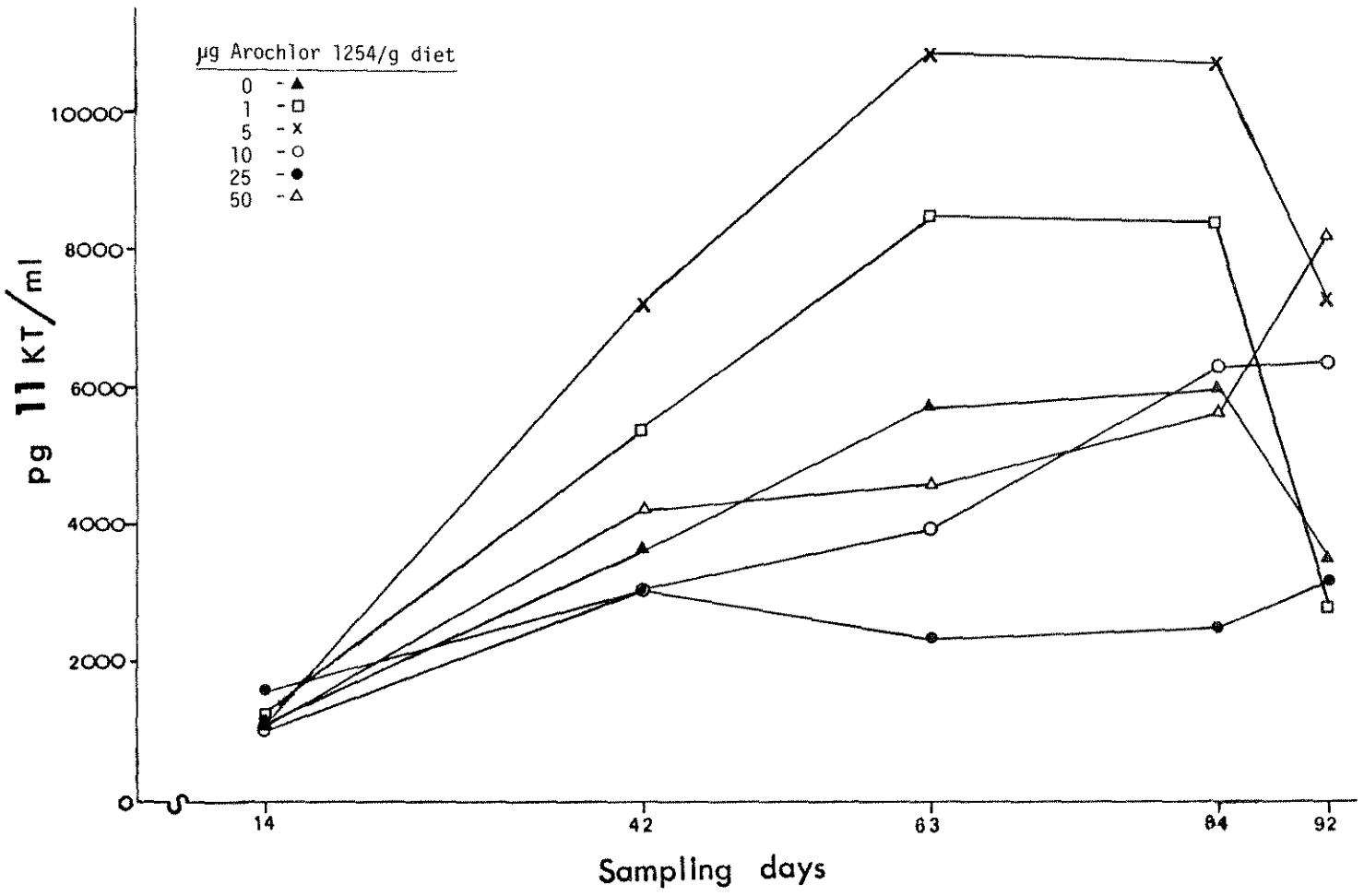


Figure 3. Level of 11-ketotestosterone in the plasma of male cod fed without and with Aroclor 1254.

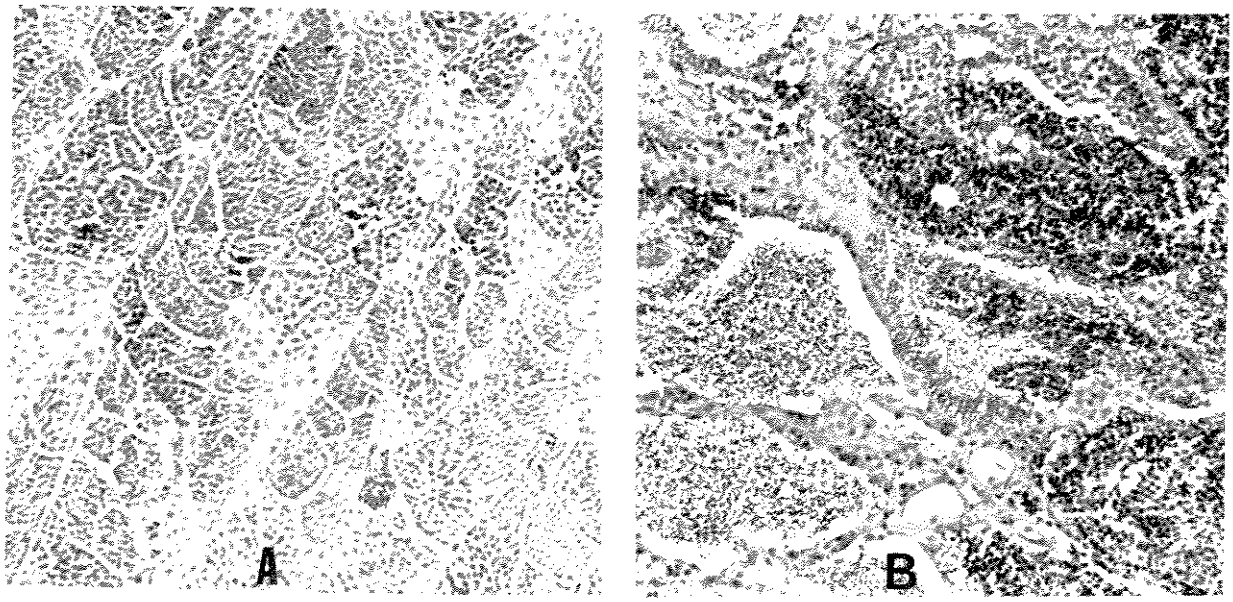


Figure 6. Photomicrographs of testes from control (A) and PCB-fed (B) cod.

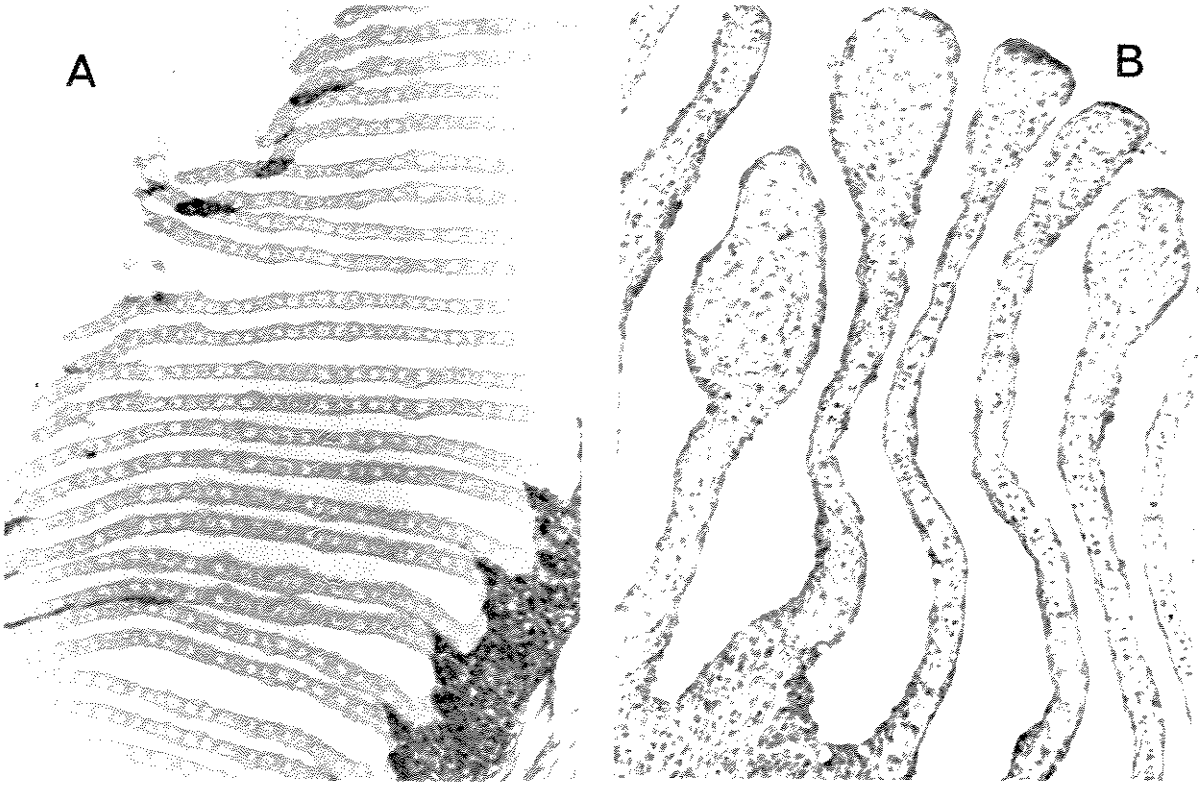


Figure 7. Photomicrograph of gills from control (A) and PCB-fed (B) cod.

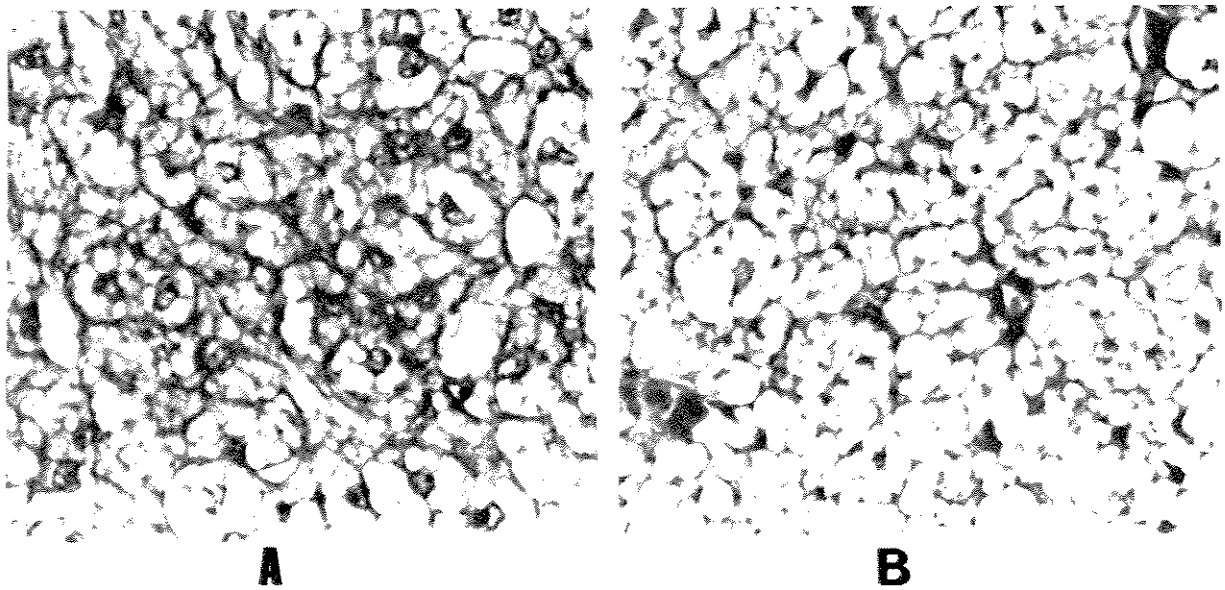


Figure 8. Photomicrograph of cod liver from control (A) and PCB-fed cod.

RELATIONSHIPS GOVERNING THE BEHAVIOR OF POLLUTANTS IN AQUATIC ECOSYSTEMS AND THEIR USE IN RISK ASSESSMENT

V. Zitko

Department of Fisheries and Oceans,
Fisheries and Environmental Sciences,
Biological Station, St. Andrews, New Brunswick, EOG 2XO

ZITKO, V. 1980 Relationships governing the behavior of pollutants in aquatic ecosystems and their use in risk assessment. Can. Tech. Rep. Aquat. Sci. 975: 243-265.

The transport of organic chemicals between water and air (volatilization), water and sediment (adsorption/desorption), and water and biota (uptake/excretion) is discussed. The transport is largely determined by the respective distribution coefficients (Henry's constant, adsorption coefficients, and bioconcentration factor). Adsorption is relatively fast and only equilibrium needs to be considered in most cases. Kinetics is important in the other two processes. In the water and biota transport, the "one compartment" model and its modifications for exponential decrease of the toxicant concentration in water, and for "saturation mechanism" are discussed. Relationships between uptake and excretion rate constants and the octanol/water partition coefficient are mentioned.

Key words: Pesticides; organic chemicals; volatilization; adsorption; accumulation

ZITKO, V. 1980 Relationships governing the behavior of pollutants in aquatic ecosystems and their use in risk assessment. Can. Tech. Rep. Aquat. Sci. 975: 243-265.

Nous analysons le transport des substances chimiques organiques entre l'eau et l'air (volatilisation), l'eau et le sédiment (adsorption/désorption) et l'eau et les biocoenoses (absorption/excrétion). Les coefficients de distribution respectifs déterminent en grande partie le transport (constante de Henry, coefficients d'adsorption et facteur de bioconcentration). L'adsorption est relativement rapide et, dans la plupart des cas, seule l'équilibre nécessitent d'être considéré. Dans les deux autres processus, la cinétique est importante. Dans le transport eau et biocoenose, nous examinons le modèle à "un compartiment" et ses modifications tenant compte de la diminution exponentielle de la concentration du toxique dans l'eau et du "mécanisme de saturation". Nous mentionnons les relations entre les constantes de taux d'absorption et d'excrétion, et le coefficient de séparation octanol/eau.

INTRODUCTION

This paper deals with pollutant transfer between three phase boundaries in aquatic ecosystems (water-air, water-sediment, and water-biota). Laboratory studies of these transfers are discussed, paying attention to the extrapolation of the results to the environment and to their application in risk assessment.

TRANSPORT BETWEEN WATER AND AIR

Transport from water to air is a phenomenon familiar to those involved in toxicity testing. In this context, transport from water to air, or volatilization, is a source of frustration since one is not able to keep the concentration of the toxicant constant when aerating water in the tanks during the experiment.

Only relatively recently was the volatilization process characterized quantitatively by MacKay and coworkers (MacKay and Wolkoff 1973; MacKay and Leinonen 1975; MacKay 1979).

Without going into much detail, the volatilization rate constant KV is given by the relationship

$$KV = \frac{C - P/H}{1/KL + RT/HKG} \text{ [mol } m^{-2}s^{-1}\text{]}, \qquad (1)$$

where C = concentration of pollutant in water [mol m^{-3}]
P = atmospheric partial pressure [atm]
H = Henry's constant [atm m^3mol^{-1}]
KL = mass transfer coefficient in water [m/s]
R = gas constant (8.206×10^{-5}) [atm $m^3mol^{-1}K^{-1}$]
T = temperature [°K]
KG = mass transfer coefficient in air [m/s]

Equation (1) may be simplified to

$$KV = K(C - P/H) \qquad (2)$$

where K = overall mass transfer coefficient.

Before introducing some simplifications into the expression (1), it may be useful to say a few words about Henry's constant H.

Henry's constant is a distribution coefficient of a chemical between a gas and a liquid (in our case between air and water), that is, a ratio between concentration in gas and in liquid, at equilibrium, and this relationship is also valid for the respective solubilities.

$$H = CG/CL \qquad (3)$$

where CG, CL = equilibrium concentrations or solubilities in gas and liquid, respectively.

Henry's law states simply that this ratio at equilibrium is a constant. Partial pressure can be introduced into the equation (3), since from the general gas equation (4) it is proportional to the concentration in the gas phase (5)

$$PV = mRT \qquad (4)$$

where P = pressure [atm]
V = volume [m^3]
m = amount of gas [mol]
R,T = as above

and $$P = mRT/V = RT\ CG \qquad (5)$$

The equation (3) can then be rewritten as

$$H = P/CL \qquad (6)$$

The actual value of H depends on the expression (3,6) and units used. Recent literature uses the expression (6) and units stated in connection with equation (1). Henry's constant is an important parameter in the assessment of movements of a chemical in the environment and its knowledge is required as a part of the information submitted according to the existing legislation. To give a few benchmark values for H, one may consider oxygen, representing a very volatile compound, and water, representing a fairly nonvolatile compound. The solubility of oxygen at 15°C is 9.76 mg/L = 0.305 mol m^{-3}. The atmospheric partial pressure of oxygen is 0.211 atm; consequently, Henry's constant of oxygen is 0.692 atm $m^3 mol^{-1}$.

The solubility of water is $10^6/18 = 5.56 \times 10^4$ mol/m^3, the partial pressure is 1.68×10^{-2} atm and, from these, Henry's constant is 3.03×10^{-7} atm $m^3 mol^{-1}$.

Low vapor pressure is not an indication of a low value of Henry's constant and, consequently, of low volatility. As discussed, Henry's constant is a ratio of vapor pressure to solubility. For example, the vapour pressure of DDT is 1.32×10^{-10} atm, but, since the solubility of DDT in water is only about 2.82×10^{-6} mol m^{-3}, Henry's constant of DDT is 4.6×10^{-5} atm $m^3 mol^{-1}$.

Returning now to equation (1), KL is usually about 5×10^{-5} m/s, and the value of KG is in the order of 10^{-2} m/s. At 15°C, RT = 2.48×10^{-2}, and equation (1) becomes

$$KV = \frac{C - P/H}{20000 + 2.5/H} \qquad (7)$$

It can be seen from equation (7) that, depending on the value of H, the second term in the denominator may, or may not, be negligible in comparison with the first one.

For H values of the order of 1E-3, 2.5/H is negligible, the denominator is small and the substance partitions fast from water into the atmosphere. For H values below 1E-5, the first term in the denominator is negligible and such compounds are volatilized to a much smaller extent.

Turning now to toxicity tests, it is worth while to note that in static experiments, under intensive aeration, the concentration of the toxicant in the bulk of the solution may be in equilibrium with its concentration in the exiting air, purging quite effectively compounds with high Henry's constants from the solution. The equation (8) describes this situation

$$LN(C/CO) = -(HG/VRT)t \qquad (8)$$

where C,CO = concentration of the chemical at time t and t_o [h], respectively
H = Henry's constant [atm $m^3 mol^{-1}$]
G = gas flow rate [$m^3 h^{-1}$]
V = volume of solution [m^3]
R = gas constant [atm $m^3 mol^{-1} K^{-1}$]
T = temperature [°K]

The difference between the rate constant in equation (8) (HG/VRT) and the rate constant given by equation (1) is due to the fact that the former assumes an equilibrium between the concentration of the chemical in water and in air in the whole system, whereas the latter assumes this equilibrium only in a thin layer at the air/water boundary.

Figure 1 demonstrates the magnitude of losses that may be expected in static experiment with compounds having H of the order of 1E-3 to 1E-5. The conditions of static tests are quite similar to those used by MacKay and coworkers (1979) to determine Henry's law constants and, with little additional effort, these constants could be determined during routine toxicity tests.

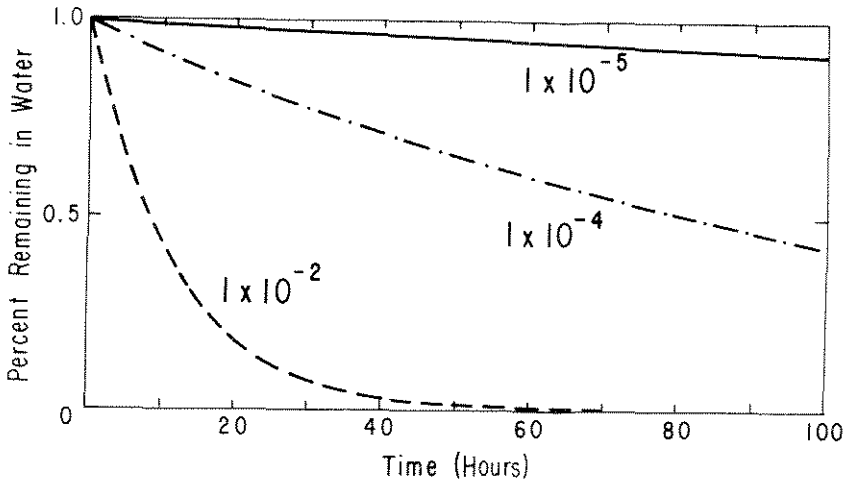


Fig. 1. Volatilization of compounds from water under "purge" conditions as a function of time and Henry's constants. Volume 3 L, gas flow 100 mL/min.

The knowledge of Henry's constants would aid the interpretation of the toxicity tests in terms of risk estimate in the environment. Chemicals with high Henry's constants are likely to volatilize quite readily and "disappear" from water. This may lead to a widespread environmental contamination if these compounds are persistent, as is the case with PCB's, or to dilution below toxic levels and eventual disappearance from the environment if the compounds are degraded. Compounds with low Henry's constants are likely to remain in the aqueous phase. Environmental contamination from a point discharge may not be as widespread as in the previous case but, due to higher persistence in water, toxic concentrations may persist for a longer time. Additional considerations have to be taken into account as above.

TRANSPORT BETWEEN WATER AND SEDIMENT

In the transport between water and sediment, the term analogous to Henry's law constant is the adsorption coefficient KS. KS is a ratio between the concentration of the chemical adsorbed on sediment or on suspended matter CA and its concentration in solution C:

$$KS = CA/C \qquad (9)$$

KS depends on the physical and chemical properties of the sediment, such as particle size, content of organic matter and, for cations, on the number and binding constants of cation-binding sites. For the majority of nonionized organic chemicals of current environmental interest, KS is very significantly influenced by the organic matter content of the sediment and variations of KS values between sediments are decreased considerably if CA is expressed on the basis of the organic carbon content of the sediment. In this case KS becomes KOC

$$KOC = CAC/C \qquad (10)$$

where KOC = adsorption coefficient on an organic carbon basis
CAC = concentration of chemical adsorbed, expressed on an organic carbon basis
C = concentration of chemical in solution.

The equations (9) and (10) are a special case of the Freundlich adsorption isotherm

$$KS = CA/C^{**}1/n \qquad (11)$$

where n = empirical constant.

The values of n are usually close to unity and at relatively low levels of C, such as those encountered under environmental conditions, the differences between (10) and (11) are negligible.

The kinetics of adsorption is more complex than that of volatilization. Both diffusion and mass flow (transport in water crossing the phase boundary) have to be considered and there are no reliable estimates of the respective mass-transfer coefficients. Fortunately, adsorption appears to

be a much faster process than any other transport or transformation of most chemicals in the aquatic environment. Consequently, the problem of kinetics can be, at least for the time being, eliminated by assuming the establishment of an equilibrium governed by relationships (9)-(11).

The adsorption coefficients are related to properties such as water solubility and octanol/water partition coefficients. In a recent paper, Kenaga and Goring (1978) derived the relationships (12) and (13)

$$LOG(KOC) = 3.64 - 0.55\ LOG(WS) \qquad (12)$$

$$LOG(KOC) = 1.377 + 0.544\ LOG(KOW) \qquad (13)$$

where WS = water solubility [mg/L]
KOW = octanol/water partition coefficient.

The relationships were derived mostly using soils rather than sediments, but appear to be applicable to aquatic sediments as well. A complication arises from the fact that with some chemicals, for example with PCB's, the adsorption is to some extent irreversible. In other words, KOC values determined by approaching the equilibrium from the adsorption side are smaller than KOC measured during desorption. Not enough data are available on this subject to make predictions about the generality of this phenomenon.

To obtain the distribution of a compound between suspended matter and the aqueous phase, the concentration of suspended matter, S, has to be considered according to (14)

$$KOC = CAC/S*C \qquad (14)$$

where CAC = concentration adsorbed [mg/g]
S = concentration of suspended matter [g/L]
C = concentration in solution [mg/L].

The fraction dissolved is then given by

$$C/CT = 1/(KOC*S+1) \qquad (15)$$

where C,CT = dissolved and total concentration of the chemical, respectively.

The rate of any process is reduced by adsorption to the extent indicated by

$$dCT/dt = K*CT/(KOC*S+1) \qquad (16)$$

A detailed discussion of this subject is given by Baughman and Lassiter (1978).

By the same reasoning, toxicity of chemicals to aquatic fauna should be decreased in a similar fashion in the presence of suspended matter. Qualitatively this is a well known fact, but quantitative data are difficult

to find in the literature. If the toxicity is proportional to the concentration of the dissolved chemical, then, from (15)

$$TX = K*CT/(KOC*S+1) \qquad (17)$$

where TX = toxicity, K = constant.

The validity of (17) should be easy to check experimentally by measuring the toxicity of a chemical in the presence of varying concentrations of suspended matter with known KOC.

TRANSPORT BETWEEN WATER AND BIOTA

The accumulation of certain chemicals in aquatic biota is well known. A large amount of literature is available on this subject (see for example, Hamelink 1977; Kenaga and Goring 1978; Zitko 1980). As a rule of thumb, the bioconcentration factors of organic compounds increase up to a point with increasing octanol/water partition coefficient and with decreasing solubility in water. The bioconcentration factor is a distribution coefficient, analogous to (1) and (9) and, according to Kenaga and Goring (1978) can be estimated from the octanol/water distribution coefficient (18), or from water solubility (19).

$$LOG(BCF) = -1.495 + 0.935\ LOG(KOW) \qquad (18)$$

$$LOG(BCF) = 2.791 - 0.564\ LOG(WS) \qquad (19)$$

where BCF = bioconcentration factor
KOW = octanol/water distribution coefficient
WS = water solubility [mg/L].

There is a discrepancy between predictions of BCF from (18) and (19). Kenaga and Goring (1978) derived equation (20) for predicting WS from KOW:

$$LOG(WS) = 4.184 - 0.922\ LOG(KOW) \qquad (20)$$

Substituting (20) into (19),

$$LOG(BCF) = 0.431 + 0.52\ LOG(KOW) \qquad (21)$$

Equation (21) gives somewhat higher values of BCF than equation (18).

One has to bear in mind that these equations give only rough estimates of BCF. They have been derived from data obtained on a variety of compounds under different conditions and by various techniques. Better predictions are likely to result from data bases limited to structurally more closely related compounds. For a quick orientation, some predicted values of BCF are given in Table 1.

Table 1. BCF predicted from water solubility (WS) and from octanol/water partition coefficient (KOW) (modified from Kenaga and Goring 1978).

Water solubility [mg/L]	KOW	LOG(KOW)	BCF from WS	BCF from KOW(18)
0.001	300,000	5.5	30,000	4,400
0.01	110,000	5	8,300	1,500
0.1	40,000	4.6	2,300	640
1	14,000	4.1	600	200
10	5,000	3.7	170	90
100	2,000	3.3	50	40

To put these numbers into perspective, the best approach may be to select a few well-studied chemicals as benchmarks.

It appears that accumulation problems are likely in case of compounds with solubilities in water below 1 mg/L and octanol/water partition coefficient > 3×10^4 (log(KOW) > 4.5). This general conclusion may have to be modified in later stages of the assessment, depending on the amount of chemical, use pattern, rate of release, etc.

Up to this point only the equilibrium of the transport of chemicals between water and biota has been considered. The most commonly used kinetic description of the process is based on the "one compartment" model (Hamelink 1977), described by (22) (Fig. 2)

$$CF = K1*CW*(1-EXP(-K2*T))/K2 \qquad (22)$$

where CF,CW = concentration of the chemical in fish and water, respectively
K1,K2 = uptake and excretion rate constants [1/time], respectively.

The bioconcentration factor is given by

$$BCF = K1/K2 \qquad (23)$$

To fit experimental data to equation (22), the rate constants K1 and K2 have to be determined.

A computer program BIOFAC (Blau and Agin 1978), performs the fitting routine and other commercially available programs [for example MLAB available as a component of CIS (Chemical Information System), carried by ISC] may be used. These programs require initial estimates of K1 and K2 that are reasonably close to the actual values. The BIOFAC user's manual describes in detail the methods used to arrive at the initial estimates. If

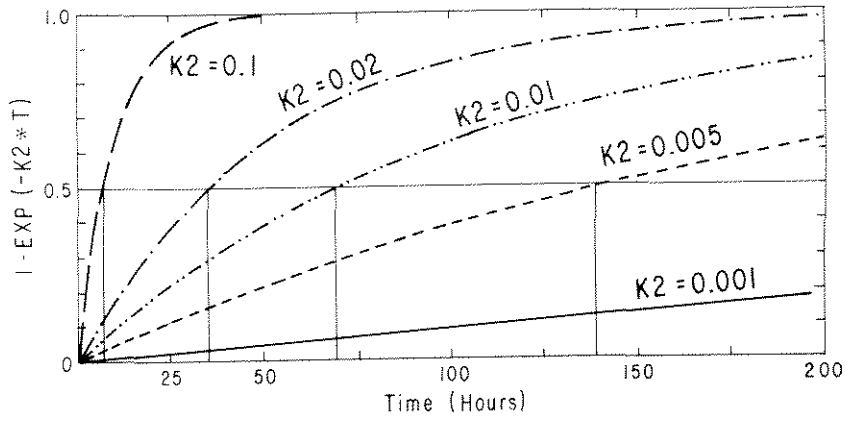


Fig. 2. Approach to equilibrium in the "one compartment" model.

excretion data are available, K2 is determined easily as the slope of LN(CF) vs. time, and K1 is obtained from (22).

Often the experimental scatter is such that the use of sophisticated fitting routines is not warranted and simpler procedures produce adequate results. For example, from (22) and (23)

$$(CF/CW)/BCF = 1-EXP(-K2*T) \qquad (24)$$

It follows from (24) that, if K2 is known, the observed bioconcentration factor CF/CW may be corrected to yield the bioconcentration factor BCF. For example, a bioconcentration factor CF/CW of fluoranthene in shrimp is 694 after a 2-d exposure (Lee et al. 1978). The authors also report that K2 = 0.00578/h. From (24)

$$(CF/CW)/BCF = 694/BCF = 1-EXP(-0.00578*48) = 0.24$$
$$\text{and} \quad BCF = 2860.$$

The equation (24) can also be used to estimate K2 if the accumulation curve shows signs of reaching equilibrium. For example, if the time required to reach 50% of the equilibrium accumulation is T50, then from (24)

$$0.5 = 1-EXP(-K2*T50)$$
$$\text{and } K2 = -LN(0.5/T50)$$

In another method, an empirical function that is easier to fit to the data than function (22) may be used. Curtis et al. (1977) compared function (22), two "two-compartment" models, and an empirical function (25) in describing the accumulation of methyl mercury in bluegill sunfish (Lepomis macrochirus). Function (25) described the data very well and was easy to fit.

$$CF = A + \frac{T}{B+C*T} \qquad (25)$$

where A,B,C = empirical coefficients
T = time

From (25)

$$CFE = A + 1/C \qquad (26)$$

where CFE = concentration in fish at equilibrium.

The disadvantage of the empirical function approach is that the parameters (A,B,C in the above example) have no physical meaning (compare to K1 and K2, the uptake and excretion rate constants in the one-compartment model).

In another approach, (22) can be approximated by (27) or (28)

$$CF = K1*CW*T \qquad (27)$$

$$CF = K1*CW*T - \tfrac{1}{2}\, K1*K2*CW*T**2 \qquad (28)$$

In static accumulation experiments the concentration of the chemical in water usually decreases exponentially

$$CW = A*EXP(-B*T) \qquad (29)$$

where A,B = empirical coefficients
T = time

In this case, equation (30) must be used instead of (22)

$$CF = K1*A*(1-EXP(-(K2-B)*T))*EXP(-B*T)/(K2-B) \qquad (30)$$

The effect of B and K2 on the shape of the function (30) is illustrated in Fig. 3 and 4. The curves have a maximum that shifts to longer times with decreasing B and K2 values. At a very low K2 (K2 = 0.005, Fig. 4) the maximum is not obvious from the "experimental" data and the curve resembles, at a first glance, a "normal" accumulation curve.

Since the experimental data result in the determination of B (one is supposed to measure CW during the test), and the time of the maximum TM can be estimated from the accumulation data, K2 can be calculated from (31). The equation contains both LN(K2) and K2 and can be solved numerically by iterations performed easily on a pocket calculator

$$K2 = LN(K2)/TM - (LN(B)-B*TM)/TM \qquad (31)$$

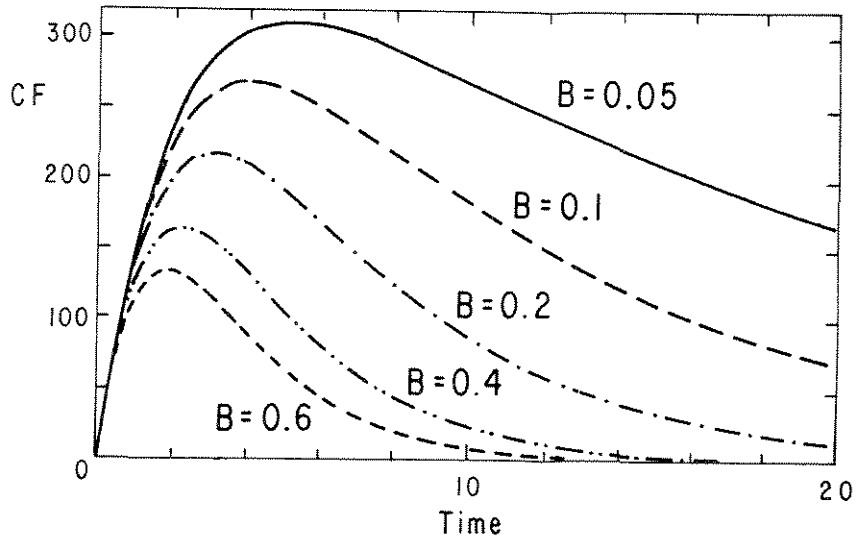


Fig. 3. The effect of B on CF (K1 = 200, K2 = 0.5, A = 1).

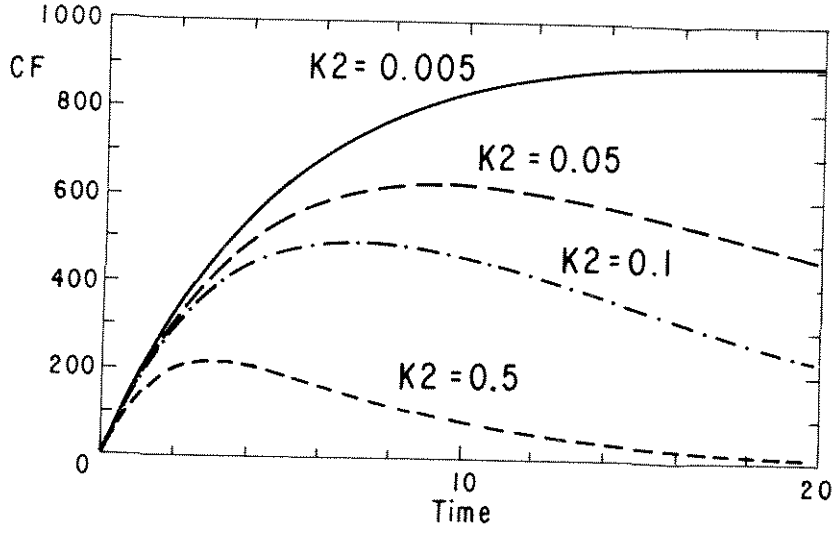


Fig. 4. The effect of K2 on CF (K1 = 200, A = 1, B = 0.2).

In this process, an initial K2 value is chosen, substituted into the right-hand side of (31) and a new K2 value is calculated. This process is repeated until the K2 value is estimated within reasonable accuracy.

With K2 estimated, K1 can be calculated from the maximum concentration of the chemical in fish, CFM, attained at TM

$$K1 = CFM*K2/(A*EXP(-B*TM)) \qquad (32)$$

Kanazawa's (1975) data on the uptake of diazinon were evaluated as described (Fig. 5).

The "one compartment" model is obviously an oversimplification. "Two and more compartment" models are available (Moriarty 1975; Robinson 1975) but the experimental data are seldom detailed and accurate enough to warrant their use.

All the discussion so far was centered on the problem of determining the equilibrium concentration of chemicals in fish (CFE) and the bioconcentration factor BCF. It was assumed that the uptake and excretion rate constants K1 and K2, and consequently BCF, are independent on concentration of the chemical. Experimental data indicate that this is frequently not the case and that the bioconcentration factor usually decreases (but sometimes increases) with increasing concentration of the chemical. References to specific examples will be given later.

Majori and Petronio (1973) demonstrated that the decrease of BCF with increasing CW follows from the law of mass action. The equilibrium constant K for the chemical reaction (33) is given by (34)

$$U + V \rightleftharpoons UV \qquad (33)$$

$$K = \frac{[UV]}{[U]*[V]} \qquad (34)$$

where [UV], [U], and [V] = concentrations of compounds UV, U, and V.

From (34) and the material balance (35)

$$[U] = [UT] - [UV] \qquad (35)$$

where [UT] = concentration of total U in the system.

$$[UV] = \frac{K*[UT]*[V]}{1 + K*[V]} \qquad (36)$$

$$BCF = \frac{[UV]}{[V]} = \frac{K*[UT]}{1+K*[V]} = \frac{[UT]}{1/K + [V]} \qquad (37)$$

As follows from (36) and (37), functions (36) and (37) are linear in coordinates 1/[UV], 1/[V], and 1/BCF,[V], respectively, thus enabling an easy determination of K and [UT].

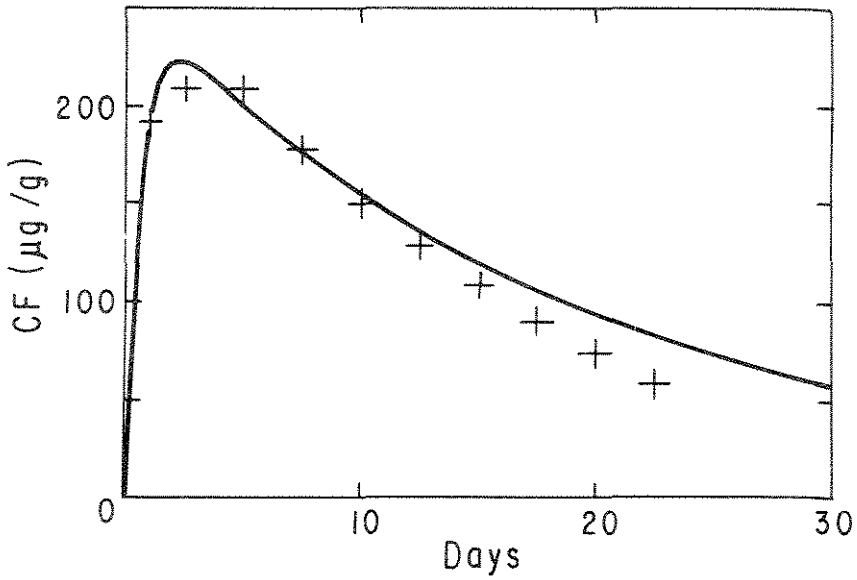


Fig. 5. Accumulation of diazinon in fish (Kanazawa 1975). Concentration of diazinon in water CW = 0.826*EXP(-0.0508*T). Plotted curve: K1 = 505, K2 = 1.62, TM = 2.2, CFM = 230.

$$1/[UV] = K*[UT] * \frac{1}{[V]} + \frac{1}{[UT]} \tag{38}$$

$$1/BCF = \frac{1}{K*[UT]} + \frac{1}{[UT]} * [V] \tag{39}$$

Relationships analogous to (36) and (37) can be derived from the "one compartment" model by assuming that the concentration of a chemical in fish (CF) cannot exceed a certain maximum concentration CT ("concentration of binding centers") and that the rate of uptake is proportional not only to the concentration of the chemical in water (CW) but to the difference CT-CF ("concentration of free binding centers") as well. Under these assumptions the equation (40) is obtained

$$CF = (K1*CW*CT/(K1*CW+K2))*(1-EXP(-(K1*CW+K2)T)) \tag{40}$$

where CT = total concentration of binding centers.

The equilibrium concentration CFE is then (41) and the bioconcentration factor is given by (42)

$$CFE = K1*CW*CT/(K1*CW+K2) \tag{41}$$

$$BCF = K1*CT/(K1*CW+K2) \quad (42)$$

The function (40) is very similar to the function (22) and would explain some of the observed changes of accumulation of a chemical with its concentration in water. One important difference between functions (22) and (40) is that according to the former the excretion rate constant K2 is the same during the accumulation (CW ≠ 0) and excretion (CW = 0) phases of the experiment. On the other hand, according to the latter equation, the effective excretion rate constant is higher during the accumulation phase (K1*CW+K2) than that during the excretion phase (K2). Depending on the values of K1, CW, and K2, this difference may or may not be significant. The applicability of the equation (40) was tested on several sets of accumulation data.

The data of Mayer (1976) on the uptake of di-(2-ethylhexyl)phthalate (DEHP) by fathead minnows (Pimephales promelas) were evaluated using equations (40) and (41). It was assumed that CFE's had been reached on day 56 and the values were fitted to the equation (41) by linear regression between 1/CFE and 1/CW. This yielded CT = 11.13 and K2/K1 = 0.0119.

The excretion data were evaluated by linear regression between LN(CF) and T, yielding an average K2 = 0.04. Consequently, K1 = 3.36. The equation (40) then becomes (43)

$$CF = 3.36*11.13*CW*(1-EXP(-(3.36*CW+0.04)*T))/(3.36*CW+0.04) \quad (43)$$

The concentrations of DEHP calculated from (43) and those measured by Mayer are given in Table 2. The agreement of the measured and calculated values is reasonably good except at short exposure times when, in most instances, the calculated values are considerably lower.

Table 2. Accumulation of di-(2-ethylhexyl)phthalate by fathead minnow (Pimephales promelas). Equation (40) fitted to the data of Mayer (1976) (see equation (43)). CF's are in µg/g.

	Mean exposure concentration (µg/L)													
	1.9		2.5		4.6		8.1		14		30		62	
Days	M[a]	C[a]	M	C	M	C	M	C	M	C	M	C	M	C
1	.26	.07	.40	.09	.48	.17	1.2	.29	1.4	.50	1.8	1.0	2.1	2.1
3	.46	.20	.77	.26	1.0	.48	1.8	.82	2.6	1.4	3.4	2.8	3.1	4.9
7	.70	.42	.93	.55	2.2	1.0	2.9	1.7	3.3	2.7	3.2	5.0	3.9	7.7
14	.87	.73	1.5	.95	2.6	1.7	2.8	2.8	2.7	4.2	3.2	6.9	3.8	9.1
28	1.4	1.1	2.2	1.4	3.1	2.5	2.9	3.8	4.8	5.5	7.5	7.8	6.9	9.3
56	1.4	1.4	2.2	1.8	4.1	3.0	3.6	4.4	5.0	6.0	8.6	8.0	9.6	9.4

[a]M,C = measured and calculated CF, respectively

According to Streit and Schwoerbel (1976) the equilibrium concentration of atrazine in the leech (Glossiphonia complanata) is given by the power function (44) for a CW range from 0.001 to 1 mg/L.

$$CFE = 8.690*CW**0.740 \quad (44)$$

The function (44) was approximated by the function (41) for CT = 6.04 and K2/K1 = 0.201. K2 = 0.183 was estimated from excretion data presented in the original paper. Consequently K1 = 0.91. After substituting these values into (40), the function (45) is obtained:

$$CF = 5.50*CW*(1-EXP(-(0.91*CW+0.183)*T))/(0.91*CW+0.183) \quad (45)$$

A comparison of experimental with calculated data is given in Table 3. The agreement is reasonable considering that the range of CW is almost three orders of magnitude.

Table 3. Accumulation of atrazine by the leech (Glossiphonia complanata); equations (40) and (41) fitted to the data of Streit and Schwoerbel (1976).

CW, µg/L	0.001	0.01	0.1	0.5	1.0
CFE, measured	0.052	0.288	1.58	5.20	8.69
CFE, calculated(41)	0.030	0.287	3.01	4.31	5.03
Exposure, h	2	4	6	8	10
CW = 0.002					
CF measured	0.05	0.07	0.08	0.08	0.08
CF calculated	0.02	0.03	0.04	0.05	0.05
CW = 1.0					
CF measured	7.0	7.5	7.5	7.5	7.5
CF calculated	4.46	4.97	5.02	5.03	5.03

From the data of Hansen et al. (1974) the CFE's of Aroclor 1016 in American oysters (Crassostrea virginica) and grass shrimp (Palaemonetes pugio) but not those in the pinfish (Lagodon rhomboides) can be expressed as a function of CW by equation (41). The results are presented in Table 4.

Turning now to the application of the laboratory-determined BCF's to risk assessment, it is obvious from the preceding discussion that care has to be exercised in evaluating BCF's given in the literature. Attention should be paid to the possibility that the reported value may not be an

Table 4. Accumulation of Aroclor 1016 by some estuarine animals. Data of Hansen et al. (1974) fitted to equation (41).

CFE (µg/g)								
C. virginica			P. pugio			C. rhomboides		
CW	M[a]	C[a]	CW	M	C	CW	M	C
0.6	4.0	4.0	0.4	1.1	1.1	0.8	2.2	2.2
7.2	32	33.8	9.4	22	20.6	6.9	21	18.2
58	95	83	38	44	50.6	56	65	112
CT		104.6			97.2			402
K2/K1		0.0151			0.0349			0.145

[a]M,C = CFE measured and calculated, respectively

equilibrium BCF. As demonstrated, this can be checked if the excretion rate constant is known. As also illustrated, the BCF's depend quite frequently on concentration of the chemical in water and it is expected that they increase with decreasing concentration.

Generally speaking, compounds that have BCF's of more than about 200-300 in laboratory tests and excretion rate constants of < 0.02 day^{-1} are potential suspects for bioaccumulation problems. Even in the absence of demonstrated toxic effects such compounds must be scrutinized carefully, because there is always a potential for subtle chronic effects when a compound resides in an organism for considerable periods of time.

A review of uptake (K1) and excretion (K2) rate constants is given in Table 5. The data were obtained on a variety of test animals. In many cases the rate constants were taken directly from the original papers, in some cases the constants were obtained by evaluating published uptake and excretion data. KOW values were taken from the literature or estimated.

For the whole set, there is a significant relationship between LOG(K2) and LOG(KOW) (46)

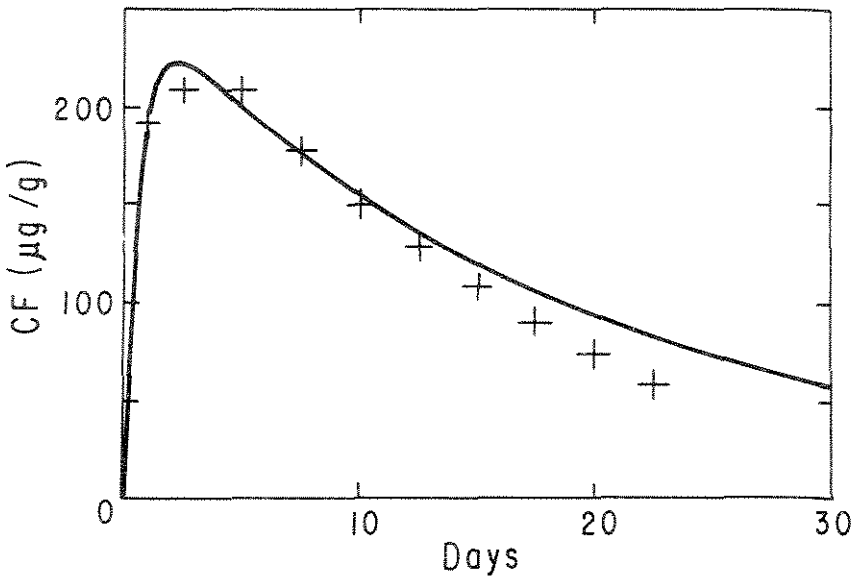
$$\text{LOG(K2)} = 1.36 - 0.34\ \text{LOG(KOW)} \qquad (46)$$
$$R = -0.476$$

and, as one would expect, between K1/K2 (bioconcentration factor) and LOG(KOW) (47)

$$\text{K1/K2} = -1650 + 761\ \text{LOG(KOW)} \qquad (47)$$
$$R = 0.373$$

Table 5. Uptake (K1) and excretion (K2) constants (per day) and log(KOW) values.

#	Compound	K1	K2	Log (KOW)	Reference
1	Chloroform	162	16	1.97	
2	Trichloroethylene	83	3	1.17	Osaki and Ogata
3	1,1,1-trichloroethane	137	3	1.63	1977
4	Carbon tetrachloride	198	7	2.64	
5	4-chlorobiphenyl	22	0.21	4.26	
6	2,2'-dichlorobiphenyl	39	0.33	4.04	
7	4,4'- "	86	0.07	5.18	
8	2,3,2',3'-tetrachlorobiphenyl	140	0.14	4.63	
9	2,3,5,6- "	123	0.19	5.46	Sugiura et al.
10	3,4,3',4'- "	147	0.11	6.04	1978
11	3,5,3',5'- "	136	0.14	6.85	
12	3,5-dibromobiphenyl	56	0.12	5.78	
13	3,5,4'-tribromobiphenyl	199	0.02	6.41	
14	3,5,3',5'-tetrabromobiphenyl	193	0.05	7.41	
15	Tetrachloroethylene	80	1.98	2.88	
16	Carbon tetrachloride	97	5.50	2.64	
17	p-dichlorobenzene	136	0.63	3.38	Neely et al. 1974
18	Hexachlorobenzene	450	0.057	6.18	
19	2,2',4,4'-tetrachlorodiphenyl oxide	293	0.024	3.40	
20	2,2',4,4'-tetrachlorobiphenyl	286	0.03	3.49	Branson et al. 1975
21	Chlorodiphenyl ether	358	0.48	4.08	Branson 1976
22	Butyl chlorodiphenyl ether	216	0.72	4.21	
23	α-BHC	92	0.86	4.44	
24	γ-BHC	75	0.75	4.04	
25	Heptachlorepoxide	578	0.34	5.03	
26	α-endosulfan	295	0.49	4.92	Ernst 1977
27	Dieldrin	490	0.31	5.19	
28	Endrin	766	0.40	5.11	
29	DDD	1270	0.14	5.81	
30	Chlordane	40	0.4	4.51	Moore et al. 1977
31	Pentachlorophenol	74	2.4	5.01	Glickman et al.
32	Pentachloroanisole	100	2.4	5.5	1977
33	Phenol	107	59	1.66	Kobayashi et al. 1976
34	Carbazole	5700	49	3.29	Southworth et al.
35	13-H-dibenzo(a,i)carbazole	13000	1.85	5.59	1979



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where CT = total concentration of binding centers.

The equilibrium concentration CFE is then (41) and the bioconcentration factor is given by (42)

$$CFE = K1*CW*CT/(K1*CW+K2) \tag{41}$$

The relationships are not very good. Widely different chemicals and test species including mussels, fish, and Daphnia may be largely responsible for the poor fit. Relationships obtained on more homogeneous sets of chemicals and test species are much better. For example, for aromatic hydrocarbons and azaarenes

$$LOG(K1) = 2.82 + 0.249\ LOG(KOW) \qquad R = 0.839 \qquad (48)$$

$$LOG(K2) = 3.78 - 0.593\ LOG(KOW) \qquad R = -0.973, \qquad (49)$$

for halobiphenyls

$$LOG(K1) = 0.786 + 0.212\ LOG(KOW) \qquad R = 0.741 \qquad (50)$$

$$LOG(K2) = 0.178 - 0.203\ LOG(KOW) \qquad R = -0.649 \qquad (51)$$

No relationships are observed for organophosphate pesticides. Lack of data may be partly responsible for this.

SUMMARY

This paper discussed the movement of organic chemicals between water and air, water and sediment, and water and biota, and reviewed some mathematical relationships describing these processes. The main parameters in these relationships are the respective distribution coefficients: Henry's constant, the adsorption coefficient, and the bioconcentration factor.

Adsorption appears to be faster than either volatilization or bioconcentration, and matters can be simplified considerably by assuming that adsorption processes are at equilibrium. On the other hand, the kinetics of volatilization and of bioconcentration must be considered in most circumstances.

In laboratory studies with aquatic animals there is no need to be concerned too much about volatilization of the toxicants, as long as the concentration of the toxicant in water is measured, but Henry's constants may be determined as well, since it does not take much additional effort.

In studies of bioaccumulation more attention should be given to the dependence of bioconcentration factors on concentration and to quantitative descriptions of uptake and excretion in general.

The search for quantitative structure-activity relationships is a continuous task, bearing in mind of course that such relationships are just one of the tools of environmental research. The same may be said about mathematical description of environmental processes and environmental modeling. These are tools that, if nothing else, help to clarify our thinking, may focus attention on, and improve understanding of, the movement and fate of chemicals in the environment.

Our primary objective is the protection of the aquatic environment. To this end there is a continuing need for toxicity data, knowledge of the mechanism of action at the molecular, organism, and ecological levels, and actual and anticipated movement of chemicals through the environment. In the regulatory process we will be more and more involved with the assessment of new chemicals and with the adoption of realistic control measures. To meet these requirements, we must be open to and use new tools from our own, as well as from related fields.

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APPENDIX I

A REVIEW OF AQUATIC TOXICITY RESEARCH ACROSS CANADA

S.L. Leonhard

Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, R3T 2N6

The following tables summarize the results of surveys solicited from researchers in the designated regions. The input was presented as a poster display at the Sixth Annual Aquatic Toxicity Workshop in Winnipeg. Sincere thanks are extended to the individuals who compiled this information and to their numerous co-operative contributors. Apologies are offered to those researchers who, inadvertently, may have been missed.

B.C. region:	D. McLeay Programme Leader Aquatic Toxicology Division of Applied Biology B.C. Research 3650 Wesbrook Mall Vancouver, Canada V6S 2L2
Prairies:	B. Hammond Alberta Environment Research Secretariat Oxbridge Place 9820-106 Street Edmonton, Alberta T5K 2J6
Ontario: (excluding Great Lakes Biolimnology Lab.)	G. Craig Ontario Ministry of Environment Limnology and Toxicology Section P.O. Box 213 Rexdale, Ontario M9W 5L1
Ontario: (Great Lakes Biolimnology Lab.)	A. Niimi Great Lakes Biolimnology Laboratory Burlington Canada Centre for Inland Waters 867 Lakeshore Road, P.O. Box 5050 Burlington, Ontario L7R 4A6
Quebec:	C. Blaise Environment Canada 1001 Pierre Dupuy Longueuil, Quebec
Maritimes:	P. Wells EPS - Environment Canada 5151 George Street Halifax, Nova Scotia

REVIEWER: D. McLEAY REGION: PACIFIC

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Dept. Fisheries and Oceans, Habitat Protection Division Water Quality Unit 1090 W. Pender Vancouver, B.C.	I.K. Birtwell	Juvenile Salmonids	*in situ* avoidance and distribution	Pulp Mill effluent
	R.M. Harbo	Pacific Oysters Flatfish Salmonids	Heavy metal accumulation	Municipal wastes
	S.C. Samis M.D. Nassichuk	Benthic Invertebrates Algae	Numerous Water Quality, Variables	Hatchery and Municipal wastewater
Fisheries and Oceans, Canada West Vancouver Laboratory	George Kruzynski	Sockeye Salmon	Effects of sublethal exposure on plasma electrolytes Uptake and tissue distribution	Dehydroabietic Acid
Fisheries and Oceans Nanaimo	G.P. Haywood	Coho Salmon	Growth and survival	NH_3^+/low O_2
	J. Jensen	Sockeye Salmon Eggs	Rate of development anomalous development survival fertilization to hatching	Unionized ammonia
	J. Jensen	Sockeye Salmon Eggs	Coagulated yolk syndrome	High NH_{3u} x low O_2
Beak Consultants Ltd. Richmond, B.C.	G.L. Hardaker	Rainbow Trout Coho Salmon	Routine monitoring	Pulp and Paper, Oil Refinery, Chemical Plant and Steel Mill effluents
E.V.S. Consultants Ltd. 195 Pemberton Ave. North Vancouver, B.C. V7P 2R4	Peter Chapman Gary Vigers Melody Farrell	Oligochaete Worms (4 marine; 7 fresh water species)	Temperature, salinity dissolved oxygen, pH	Mercury, cadmium, pentachlorophenol, chlorinated sewage effluent, Pulp Mill effluent
	Eric McGreer Dave Munday Gary Vigers	Chum, Coho and Rainbow Trout Fry, Juvenile Herring, Coho Smolts, Three-spine Stickleback Zooplankton and Ichthyoplankton	Temperature, salinity, dissolved oxygen, pH *in situ* and laboratory	Sulfite Mill effluent
	Gary Vigers Jeffrey B. Marliave	Herring Larvae Rainbow Trout	Light, salinity, temperature	Oil/oil dispersant mixtures. Bleached Kraft Mill effluent
*Can Test Ltd. 1650 Pandora St. Vancouver, B.C.	Gary Vigers Allan W. Maynard*	Staghorn Sculpin, Prickly Sculpin, Clam *(M. balthica)*, Starry Flounder, Dungeness Crab	Sediment, water and body tissue levels of contaminants *in situ*	Chlorophenols and chlorinated aromatics
E.V.S. Consultants Ltd.	Eric McGreer Gary Vigers	Clam *(M. balthica)*, Dungeness Crab, Chinook Smolts, Starry Flounder	Sediment, sewage effluent and body tissue levels of contaminants *in situ*	24 heavy metals; emphasis on mercury and arsenic
	Eric McGreer Peter Chapman	Shore Crab, Coho Smolts	Salinity, temperature, *in situ* and laboratory	Copper ore leachates
	Gary Vigers	Rainbow Trout	Routine monitoring	Pesticides, Mine tailings effluents, Pulp Mill effluents, Chemical products, oil refinery effluents
Dept. of Biological Sciences Strategic Grant Simon Fraser University	Drs. Geen, Albright, Oloffs, McKeown	All Trophic Levels	Uptake, clearance, respiration, swimming performance, blood parameters (Fish)	Orthene
	B.A. McKeown	Fish	Uptake, clearance, toxicity, blood parameters	Crude Oil, Dispersants
	B.A. McKeown	Fish	Endocrine pancreatic hormones	Zinc

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Environmental Toxicology Group University of Victoria (a multidiscipline group involving Dept. of Biochemistry and Microbiology, Dept. of Chemistry and Dept. of Biology)	A.T. Matheson (Dept. of Biochemistry and Microbiology) A. McAuley (Dept. of Chemistry) (Co-ordinators) Others J.T. Buckley (Group I) T.J. Trust W.W. Kay E.E. Ishiguro J.L. Nichols P.R. West (Group II) R.H. Mitchell M.J. Ashwood-Smith G.R. Brunton D. Ellis (Group III)	Marine and aquatic organisms - mainly Salmonids	Gp. I (a) Effects of prolonged exposure of Coho Salmon to sublethal levels of copper and zinc (b) Effect of heavy metal contamination on the susceptibility of salmonids towards infectious disease Gp. II Industrial hydrocarbons in marine environments Gp. III Rehabilitation of Marine Ecosystems	(a) heavy metals - mainly copper and zinc (b) pathogenic bacteria - *Vibrio anguillarum*, *Aeromonas salmonicida*. (c) polynuclear aromatic hydrocarbons
Environmental Carcinogenesis Unit, B.C. Cancer Research Centre	H. F. Stich (Group)	Bottom sediments	Levels of Polycyclic Aromatic Hydrocarbons in Arctic Waters	
	D. Walton	4 tissue culture lines of fish	DNA repair synthesis (unscheduled uptake of ^{3}HTdR)	MNNG, 4NQO, aflatoxin B_1, N-acetoxy-AAF
	R. San, A.B. Acton and H.F. Stich	- *Salmonella typhimurium* - mammalian cell cultures (human and hamster)	- mutagenicity in *Salmonella* - DNA repair synthesis - cytogenetic changes (chromosome aberrations, sister chromatid exchanges)	- sewage - fecal extracts
	B.P. Dunn	- commercial seafoods	- levels in tissues of polycyclic aromatic hydrocarbons	- PAH
MacMillan Bloedel Ltd.	Environmental Control Dept.	RAINBOW TROUT (*Salmo gairdneri*)	Freshwater Effluent Streams Acute (LC_{50}) Responses and Sublethal (chronic) Responses	Landfill Leachates Entering Freshwater 1° and 2° Treatment Effluents
		HERRING (*Clupea harengus pallasi*)		1. Bark Press Effluents 2. Saltwater 1° Treatment Streams
		STICKLEBACK (*Gasterosteus aculeatus*)	Saltwater Effluent Streams	3. Kraft Mill Diffuser Effluents
		JUVENILE COHO (*Oncorhynchus kisutch*)	Acute and Sublethal Responses	4. *Debarker Effluents* 5. Resin Acids and Other Wood Derivatives 6. Caustic Extraction Effluents (Pulp Bleaching)
	MacMillan Bloedel Research	*Daphnia pulex*	Acute Toxicity of Bleaching Compounds	Bleaching Compounds
International Pacific Salmon Fisheries Commission	J.A. Servizi	Sockeye Salmon Pink Salmon	Acute toxicity Acute and chronic toxicity	Municipal sewage 2,4-D
Seakem Oceanography Ltd. 9817 W. Saanich Rd. Sidney, B.C. V8L 3S1	D.A. Brown W.A. Heath K.A. Thompson	*Mytilus edulis* *Macoma inconspicua*	Bioaccumulation of PAH's, Histopathology	Polycyclic aromatic Hydrocarbons
	P.G. Berrang A.J. Cribb	Artificial Mussels	Accumulation from seawater of toxicants	Cadmium, Copper and Lead
Artic Laboratories Ltd. P.O. Box 1070 Inuvik, N.W.T. X0E 0T0	D.A. Brown D.J. Thomas W.A. Heath K.A. Thompson	Polychaete Worms	Histopathology and metallothionein production	Oil Drilling Mud
B.C. Research Vancouver	D.J. McLeay M.R. Gordon	Rainbow Trout	Avoidance/preference Thermal tolerance Sealed jar bioassays Acute stress tests	Cadmium, pentachlorophenol, benzene hexachloride

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
B.C. Research Vancouver	D.J. McLeay M.R. Gordon	Rainbow Trout Coho Salmon	Partial life-cycle studies	Herbicides
	J.M. Leach	Rainbow Trout	Chemical Analyses, Toxic constituents	Pulpmill effluent
	J.M. Leach D.J. McLeay	Pacific Oyster Common Mussel Sculpin	Bioaccumulation of chlorinated organics	Kraft Pulpmill effluent
	J.C. Mueller E.G.H. Lee	*Salmonella typhimurium*	Mutagenicity (Ames Test)	Industrial effluents misc. chemicals
	M.R. Gordon J.C. Mueller	Rainbow Trout	Fish flesh tainting	Pulpmill effluent
	D.J. McLeay	Rainbow Trout Coho Salmon	Routine monitoring	Municipal, Pulpmill, Mining and Oil Refinery wastes; Chemical products
EPS, West Vancouver in conjunction with Resources Services Branch, D.F.O.	Freshwater Group	*Salmo gairdneri*	Plasma cortisol Histopathology 96 hr LC_{50}-static	Sanitary landfill Leachate
EPS, West Vancouver in conjunction with D.F.O.	Freshwater Group	*Salmo gairdneri*	96 hr LC_{50}-static identification and isolation of toxic fractions	Woodwaste leachate

REVIEWER: B. HAMMOND REGION: ALBERTA/SASKATCHEWAN

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Environment Canada Environmental Protection Service Northwest Region Edmonton, Alberta	Aquatic Toxicology Lab. 14317-128 Avenue Edmonton, Alberta T5L 3H3	*Salmo gairdneri*	Acute Lethality	1. Industrial effluents 2. Industrial chemicals and products
		Gasterosteus aculeatus	Acute Lethality	1. Oil exploration drilling fluid wastes 2. Drilling fluid components
		Daphnia pulex	Acute Lethality	1. Developmental work using standard chemical toxicants eg. NH_4Cl, Phenol, etc. 2. Industrial effluents
Alberta Environmental Centre - Vegreville	W. Lake		Recycle Water Treatment System for Toxicity studies and using Fish and Aquatic Invertebrates	
Water Quality Control Branch - Edmonton	J. LeFebvre	Rainbow Trout	pH	Heavy metals and ammonia
Alberta Environmental Centre/Water Quality Control Branch Edmonton	W. Lake E. McGuinnes	Rainbow Trout		Monitoring and surveillance of industrial liquid effluents
University of Calgary Biology	R. Davies	Fish and Invertebrates	LC50 metabolism	Chlorine and chlorinated organics (sewage)
	D.M. Reid	Phytoplankton	Reproduction	Low levels of Ethylene
University of B.C.	K. Sanderson	Ames testing	Carcinogens/mutagens	Polycyclics in used oil
Chemistry	L.A. Beahie	Daphnids	Population	Surfactants/pulp mill effluents Spent sulfide liquors

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
University of Calgary Pathology	R.L. Moore	Ames testing	Carcinogens/mutagens	General mutagenicity
University of Calgary/ Kananaskis Centre	E. Peake	Ames and Chemical Identification	Air and Water	All mutagenic compounds
	B.L. Baker	Ames	Carcinogens	Land fill leachates
	M.T. Strosher	Luminescent bacteria		Drilling fluids
University of Alberta Pharmacology	D.F. Biggs	Rainbow Trout	Electrophysiology	Organophosphates
University of Alberta Zoology	W. Mackay	Goldfish	Transport physiology	Metals
University of Alberta	S. Hrudey	Rainbow Trout	pH and hardness on LC50	Long chain fatty acids
AOSERP FUNDED RESEARCH				
Alberta Environment Pollution Control Division	W. Lake W. Rogers	Rainbow Trout Trout-Perch	96h-LC50	Mine depressurization water
Aquatic Environments Ltd., Calgary	P. McCart P. Tsui B. McMahon	Lake Chub White Sucker Rainbow Trout Lake White Fish *Caenis simulans* *Paraleptophlebia bicornuta* *Hyalella azteca*	96h-LC50 90d-LC50 Metamorphosis Growth Osmoregulation Coughing reflex Hematocrit Blood and Tissue ion Levels Gill histology Fertilization success	Mine depressurization water
University of Guelph Guelph	J.B. Sprague D.A. Holdway D. Stendahl	Rainbow Trout American Flagfish Zebra fish	96h-LC50	Vanadium
FUNDED BY SYNCRUDE *				
*Aquatic Environments Ltd.	B. McMahon P. McCant A. Peltznor G. Walder	Rainbow Trout Mountain Whitefish Arctic Grayling Lake Chub Fathead Chub White Sucker Trout-Perch Walleye *Heptagenia marginalis* *Paraleptophlebia bicornuta* *Isogenus (Isogenoides)* sp. *Hydropsyche bifida*	96h-LC50 Swimming performance	Mine depressurization water
Toxicology Research Group College of Graduate Studies University of Saskatchewan	Dr. B. Schiefer			
Pharmaceutical Research and Analyses Laboratory College of Pharmacy University of Saskatchewan Saskatoon, Sask.	Dr. D.K . Gorecki			
College of Veterinary Medicine University of Saskatchewan Saskatoon, Sask.	Dr. G. Wobesser P. Daoust	Trout		
Regina Water Research Institute University of Regina	Dr. D.R. Cullimore	Several species Algae		Herbicides

REVIEWER: S. LEONHARD REGION: MANITOBA

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Fisheries & Oceans Freshwater Institute Limnology Section Northern Reservoir Biology & Fisheries	D. Bodaly	Lake whitefish, walleye, northern pike, longnose sucker	Muscle levels of Hg	Hg
Experimental Limnology	D.W. Schindler	Whole lake systems	Limnology	Acid
	R. Hesslein C. Kelly	Microbial systems	Methane production	Acid, heavy metals and their interactions
	E. Fee J. Shearer D. DeClerco	Algae	Primary production	Acid
	D. Findlay	Algae	Community composition	Acid
	K. Mills S. Chalanchuk	Lake trout, suckers	Population size and age structure; growth	Acid
	I. Davies	Crayfish *(Orconectes virilis)*	Population size and age structure	Acid
	D. Malley P.S.S. Chang	Crayfish *(O. virilis)*	Ca^{++} balance	Acid, Cd, Se
	W. Findlay	Zooplankton	Population responses; community composition	Acid
	R. France (Grad. student with D.W. Schindler)	Crayfish *(O. virilis)*	Growth and reproduction	Acid
	R. Nero (Grad. student with D.W. Schindler)	Opposum Shrimp *(Mysis relicta)*	Population size and age structure	Acid
	D. Ramsay (Grad. student with K. Patalas)	Zooplankton	Population responses; community composition	Acid
Ecosystem Toxicology Section Organic Chemicals Toxicology	W.L. Lockhart D.A. Metner A.P. Blouw D.A.J. Murray	*Salmo gairdneri* (Rainbow Trout)	Uptake and loss and metabolism of various organics Blood Chemistry Tissue Enzymes	Pesticides ie. Ethalfluralin, Terbutryn, Fluridone, Methoxychlor, Niclosamide, Fenitrothion, Abate, Permethrin, Krenite, Glyphosate, 2,4-D Other Organics Hexachlorobiphenyl, Terphenyl Coolant, Atlox Corexit Surfactants Others Methyl-Mercury
		Lemna minor (Duckweed)	Uptake of various organics Growth	
		Occasional use of Invertebrates ie. Chironomids, Blackflies, Stoneflies	Uptake of organics Enzyme Inhibition	
		Analyses of Field-Captured species of Fish		
Ecotoxicology	E. Scherer S. Harrison B. deMarch M. McLean R. McNicol	Rainbow Trout *(Salmo gairdneri)* Whitefish *(Coregonus clupeaformis)* Walleye *(Stizostedion vitreum)* Arctic Char *(Salvelinus alpinus)* Creek Chub *(Semotilus atromaculatus)* *Hyalella azteca* *Gammarus lacustris* Misc. ephemeroptera trichoptera plecoptera	Preference/avoidance responses; locomotor activity; optomotor response; rheotaxis; visual activity and orientation Whole-life cycle studies; preference/avoidance; locomotor behaviour; drift	Acid waters Hg, Cd, Cu, Zn Organophosphate pesticides Experimental herbicides
	W. Franzin	Wild Fish Populations	Growth, Numbers, Survival, Recruitment, Fecundity, Bioaccumulation, Feeding Ecology	Metals especially base metals

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Fisheries and Oceans Freshwater Institute Ecosystem Toxicology Section Organic Chemicals Toxicology	M. Povoledo		1. Establish criteria for the selection of chemicals (organic and inorganic) to be placed on a priority chemicals list for further study	
			2. Interaction of humics with herbicides	Paraquat
Acidification Ecotoxicology	J. Rudd	Whole ecosystem	Dynamics of mercury movement into biota	Mercury and selenium
		Bacteria	Methylation and demethylation sites of activity and factors controlling rates	Mercury
Ecotoxicology	J.F. Flannagan	Aquatic Invertebrates	Ecological impact of treatments	Pesticides
	D.G. Cobb	Plecopterans: - *Acroneuria abnormis*, *Acroneuria lycorias*	Cholinesterase activity in brain	Organophosphate insecticides
	M.K. Friesen	Mayfly - *Hexagenia rigida*	Uptake from water and sediment Effects on moulting	Permethrin (radio-labelled)
Fish Toxicity Mechanisms	M.A. Giles R. Danell H. Majewski	Rainbow Trout	Cardiovascular-Respiratory function Kidney function Ionic regulation	Acid Cadmium
Fisheries and Oceans Freshwater Institute	M. Lawrence	Lake Whitefish Rainbow Trout	Avoidance	Drilling muds
Fisheries and Oceans Freshwater Institute Ecosystem Toxicology Section	S.G. Lawrence M.H. Holoka	*Tetrahymena vorax* - *Chlamydomonas reinhardti*	Biomass fluctuations, uptake of toxicant, morphological changes	Cadmium
Acidification Ecotoxicology	S.G. Lawrence M.H. Holoka	Lake zooplankton	Reproductive changes species diversity, biomass fluctuation	Cadmium; acid
	S.G. Lawrence M.H. Holoka	Lake zooplankton	Food web structure and function	
	S.L. Leonhard	*Oreomectes virilis*	Molting cycle and calcium uptake	Acid, heavy metals, pesticides
		Daphnia sp.	Reproductive impairment	Acid, heavy metals
		Artemia sp.	Reproductive impairment	Heavy metals, pesticides
		Hexagenia rigida	Acute mortality in yearling nymphs	Cadmium
Zoology Department University of Manitoba	Dr. J.W.T. Dandy (1 student)	Rainbow Trout	Effects of Gll cholinesterase activity and on respiratory rates and swimming speed	Fenetrothion
	Dr. M. Samoiloff (3 students)	Free-living nematode, *Panagrellus redivivus*	Mutagenesis and Carcinogens	A simple assay for heavy metals, pesticides, Industrial wastes, Biological products
Pesticide Research Laboratory Dept. of Soil Science University of Manitoba	G.R.B. Webster G.P. Rawn	Mosquitoes Fathead Minnows	Degradation, residues Fate	Permethrin
Department of Microbiology University of Manitoba Winnipeg, Manitoba R3T 2N2	N.E.R. Campbell R.D. Hamilton	Nitrogen Cycle Events	Nitrogen Fixation Denitrification	None
Government of Manitoba Water Pollution Control (Water Quality)	M. Morelli D.J. Brown		Surface Waters North of 53° Parallel	Cu, Zn, As, Cd, Pb, Hg

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
University of Manitoba and Manitoba Department of Agriculture	R. Brust D. Smith A. Kolach	Blackflies	Effects of larvicide treatment on blackflies % control and distance downstream control is effective	Methoxychlor 0.01 ppm for 15 minutes
The City of Winnipeg Insect Control Branch	R.A. Ellis	Mosquitos: *Aedes* *Culex* *Culiseta*	Larvicides	Organophosphates Bacteria Chitin inhibitors Juvenile hormone analogs
	J. Buth		Sublethal effects	Precocene Antijuvenile hormone BaySir *Bacillus sphaericus*

REVIEWER: G. CRAIG REGION: ONTARIO

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Ontario Ministry of the Environment Rexdale, Ontario	K. Suns C. Curry	Fish	Identify mercury dynamics in Wabigoon River and sources from natural sinks	Mercury
	K. Suns C. Curry	Fish	Establish background information and trends of tributary impact on Great Lakes fishes	Pesticides, PCB's Heavy Metals
	K. Suns C. Curry	Fish	Contaminant source identification and possible bioaccumulation in fish	Sewage Treatment Plant effluents (Industrial organics)
Ontario Ministry of the Environment Head - Toxicity Unit C. Craig			Identification of the toxic and chemical nature of various industrial effluents including:	
	J. Munro B. Flock	Rainbow Trout		Ontario Pulp and Paper Mills
	J. Munro K. Flood	Rainbow Trout		St Clair River Industrial Complex (Organics)
	K. Holtze	Rainbow Trout *Daphnia magna*		Gold and Uranium Mining Operations
	K. Suns	Fish	Comparison of fish, sediment and water to establish relationships of transport	PCB's
	K. Suns	Fish	Impact of road oiling on PCB contamination of inland fish	PCB's
	K. Suns C. Curry	Fish	Mercury accumulation in fish in acid stressed lakes	Mercury - Acid Precipitation
University of Ottawa	R. Engelhardt P. Weinberger S. Qadri F. Brillon	Rainbow Trout Blackfly and Mosquito Larvae Algae	Behaviour, reproduction Reproduction Growth	Metacil (Carbamate Insecticide) "
	"	Algae	Growth, survival	Seniprothion (organo-phosphate insecticide)
	R. Engelhardt	Rainbow Trout, Seals	Acute toxicity, Sublethal effects, feeding, reproduction, uptake	Petroleum Hydrocarbons
	Dr. D. Kushner	Blue-green algae, bacteria	Acute Toxicity	Heavy Metals

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
University of Western	Dr. D. Ogilvie and	Brook Trout	Thermal tolerance	Acidic water
University of Waterloo Waterloo, Ontario	C. Mayfield	Rainbow Trout	Rheotropic response as indicator of toxicity	Aquatic Herbicides
	"	Bacteria	Toxic effects	Chlorinated Organics in Sediments
	"	Bacteria	Effects of acid rain on growth and activity Effect of bacterial action on release of toxic compounds from sediments	Acid Rain
	"		Degradation and cycling of aquatic herbicides in freshwater systems	Diquat, Fluridone
University of Waterloo Waterloo, Ontario	C. Mayfield J.E. Thompson W.E. Inniss		New methods to determine sublethal damage on algal cells	Pentachlorophenol
		Algae	Membrane properties as indicators of toxic effects	Heavy metals
	A.J. Carty		Methylation of metals	Heavy metals
York University	D.J. McQueen		Hypolimnetic aeration for removal of phosphates and nitrates	Agricultural Waste
University of Toronto Toronto, Ontario	G. Holeton J. Booth L. Hofmann	Trout	Effects on acid-base balance and iono regulation	Hydrogen ion (H^+)
	H. Harvey	Fish Benthos	Effects on aquatic ecosystems	Acid Precipitation
	H. Harvey G. Booth	Salmonids	Effects on development and reproduction	Acid Precipitation
	H. Harvey G. Fraser	Freshwater Fish	Effects on respiration and iono regulation	Acid Precipitation
York University Toronto, Ontario	B. Coleman N. Bermingham	Algae	Effects on growth photosynthesis	2,4-Dichlorophenoxyacetic acid
Laurentian University Sudbury, Ontario	J.R. Morris C. Telarico K. Kelly	Rainbow Trout fry Minnows	Toxicity - interactions between metals and pH	Heavy metals (Cu, Ni, Zn)-low pH
	P. Bolger	Aquatic organisms	Community analyses and water chemistry	Metals - low pH
	J. Ordendorf	Macro-invertebrates	Colonization of leaf packs	Acid precipitation
	B. McKillop	Fish	Toxicity	Lime-treated acid lakes
Queens University Kingston, Ontario	S.R. Brown	Algae	Accumulation	Heavy metals
	W. Breck	Algae invertebrates	Heavy metal concentration in aquatic organisms	Heavy metals
University of Guelph Guelph, Ontario	J.B. Sprague	Rainbow Trout	Acclimation effects on toxicity	Copper
	G. Dixon W. Bannis J.B. Sprague	Rainbow Trout	Acclimation effects on toxicity	Organophosphate insecticide
	R. Bradley J.B. Sprague	Fish	pH, hardness and alkalinity effects on toxicity	Zinc
	N. Hutchinson	Fish	Effects of heavy metals in rehabilitated acid lakes	Heavy metals
Lakehead University Thunder Bay, Ontario	G.W. Ozburn D. Orr A. Smith	Flagfish	Acute and chronic toxicity Fecundity, Reproduction Survival, Bioconcentration	PCB substitutes eg. 1,2,3-trichlorobenzene 1,2,4-trichlorobenzene -butylated monochloro diphenyl oxide -isopropyl biphenyl -poly alpha olefin (PAO)
		Rainbow Trout	Determination of uptake in the gut	

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
McMaster University Hamilton, Ontario	G. Harris S. Goudey	Algae	Toxic mode of action	Heavy metals (Zn, Cu)
	R. Sonsetgard (McMaster) J. Leatherland (Guelph)	Great Lakes fishes	Chronic toxicity tumor frequencies, feeding trials, endocrinology, electrobiology	Environmental carcinogens
		Rats	Effects of ingestion of contaminated fishes in white rats	
Life Sciences Scarborough College University of Toronto West Hill, Ontario	A.H. Weatherley S.C. Rigers	Fish	Energetics of activity in zinc polluted water by using telemetry to record electromyograms from swimming muscles and opercular muscles and calibration of these signals in terms of oxygen consumption	Zinc

REVIEWER: A NIIMI

REGION: ONTARIO (GREAT LAKES BIOLIMNOLOGY LABORATORY, BURLINGTON)

Agency and Location	Individual or Group	Organism	Parameters under Investigation
Great Lakes Biolimnology Laboratory Burlington, Ontario	P. Wong	Algal studies	Toxicity of Metal Mixtures to Freshwater Algae It has been previously demonstrated in our laboratory that a mixture of the 10 metals at the Great Lakes Water Quality Objective levels was toxic to several species of freshwater algae. Studies are continuing to determine which combinations of metals are the most toxic at the sublethal level to natural phytoplankton. The effects of modifying factors such as hardness, alkalinity, and complexing capacity are also examined. This information will be integrated, and recommendations of changes to the Water Quality Objectives will be made where necessary.
	P. Wong	Algal studies	Effects of Arsenic on Algae The sublethal effects of inorganic and organic compounds of arsenic on laboratory and natural phytoplankton populations are being examined. Factors that are monitored includes changes in primary productivity, growth, and morphology as well as the bioaccumulation of arsenic by algae.
	P. Wong	Algal studies	Cadmium Toxicity on Algae Laboratory and natural populations of algae are being exposed to cadmium compounds at sublethal concentrations. The effects of environmental variables such as hardness and temperature are also being examined.
	P. Wong	Algal studies	Toxicity of Pentachlorophenol (PCP) on Algae PCP is one of the few environmental contaminants that has been shown to have a direct physiological effect on an organism. It is generally suggested that PCP acts by uncoupling oxidative phosphorylation which causes a rapid breakdown of ATP resulting in an increased energy expenditure by the organism. To examine the sublethal effects of PCP on phytoplankton, indicators such as primary productivity and growth are being measured.
	P. Wong	Algal studies	Algal Enzyme and Cell Membrane Changes as Indicators of Sublethal Toxicity Subcellular methods are presently being developed to measure the effects of toxicants on algae. A series of extracellular and intracellular enzymes are being examined as potential indicators of sublethal stress. Changes in X-ray diffraction of the cell membrane is also being examined as a toxicological indicator.

Agency and Location	Individual or Group	Organism	Parameters under Investigation
Great Lakes Biolimnology Laboratory Burlington, Ontario	A. Niimi	Fish studies	Hexachlorobenzene (HCB) Levels in Lake Ontario Salmonids Adult lake trout, rainbow trout, and coho salmon were collected from Lake Ontario and analyzed for HCB to provide information on the status and probable kinetics of HCB in the Great Lakes environment. Mean HCB levels for the three species were 80 ng/g, 62 ng/g, and 36 ng/g respectively. HCB levels increased significantly with body weight for each species suggesting this compound will bioaccumulate. There were distinct differences in the body burden to weight relationship among the three salmonid species that would suggest food habits and/or age may be contributing factors to body residue levels. Canada has no guidelines regarding HCB levels in food products while the U.S. Environmental Protection Agency have adopted an interim action guideline of 500 ng/g in the fat of domestic animals. To put the significance of the HCB levels of the three species examined into perspective, the HCB levels in the fat of the fish tested were calculated. The expected HCB in fat averaged 500 ng/g for lake trout, 700 ng/g for rainbow trout, and 500 ng/g for coho salmon.
	A. Niimi	Fish studies	Uptake of Hexachlorobenzene (HCB) from Food by Rainbow Trout Field sampling studies have established HCB levels of 40-80 ng/g in Lake Ontario salmonids. Since HCB levels in Lake Ontario waters range from 0.005-0.1 µg/l, the most probable pathway for bioaccumulation would be through the food chain. To examine this pathway, subadult rainbow trout were fed a dried diet containing 0, 0.5, 1 ng/g, 0.5 and 1 µg/g HCB daily for up to 57 days. Initial results suggest fish fed the 1 µg/g diet for 57 days contained a mean body burden of 660 ng/g HCB.
	A. Niimi	Fish studies	Uptake of Waterborne Pentachlorophenol (PCP) by Rainbow Trout PCP is a industrial chemical that is highly soluble in water. The solubility of 14 mg/l for PCP is increased to 4000 mg/l when converted to its sodium salt Na-PCP. This compound is used in significant quantities by the forest products industry. Levels of nearshore Great Lakes waters average 5-20 ng/l but can increase to 2 µg/l in areas such as the Bay of Quinte and Detroit River. In view of these properties, the uptake of waterborne PCP is being examined. Adult rainbow trout are being exposed to waterborne PCP at concentrations of 30 ng/l and 500 ng/l for up to 115 days. These fish will be analyzed for PCP, and its metabolites pentachloro-anisole and tetrachlorophenol.
	A. Niimi	Fish studies	Uptake of Pentachlorophenol (PCP) from Food by Rainbow Trout Initial results from GLBL's Great Lakes Contaminants Surveillance Program indicate PCP levels in the 1-10 ng/g range in fish. To examine the kinetics of this compound, subadult trout were fed diets containing 0, 0.5, 1 ng/g, and 1 µg/g PCP to satiation daily for up to 115 days. These fish will be analyzed for PCP and its metabolites.
	P. Hodson	Fish studies	Interaction between Dietary Fat and Pentachlorophenol (PCP) in Fish In view of the effect PCP has on the oxidative phosphorylation process, hence the energy requirements of an organism, a study was conducted to examine the effect of dietary fat on PCP toxicity. The results suggest no apparent effect was evident for short-term exposures.
	P. Hodson	Fish studies	Effects of Pentachlorophenol (PCP) on the Early Life Stages of Rainbow Trout The occurence of PCP at low concentrations has been monitored in many water courses that is used for effluent discharges by the forest products industry. To examine the potential effect of this compound on self sustaining populations of fish like rainbow trout that use these waters, the effects of PCP on the early life stages were examined.

Agency and Location	Individual or Group	Organism	Parameters under Investigation
Great Lakes Biolimnology Laboratory Burlington, Ontario	P. Hodson	Fish studies	Effect of Temperature on Lead Uptake by Rainbow Trout The effects of temperature on physiological rates has been well documented for processes such as respiratory metabolism and growth in poikilotherms. To demonstrate this effect on the response of fish to environmental toxicants, trout were thermally acclimated to temperatures of 5-20 C, and exposed to lead. Preliminary results suggest chronic toxicity should increase with temperature based on increased uptake.
	P. Hodson	Fish studies	Impact of Lead on Great Lakes Fishes To confirm the results of lead toxicity on fish that has been derived from laboratory studies, specimens are now being sampled from the field. Salmonids from Lake Ontario, and carp, bullheads, and channel catfish from Hamilton Harbor are being analyzed for levels of blood lead and erythrocyte amino levulinic acid dehyratase (ALA-D) activity as indicators of lead exposure.
	P. Wong	Fish studies	Methylation of Lead by Rainbow Trout Earlier studies had demonstrated the presence of methylated lead compounds in several species of fish collected from the Great Lakes. To establish the probable source of these compounds, rainbow trout are being exposed to organic and inorganic compounds of lead to examine the methylation process, and to monitor the uptake and depuration of tetramethyllead. The contribution of the microorganisms that inhabit the fish's intestine to the methylation process is also being examined.
	J. Hilton (Visiting Fellow with P. Hodson)	Fish studies	Interactive Study of Waterborne Lead Toxicity and Tryptophan Metabolism in Rainbow Trout Earlier studies in this laboratory have observed that certain symptoms of waterborne lead toxicity in trout suggests a tryptophan deficiency. This may indicate that lead interferes with the metabolism of tryptophan by possibly blocking tryptophan uptake in certain organs or by directly blocking metabolism of this amino acid. A study is in progress to determine the effect of increased levels of tryptophan in the diet on lead toxicity in trout. An investigation of the metabolism of ^{14}C-labelled tryptophan in the trout brain will also be attempted.
	J. Hilton (Visiting Fellow with P. Hodson)	Fish studies	Isolation and Identification of Seleno-Compounds in Rainbow Trout Studies in this laboratory indicate that trout can store very high levels of selenium in the liver (100 µg/g dry weight) without any apparent histopathological or physiological effects. This suggests that these fish have an efficient detoxification mechanism. A study is in progress to determine how trout detoxifies selenium and what compounds or transformation products of selenium are formed. The study will attempt to locate and identify these seleno-compounds in trout and how they relate to low and toxic levels of selenium in diets.
	J. Hilton (Visiting Fellow with P. Hodson)	Fish studies	Chronic Dietary Toxicity of Triphenylphosphate to Rainbow Trout Previous studies have established that certain waterborne triarylphosphates are extremely toxic to trout and produce several physiological responses such as lens cataracts and spinal deformities. A study is in progress to determine the toxicity of triphenylphosphate to rainbow trout administered through the feed. The study will examine the physiological response of the trout as well as the metabolism of this compound in the trout.
	U. Borgmann	Invertebrate studies	Effect of Metal Mixtures on Natural Copepod Populations To relate the results of laboratory studies of metal toxicity on *Daphnia* to natural conditions, the effect of varying concentrations of individual metals and mixtures of Cd, Cu, Hg, Pb, As, and Zn on the production and growth rate of Lake Ontario copepods are being examined. Weekly samples of copepods are also being collected from Lake Ontario and exposed to Cd, Cu, and Hg to determine seasonal variations in toxicity, and probable factors that may cause these variations.

Agency and Location	Individual or Group	Organisms	Parameters under Investigation
Great Lakes Biolimnology Laboratory Burlington, Ontario	U. Borgmann	Invertebrate studies	Examination of Mixed Function Oxidases (MFO) Activity in Aquatic Organisms MFO activity can be used as a measure of the ability of organisms to detoxify aromatic hydrocarbons. an assay for benzo(a)pyrene hydroxylation in crustaceans is being developed to monitor the effects of compounds such as PCB's and HCB on aquatic organisms.
	B. Wilson (PhD candidate with U. Borgmann)	Invertebrate studies	Acute Effects of Lead and Cadmium on Various Species of Freshwater Crustacean Zooplankton A 10-stage, one order-of-magnitude microdilutor system is being used to study the acute 96h effects of the nitrate salts of Pb and Cd on four species of crustacean zooplanktors. The species being examined include the often-studied and lab-cultured *Daphnia magna*, as well as three common field species *Cyclops bicuspidatus thomasi*, *Diaptomus sicilis*, and *Daphnia galeata mendotae*. All species are being exposed at a series of at least three temperatures covering the normal *in situ* temperature range of each animal. The purpose of this project is to establish the applicability of *D. magna* test results to other species, and to examine the effect of temperature on heavy metal toxicity.
	B. Wilson (PhD candidate with U. Borgmann)	Invertebrate studies	Response of Various Species of Freshwater Crustacean Zooplankton to Light The phototaxis of crustacean zooplankton is a behavioral response which is often cited in studies of vertical diurnal migration, but which has been only poorly quantified. Experiments are being carried out using the same species and temperatures utilized for the acute toxicity study, to quantify this response. Dark-adapted animals are exposed to specific combinations of wavelength, intensity, and temperature for a pre-determined, optimal time period in a one meter horizontal (to negative geotactic responses) chamber. Phototaxis is quantified as a percentage shift in the mean position of the sample of 25 animals. Results are fitted to multivariate relationship using wavelengths, intensity, and temperature interactions. This project is also used to establish the use of phototactic response as a indicator of sublethal heavy metal toxicity.
	B. Wilson (PhD candidate with U. Borgmann)	Invertebrate studies	Chronic Effects of Lead and Cadmium on the Light Response of Freshwater Crustacean Zooplankton One laboratory and three zooplankton species are being exposed to low concentrations of lead and cadmium for periods up to 16 days to study the toxic effects of these heavy metals on phototaxis. A single temperature common to all species is being used for exposure and testing. Light response experiments utilize the wavelength of maximum sensitivity and on intensity ensuring maximum response in control animals. The results suggest a reduction in light response resulting from both increasing concentrations of, and time of exposure, to each metal.
	S. Millard	Model ecosystems	Lake Column Simulator System The lake column simulator complex at GLBL is one of the largest laboratory based model ecosystems. Each of the eight columns is 4 meters in height and 1 meter in diameter with a working capacity of 3300 liters. A peripheral cooling system allows the water column to be thermally stratified if desired. Each column is illuminated with a 1000 watt halogen lamp which produces a surface intensity that is approximately 40% of sunlight, and is sufficient to enhance algal growth. This system can maintain a biological community containing 3 to 4 trophic levels for experimental purposes. These columns have been used for studies on the kinetics of atrazine, phosphorus, mercury, and PCB's.
	S. Millard	Model ecosystems	Partitioning of PCB's in a Model Planktonic Ecosystem: Influence of PCB Loading Rate, Ecosystem Productivity, and Inorganic Particles Studies on the kinetics of PCB's in a planktonic community have been initiated using the lake column simulator system. The results suggest particulates such as clay particles and algae play a significant role in the movement of PCB's from water into the food chain. Factors that could affect the rate of transfer includes the size and density of clay, algae, and zooplankton.

Agency and Location	Individual or Group	Organism	Parameters under Investigation
Great Lakes Biolimnology Laboratory Burlington, Ontario	A. Niimi	Other studies	An Oxidative Combustion Method for Preparing ^{14}C-Labelled Samples for Liquid Scintillation Analysis The use of radioisotopic compounds has greatly increased research efforts in pollutant kinetics studies by providing the ability to detect residue levels in materials at nanogram and picogram concentrations in small sample sizes. Tissue solubilizers such as NCS and Hyamine have been used to prepare a sample for scintillation analysis but this procedures does have some limitations. An oxidative combustion method may provide an alternate method of sample preparation. This method oxidizes the sample to carbon dioxide and water, and the $^{14}CO_2$ emitted is trapped in a ethylamine solution. The operating conditions of the instrument used (Biological Material Oxidizer, R.J. Harvey Instrument Corporation, Hillsdale, New Jersey) which oxidizes the samples at 900 C with a catalyst in an oxygen atmosphere, is adequate to combust compounds such as PCB's and HCB. Recovery rates of 96-98% can be expected. A comparison of the two procedures indicated the oxidative combustion method had a lower detection limit, and the variation among replicates was less than the tissue solubilizer method.
	D. Maraldo (contractee with P. Hodson)	Other studies	Effects of Toxicants on the Behavior of Rainbow Trout An experimental stream channel has been designed to examine the sublethal effects of toxicants on the social behavior of fish. After observing the effects of different feeding levels on behavior, and establishing ethological criteria, fish were exposed to lead. Initial results suggest the lead exposed fish were more socially dominant than the control fish, this response was consistent even when a different substrate configuration was used.
	M. Baker (PhD candidate with P. Wong)	Other studies	Effect of pH on the Release of Metals from Sediments The increasing awareness on the effects of acidification on aquatic ecosystems has raised some serious questions on the increased availability of pollutants from sediments. A study has been initiated to examine the effects of pH on the release of Pb, As, Se, Cd, and Hg from sediments, and the effects of microbial actions on their release.
	P. Hodson	Other studies	Potential Utilization of Lake Ontario Fish as Fish Feed The present fish stocks in Lake Ontario cannot be harvested commercially because levels of contaminants such as PCB's and Mirex which exceed human health guidelines. To examine ways that this renewable resource can be utilized, a study has been initiated to examine the use of this product as a source of fish feed. Lake fish will be processed into diets containing whole fish (moist diet), fish meal, and protein concentrates, fed to laboratory fish, and residue levels monitored.

REVIEWER: C. BLAISE REGION: QUEBEC

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Concordia University Dept. Biological Sciences Sir George Williams Campus Montreal, Quebec	P.D. Anderson	Zebra Fish/Rainbow Trout	LC50/Reproduction, multiple toxicity	Heavy Metals
	S.M. Ruby	Flagfish/Rainbow Trout	Reproduction oogenesis	Cyanide, Acid Water
	E. Maly	*Daphnia*-Rainbow Trout	Growth/reproduction	Cadmium
	G. Leduc	Rainbow Trout	Physiology-biochemistry	Cyanide
	P.D. Anderson	Rainbow Trout	96H-LC50	Vanadium
	P. Spear	Rainbow Trout	incipient lethal levels	
	S. D'Apollinia		sub-lethal tests	Nickel
	S. Perry			
	J. deLuca		multiple toxicity tests	Phenol
	J. Dick			

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Noranda Research Centre	M.R. Speyer R.L. Levaque-Charron C. Wood R. Prairie	Rainbow Trout *Daphnia*	Regulatory bioassays, Hg uptake Toxicity of contaminated sediments Impact studies at various smelters and mines	Heavy metals, effluents, Flotation reagents Heavy metals
McGill University	L.D. Spraggs L. Hadjinicoloun	Rainbow Trout Algae	Toxicity avoidance with relation to fluid mechanic problems, jets, diffusers	Selected Industrial Wastewaters
EPS (E.C.)	C. Blaise N. Bermingham R. Legault	Trout Algae Photoluminescent bacteria	Trout bio-assays Algal bio-assays Microtox	Industrial effluents Contaminants
Eco Recherches (C.I.L.)	C. Thellen R. Vancoillie R. Schoeneit	Poissons (Fish) Daphnies *(Daphnia)* Algues (Algae)	LC50 LT50 Récupération (Recovery) CL50 (Daphnies) Fertility test Delayed Toxicity Sublethal Toxicity	Effluents Industriels (Industrial Effluents) Produits chimiques (Chemical Products) Effluents municipaux (Municipal Effluenrs)
Environment Québec	G. Joubert R. Cardin L. Tremblay	*Daphnia* Algae *(Selenastrum capricornutum)* Microtox	*Daphnia* bioassay IC50 Algae bioassay - IC50 Primary productivity test LC50	Potassium dichromate
INRS-EAU Université du Québec	A. Tessier P. Campbell D. Couillard M. Bisson P. Couture G. Croteau	Algues (Algae) Daphnies (Daphnids) Poissons (Fish)	Algues: CI50 Daphnies: CI50 Poissons: CI50 Spéciation Bioaccumulation Paramétre physiologique (Physiology)	Substances inorganiques (Inorganics) EX: Effluents Urbains Substances organiques (Organics) EX: Pesticides
Ecole Polytechnique University of Montréal	C. Delisle (and 3 graduate students)	Rainbow Trout, Perch, Plankton	LC50, LT50 sub.-lethal effects, avoidance behavior	Hg, Cd, Zn, As sediment studies

REVIEWER: P. WELLS REGION: MARITIMES

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
New Brunswick Department of Natural Resources (Montreal Engineering Company, Limited) Fredericton, N.B.	G.F. Gillis	Stream Fish particularly Salmonids	Behaviour of salmonid fishes following exposure to Matacil, applied during the 1979 N.B. Spruce Budworm Control Program, also effects of Matacil on growth and population structure of stream fish populations	Matacil
Government of Canada Marine Ecology Laboratory Bedford Institute Dartmouth, N.S.	R.F. Addison *et.al.*	Brook Trout *(Salvelinus fontinalis)* Marine invertebrates *(Mytilus edulis, Carcenus maenas)*	Hepatic mixed function oxidase (MFO) responses MFO's ATPases	Various organics, including PCB replacement Organics
	C.C.H. Harding	Copepods *(Calanus spp.)* Euphausiids *(Thysanoëssa)*	Uptake and clearance rates of labelled compounds	PCB's DDT

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Government of Canada Marine Ecology Laboratory Bedford Institute Dartmouth, N.S.	J.H. Vandermeulen *et.al.*	*Mya arenaria*	Metabolism, uptake, bio-availability, physiological perturbations	Petroleum hydrocarbons (PH's)
		Chlamydomonas sp.	Mutagenic potential	PH's
		Marine bacteria	Mutagenic potential (Ames study)	PH's
		Patella	Metabolism and bio-availability	PH's
		Trout	Uptake and metabolism	PH's
		Marine Alga *(Monochrysis lutheri)*	Uptake Physiological responses	PH's Chlorinated hydrocarbons Metals PCB's
Government of Canada Fisheries and Oceans Fisheries and Environmental Sciences Resource Branch P.O. Box 550 Halifax, N.S.	J. Uthe G. Sirota *et.al.*	Fin Fish	Relationships between muscle and liver levels Changes in populations over time	PCB's, DDE, HCB, HCH, Se, As, Hg, Pb, Cu, Zn, Cd
		Shellfish	Uptake and depuration	PAH's
		Shellfish Molluscs	"Mussel Watch" Usage	Cd, Zn, Ag, Cu, Mn, As, Fe, Mg, Pb
		All Marine Species	Analytical methodology	Phthalates PAH's Aminocarb (carbamate insecticide)
		Lobster Mussel	Assessment of antropomorphic activity	Common toxic elements PCB's PAH's
	H.C. Freeman	Atlantic Cod Brook Trout	Sublethal effects on steroidogenesis and reproduction	Diethylhexyl phthalate Matacil
	C.M. Morrison	Brook Trout *(Salvelinus fontinalis)*	Pathological effects on gill, liver, intestine and kidney	Cadmium
		Mussel *(Mytilus edulis)*	Histology and pathology of mussels around Nova Scotia Mussels as pollution indicator	Various pollutants (oil, PCB's, metals, etc.)
Government of Canada Fisheries and Oceans Fish Disease and Nutrition Section Resource Branch P.O. Box 550 Halifax, N.S.	M.F. Li	Cultivated mammalian cells (L-cells) and fish cells	Growth Respiration Cytotoxic changes	$HgCl_2$, $CdCl_2$, Fenitrothion, Matacil
		Oysters Mussels Clams	Histo-pathological changes	Fenitrothion Matacil
Government of Canada Fisheries and Oceans Fish Culture Section Resource Branch P.O. Box 550 Halifax, N.S.	G.J. Farmer T. Goff	Atlantic Salmon *(Salmo salar)*	Mortality at various stages in a hatchery (egg, sac fry, fingerling, smolt)	Low pH (acidity)
Government of Canada Environment Canada Environmental Protection Service Bedford Institute Dartmouth and Halifax N.S.	Laboratory Division W.R. Parker *et.al.*	Rainbow Trout	Acute lethality	Industrial effluents Industrial chemicals
		Daphnia pulex	Acute lethality	Industrial effluents
		Striped Bass	Hatchability of eggs Survival, growth of larvae	Effluents Contaminated river water
		Threespine stickleback	Acute lethality	Marine effluents Industrial chemicals
		Clam *(Macoma balthica)*	Uptake from sediments	Metals PCB's
	A. Menon	Algae *(Selenastrum capricornutum)*	Productivity and inhibition of photosyn.	Industrial wastes Natural waters
	G.F. Westlake	Rainbow Trout	Preference/avoidance	Pulpmill effluent

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Government of Canada Environment Canada Environmental Protection Service Bedford Institute Dartmouth and Halifax N.S.	P.G. Wells G.W. Harris	Threespine stickleback *(Gasterosteus aculeatus)*	Lethality	Fuel oil (No. 2) Corexit 9527 (Dispersant) Mixtures of both
		Rainbow Trout	Lethality	" "
		Crustacean Larvae	Lethality, Behaviour	" "
	P.G. Wells	Rainbow Trout	Revised bibliography	Response to toxicant and biology
N.S. Research Foundation Corp. Dartmouth, N.S.	K. Hellenbrand	Rainbow Trout	Lethality	Industrial effluents
National Research Council Atlantic Regional Lab. Halifax, N.S.	W.D. Jamieson		Pollutant analytical chemistry - development of standard reference materials	Organic and inorganic pollutant
Trace Analysis Research Center Dept. of Chemistry Dalhousie University Halifax, N.S.	A. Chatt	Fish	Various factors influencing trace element content	Metals
	R.D. Guy	Algae *(Selenastrum capricornutum)* (for model purposes only)	Speciation of the chemicals Toxicity Analytical methods, for speciation	Cu, Cd, Pb, Hg Organocations such as paraquat, diquat, etc.
Department of Biology Dalhousie University Halifax, N.S. B3H 4J1	E.T. Garside P.J. Rombough	Atlantic Salmon Brook Trout (embryos/alevins)	Tolerance levels Resistance times Teratogenesis Bioaccumulation Amelioration of effects by Calcium and Magnesium	Cadmium (solute)
	E.T. Garside W. Ernst	Brook Trout (fingerlings)	Tolerance levels Resistance times Bioaccumulation	Vanadium (solute)
	E.T. Garside S. O'Neil	Brook Trout (embryos; maturing post-yearlings)	Survival of ova and sperm Inhibition of fertilization Influence on gonadal maturation and release	Chronic acidity
	E.T. Garside J. Appleby	Brook Trout (fry and fingerlings)	Tissue regeneration during and following exposure	Chronic acidity
	E.T. Garside G.B. Croft	Mummichog *(Fundulus heteroclitus)*	Tolerance levels Resistance times Histopathogenesis	Lower lethal temperature in relation to ambient salinity
Government of Canada Fisheries and Oceans Fisheries and Environmental Sciences Biological Station St. Andrews, N.B. E0G 2X0	V. Zitko	Atlantic Salmon	Lethal toxicity Bioaccumulation Structure-activity relationships	Industrial chemicals (flame retardants, alkylphenols)
		Various spp.	Residues	Industrial chemicals Pesticides
	K. Haya	Fish Lobsters	Biochemical indicators of sublethal xenobiotic stress. Redox state of pyridine nucleotides and adenylate energy charge	Industrial chemicals Pesticides
	S. Ray (partly with D.W. McLeese)	Marine Invertebrates (Lobsters, *Pandalus montagui* *Nereis virens* *Macoma balthica)* Atlantic Salmon	Uptake and excretion	Heavy metals
	D.W. McLeese (alone, or in cooperation with V. Zitko and S. Ray)	*Crangon* *Homarus*	Lethality (water, sediments) Food chain accumulation	Chlorinated hydrocarbons
		Nereis *Macoma* *Crangon* *Pandalus*	Lethality Bioaccumulation Interelement effects Chelation effects	Cd, Cu, Zn
		Mya *Mytilus* *Anodonta*	Lethality Bioaccumulation	Fenitrothion
		Crangon *Scallops* *Clams*	Lethality	Fenitrothion

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Government of Canada Fisheries and Oceans Fisheries and Environmental Sciences Biological Station St. Andrews, N.B. EOG 2XO	D.W. McLeese (alone, or in cooperation with V. Zitko and S. Ray)	*Nereis*	Bioaccumulation	PCB's
		Crangon	Flux	
		Mytilus	Bioaccumulation	Aminocarb, Nonylphenol, Diluent 585 oil; formulation
		5 species	Lethality	Same
		Homarus	Lethality	Creosote
		Crangon	Accumulation	
		Crangon	Joint lethality	Organophosphates
		Salmo salar	Lethality	Pyrethroids
		Homarus	Persistence	
		Crangon		
	A.W. White	Adult fish: Herring Pollock Cod Flounder Larval fish: Herring Zooplankton: Copepods Barnacle nauplii etc.	Toxin accumulation Toxin retention Behaviour Lethality - dose response LD_{50} i.p.x oral	Marine dinoflagellate toxins ("Paralytic shellfish" toxins)
	J.L. Metcalfe	Atlantic Salmon	Early development	Cadmium
	D.J. Wildish (Applied Ecology Group)	Herring - *Clupea harengus harengus*	Avoidance	Dredged dump spoil
Government of Canada I.W.D., Moncton C.W.S., Fredericton N.B.	E.L. Brun P.A. Pearce		Contamination of precipitation by forest insecticide	Fenitrothion 1978 Aminocarb 1979
Maritime Forest Research Center C.F.S., Environment Canada P.O. Box 4000 Fredericton, N.B.	D.C. Eidt	Stream insects	Drift, survival, populations, spp. comp., biomass, recovery	Fenitrothion Aminocarb
		Decomposition fungi		Fenitrothion Aminocarb
University of New Brunswick Saint John, N.B.	M.L.H. Thomas B. MacDonald	Intertidal communities	Long-term effects on communities Growth disturbances in *Mya arenaria*	Petroleum hydrocarbons
Université de Moncton Moncton, N.B.	V.N. Mallet		Analytical determination in water, other substrates	Matacil Fenitrothion Degradation products of M + F
	B. Trottier	Various spp.	Binding of pesticides to various tissues	Fenitrothion
New Brunswick Research and Productivity Council, P.O. Box 6000 Fredericton, N.B. E3B 5H1	P.J. Silk (Chemistry Dept.)	Eastern Oyster *(Crassostrea virginica)*	Chronic sublethal effects on spawning, development morphology	Commercial mixtures of pentachlorophenol (includes impurities and by-products)
Government of Canada Fisheries and Marine Service Fisheries and Oceans Box 5667 St. John's, NFLD.	J.W. Kiceniuk	Cunner *(Tautogolabrus adspersus)*	Blood pressure; heart rate	Oil dispersants detergents
	J.W. Kiceniuk R. Khan (M.S.R.L.)	Cunner Cod Flounder Lobster	Feeding Various physiological and morphological	Crude oil
		Sculpin Scallop	Infectivity of blood parasites, and oil-parasite interaction	Crude oil
	J. Payne, with D. Idler G. Fletcher A. Rahimtula (1^{o} activity)	Marine animals common to the coastal N.W. Atlantic; emphasis on flounder, salmon, bivalves	a) MFO induction b) hydrocarbon metabolism c) mutagen activation-deactivation	a) Petroleum hydrocarbons b) Wood chip leachate c) Decomposing animal and vegetable matter

Agency and Location	Individual or Group	Organism	Parameters under Investigation	Toxicant
Government of Canada Fisheries and Marine Service Fisheries and Oceans Box 5667 St. John's, NFLD.	J. Payne I. Martins	Ames strains of bacteria	Mutagenicity	a) Compounds from water chlorination b) Environmental sources of polycyclic aromatics
	J. Payne G. Fletcher R. Thompson J. Kiceniuk	Lobsters Scallops Flounder	Histological change (J. Payne) Plus various biochemical, physiological, and whole organism effects	Petroleum hydrocarbon (chronic exposure)
	W.R. Penrose	Cunner (*Tautogolabrus adspersus*)	Inducible aryl hydrocarbon hydroxylase	Petroleum
Government of Canada Environmental Protection Service St. John's, NFLD. (Bioassay Lab.)	R. McCubbin	Rainbow Trout Atlantic Salmon (proposed)	LT50's 4-day LC50's (static + flow-through)	Industrial effluents Persistent contaminant
Memorial University of Newfoundland (M.U.N.) (Marine Sciences Research Lab., Logy Bay)	G.L. Fletcher	Winter Flounder	Tissue levels of metals in normal and contaminated areas	Zn^{+2} Cu^{+2}
	M.A. Shears G.L. Fletcher	Winter Flounder	Uptake regulation by the gastro-intestinal tract Effects of other heavy metals on Zn^{+2} regulation	Zn^{+2} Cu^{+2}, Hg^{+2}, Cd^{+2}
	P.E. Fletcher G.L. Fletcher	Winter Flounder	Transport of Zinc in the blood Effects of other metals	Cu^{++}, Cd^{++}
	D. Idler	Salmonids	Endocrine metabolism	Petroleum
M.U.N. Biochemistry Dept. St. John's, NFLD.	A. Rahimtula J. Payne	Ames strains of bacteria	Mutagenicity testing (various conditions)	Selected toxicants from Federal Contaminants Act
	K. Keough	Sea Urchins (*Strongylocentrotus*)	Membrane function	Petroleum hydrocarbons 1) gasolines 2) crude oils
M.U.N. Chemistry Dept. St. John's, NFLD.	M. Newlands	Marine biota generally	Occurrence and sources of of metals	Heavy metals, especially Cadmium
	M. Mackie Water Analysis Unit	Water	Occurrence and levels	Petroleum hydrocarbon Chlorinated hydrocarbons
M.U.N. Biology Dept. St. John's, NFLD.	J. Green	Marine fish	Chronic behavioural effects on fish *in situ*	Petroleum
	J. Gow	Marine bacteria	Heterotrophic potential of oil-degrading bacteria	Petroleum
	J. Gow W.R. Penrose	Marine bacteria	Presence of specific oil-degrading species in coastal waters	Petroleum

APPENDIX II

PARTICIPANTS LIST

R. Allan
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

A. Avramenko
Alberta Oil Sands Environmental
Research Program
15th Floor, 9820-106 Street
EDMONTON, Alberta T5K 2J6

P.S. Barker
Research Station
Agriculture Canada
195 Dafoe Road
WINNIPEG, Manitoba R3T 2M9

V. Beaubien
Environmental Protection Service
Box 2310
YELLOWKNIFE, N.W.T.

A. Beckett
Aquatic Toxicology Laboratory
14317-128 Avenue
EDMONTON, Alberta T5L 3H3

P.J. Beckett
Biology Department
University of Winnipeg
WINNIPEG, Manitoba R3B 2E9

E.C. Birchard
Imperial Oil Limited
111 St. Clair Avenue, West
TORONTO, Ontario M5W 1K3

C.R. Blaise
Environment Canada
1001 Pierre Dupuy
LONGUEUIL, Quebec

A.P. Blouw
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D.J. Brown
Dept. Mines, Nat. Res. & Env.
Box 7, Bldg. 2
139 Tuxedo Avenue
WINNIPEG, Manitoba R3N 0H6

S. Brown
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

J.T. Buckley
Biochem. & Microbiol.
University of Victoria
VICTORIA, B.C.

A. Bulich
Beckman Instruments, Inc.
6200 El Camino Real
CARLSBAD, California
U.S.A. 92008

R.E. Burrell
Department of Environmental Biology
University of Guelph
GUELPH, Ontario N1G 2W1

V.T. Chacko
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

P. Chang
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

R. McV. Clarke
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D. Cobb
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

L.P. Cooper
Indian and Northern Affairs
Water Resources Division
YELLOWKNIFE, N.W.T.

R. Corbet
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

G.R. Craig
Ontario Ministry of Environment
Toxicity Unit
P.O. Box 213
REXDALE, Ontario M9W 5L1

W. Cretney
Department of Fisheries & Oceans
Institute of Ocean Sciences
SIDNEY, B.C. V8L 4B2

R. Danell
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

B.G.E. deMarch
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D.G. Dixon
Department of Zoology
College of Biological Sciences
University of Guelph
GUELPH, Ontario N1G 2W1

J. Donetz
Lake of the Woods
RAINY RIVER, Ontario

D.A. Duncan
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

R. Evans
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D. Fagergren
409 East Harvard Street
SHELTON, Washington
U.S.A. 98584

R. France
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

W. Franzin
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

M.K. Friesen
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

C. Garrett
EPS, Kapilano 100
Park Royal South
WEST VANCOUVER, B.C. V7T 1A2

M. Giles
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

A.P. Gilman
Canadian Wildlife Service
Department of the Environment
OTTAWA, Ontario K1A 0E7

M. Gordon
Environmental Protection Service
800-275 Portage Avenue
WINNIPEG, Manitoba R3B 2B3

J.E. Guthrie
Atomic Energy of Canada
Whiteshell Nuclear Research Est.
PINAWA, Manitoba R0E 1L0

R. Hamilton
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

B. Hammond
Alberta Environment
Res. Secretariat
9820-106 Street
EDMONTON, Alberta T5T 1B8

P. Hanson
Biology Department
Lakehead University
THUNDER BAY, Ontario P7B 5E4

T. Hara
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

R. Harbo
Fisheries and Oceans
Habitat Protection Group
1090 West Pender Street
VANCOUVER, B.C. V6E 2P1

N. Heroux
Laval Industrial Association
12500 Boul. Industriel
POINTE-AUX-TREMBLES, Quebec H1B 5P5

B. Hobden
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D. Hodgins
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

P.V. Hodson
Great Lakes Biolimnology Lab.
CCIW, P.O. Box 5050
BURLINGTON, Ontario L7R 4A6

B. Hummel
Beak Consultants Ltd.
Suite 1717 - 1155 Metcalfe
MONTREAL, Quebec H3B 2X3

G. Joubert
Quebec EPS
Complexe Scientifique
2700 Einstein
STE-FOY, Quebec G1P 3W8

R. Kamchen
41 Matheson Avenue East
WINNIPEG, Manitoba R2W 0C1

C.C. Kehr
Box 708
GREENFIELD, Indiana
U.S.A. 46140

L. Kennedy
128-107th Street
SASKATOON, Saskatchewan

M. Kern
293 Twin Lakes Drive
SARNIA, Ontario

N.Y. Khan
7th Floor, 240 Sparks Street
OTTAWA, Ontario K1A 0E6

D. Klaprat
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

J.F. Klaverkamp
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

W. Lake
Alberta Environmental Centre
Box 399
VEGREVILLE, Alberta T0B 4L0

E.K. Langtry
Environmental Assessment Division
Department of Indian and Northern Affairs
OTTAWA, Ontario K1A 0H4

G.H. Lawler
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

S.G. Lawrence
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

G. Leduc
Department Biological Sciences
Concordia University
1455 de Maisonneuve Blvd., W.
MONTREAL, Quebec H3G 1M8

J. LeFebvre
Water Quality Control Branch
11237-120 Street
EDMONTON, Alberta

R. Legault
135 Des Sorbiers
POINTE-AUX-TREMBLES, Quebec H1B 3C2

S.L. Leonhard
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

W.L. Lockhart
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

A. Lutz
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

C.R. MacDonald
Faculty of Engineering Science
University of Western Ontario
LONDON, Ontario N6A 5B9

W. Macdonald
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

H. Majewski
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D. Malley
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

K. Malone
Biology Department
University of Winnipeg
WINNIPEG, Manitoba R3B 2E9

J.S. Marshall
Radiological & Env. Res. Div.
Argonne National Laboratory
9700 South Cass Avenue
ARGONNE, Illinois
U.S.A. 60439

W.K. Marshall
Fisheries and Oceans
240 Sparks Street
OTTAWA, Ontario K1A 0E6

J.E. McComiskey
Site 17, Box 72
YELLOWKNIFE, N.W.T.

J.H. McCormick
U.S. Environmental Protection
Env. Research Lab - Duluth
6201 Congdon Road
DULUTH, Minnesota
U.S.A. 55804

G.A. McFarlane
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

E.R. McGreer
E.V.S. Consultants Limited
195 Pemberton Avenue
NORTH VANCOUVER, B.C. V7P 2R4

M. McLean
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D. McLeay
B.C. Research
3650 Westbrook Mall
VANCOUVER, B.C. V6S 2L2

R.E. McNicol
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

F.V. Mercer
MacQuarie University
NORTH RYDE, N.S.W. 2069
Australia

S. Metikosh
Environmental Protection Service
CCIW, P.O. Box 5050
BURLINGTON, Ontario L7R 4A6

B. Mueller
209-279 River Avenue
WINNIPEG, Manitoba

D. Muir
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

J.R. Munro
Ontario Ministry of Environment
P.O. Box 213
REXDALE, Ontario M9W 5L1

B.A. Munson
Alberta Oil Sands Environmental
Research Program
15th Floor, 9820-106 Street
EDMONTON, Alberta T5K 2J6

J. Murphy
Biology Department
Lakehead University
THUNDER BAY, Ontario P7B 5E4

D.A.J. Murray
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

A.J. Niimi
Great Lakes Biolimnology Lab.
CCIW, P.O. Box 5050
BURLINGTON, Ontario L7R 4A6

P.F. Olesiuk
2-479 Beresford Avenue
WINNIPEG, Manitoba R3L 1J4

G. Ozburn
Biology Department
Lakehead University
THUNDER BAY, Ontario P7B 5E4

W.R. Parker
Environmental Protection Service
16th Floor, 5151 George Street
HALIFAX, Nova Scotia B3J 1M5

G. Persoone
Artemia Reference Center
State University of Ghent
J. Plateaustraat 22, B-900
GHENT, Belgium

D. Povoledo
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

G. Rawn
Pesticide Research Lab.
University of Manitoba
WINNIPEG, Manitoba R3T 2N2

K. Reid
Atomic Energy of Canada
Whiteshell Nuclear Research Est.
PINAWA, Manitoba R0E 1L0

J.T. Retallack
Syncrude Canada Limited
10030-107 Street
EDMONTON, Alberta T5J 3E5

S.C. Rogers
Life Science Division
Scarborough College
University of Toronto
WEST HILL, Ontario M1C 1A4

S.M. Ruby
Department of Biology
Concordia University
1455 de Maisonneuve Blvd., West
MONTREAL, Quebec H3G 1M8

J.W.M. Rudd
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

M.R. Samoiloff
Department of Zoology
University of Manitoba
WINNIPEG, Manitoba R3T 2N2

E. Scherer
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D. Schindler
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

D.P. Scott
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

R.T. Seidner
Alberta Oil Sands Environmental
Research Program
15th Floor, 9820-106 Street
EDMONTON, Alberta T5K 2J6

A. Smith
Biology Department
Lakehead University
THUNDER BAY, Ontario P7B 5E4

E.A.H. Smith
107-2220 Halifax Drive
OTTAWA, Ontario

K.E. Smith
Dept. of Environmental Biology
University of Guelph
GUELPH, Ontario N1G 2W1

K.R. Solomon
Dept. of Environmental Biology
University of Guelph
GUELPH, Ontario N1G 2W1

M.R. Speyer
Noranda Research Centre
240 Hymus Blvd.
POINTE CLAIRE, Quebec H9R 1L9

M.J. Stannard
Betz Laboratories
4636 Somerton Road
TREVOSE, PA
U.S.A. 19047

G.W. Stratton
Dept. of Environmental Biology
University of Guelph
GUELPH, Ontario N1G 2W1

M.C. Taylor
Environment Canada
Water Quality Branch
7th Floor, Place Vincent Massey
OTTAWA, Ontario K1A 0E7

C. Thellen
ECO Research Ltd.
121 Hymus Blvd.
POINTE CLAIRE, Quebec H9R 1E6

J. Todd
Biology Department
Lakehead University
THUNDER BAY, Ontario P7B 5E4

E.M. Tokar
Rayonier Canada (B.C.) Ltd.
701-1111 West Georgia Street
VANCOUVER, B.C. V6E 3H1

J.F. Uthe
Department Fisheries & Oceans
Fisheries & Env. Sciences Div.
P.O. Box 550
HALIFAX, Nova Scotia B3J 2S7

R. Van Coillie
ECO Research Ltd.
121 Hymus Blvd.
POINTE CLAIRE, Quebec H9R 1E6

G.A. Vigers
E.V.S. Consultants Limited
195 Pemberton Avenue
NORTH VANCOUVER, B.C. V7P 2R4

R. Wagemann
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

B. Walbourn
Beckman Instruments, Inc.
6200 El Camino Real
CARLSBAD, California
U.S.A. 92008

A. Walichnowski
30 Pearce Avenue
WINNIPEG, Manitoba R2V 2K4

M.T. Wan
Kapilano 100
Park Royal South
WEST VANCOUVER, B.C. V7T 1A2

W.F. Warwick
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

J. Watson
Department of Fisheries & Oceans
Scientific Information & Publ. Br.
240 Sparks Street
OTTAWA, Ontario K1A 0E6

A.H. Weatherley
Life Science Division
Scarborough College
University of Toronto
WEST HILL, Ontario M1C 1A4

G.R. Webster
Pesticide Research Lab.
University of Manitoba
WINNIPEG, Manitoba R3T 2N2

H.E. Welch
Freshwater Institute
501 University Crescent
WINNIPEG, Manitoba R3T 2N6

J.B. Wilson
Department of Zoology
University of Guelph
GUELPH, Ontario N1G 2W1

C. Wren
505-120 Edinburgh Road
GUELPH, Ontario

V. Zitko
Biological Station
Fisheries & Marine Service
ST. ANDREWS, N.B. E0G 2X0