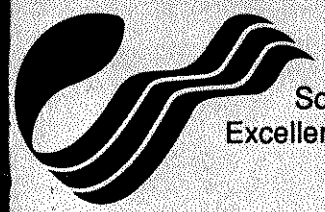

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Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop: November 28–30 1988, Montreal, Quebec

Comptes rendus du quinzième colloque annuel sur la toxicologie aquatique : 28–30 novembre 1988, Montréal, Québec

Editors/Editeurs
R. Van Coillie, A. Niimi, A. Champoux and/et G. Joubert

November 1989

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R. Van Coillie[1], A. Niimi[2], A. Champoux[3] and/et G. Joubert[4]

[1]Environnement Canada, Conservation et Protection, région du Québec, Centre Saint-Laurent, 105 rue McGill, Montréal, Québec, H2Y 2E7.

[2]Department of Fisheries and Oceans, Bayfield Institute, Canada Centre for Inland Waters, Burlington, Ontario, L7R 4A6.

[3]Environnement Canada, Direction des relations internationales, 10 rue Wellington, Hull, Québec, K1A 0H3.

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PREFACE/PREFACE

The 15th Annual Aquatic Toxicity Workshop was held at the DELTA HOTEL in Montreal on November 28, 29 and 30, 1988.

This Workshop was scheduled later than usual in order to coincide with the Annual Meeting of the Society of Toxicology of Canada (CST), which took place at the same location on November 30 and December 1. Workshop registrants attended the CST Meeting free of charge, and vice-versa.

As in previous years, the Workshop focussed on aquatic toxicology and ecotoxicology. There was a discussion of fundamental concepts in aquatic toxicology, as well as their application to environmental monitoring, formulation of rules and guidelines, and development of water quality guidelines.

In selecting presentations for the Workshop, priority was given to work addressing the following areas (in no order of preference):

Ecotoxicology of aquatic sediments;
Ecotoxicology of acidic precipitations and discharges;
Micro-bioassays (bacteria, algae, zooplankton, cell cultures, genotoxicity and biotechnology in aquatic toxicology);
Toxic effects in aquatic organisms (fate and efects of PAH's, PCB's, heavy metals, etc).

The Workshop included 17 platform presentations in plenary sessions, and 18 papers in poster sessions. Four sessions were devoted to special topics with 10 presentations.

Le 15e colloque annuel sur la toxicologie aquatique a eu lieu les 28, 29 et 30 novembre 1988 à l'hôtel DELTA de Montréal.

La tenue de cet colloque annuel a été délibérément retardée afin de le faire coincider avec la réunion annuelle de la Société de toxicologie du Canada (STC) qui s'est tien au même endroit, les 30 novembre et 1er décembre 1988. Les participants à l'colloque pourront assister gratuitement à la réunion de la STC et vice versa.

Comme par les années passées les discussions étaient centrées sur la toxicologie aquatique et l'écotoxicologie. Il But bien sûr question des notions fondamentales de la toxicologie aquatique mais aussi de son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critères pour la qualité de l'eau.

La préférence a été donnée aux travaux qui portent sur les sujets suivants, considérés tous aussi prioritaries les uns que les autres:

Écotoxicologie des sédiments aquatiques;
Écotoxicologie des précipitations et/ou des déversements acides;
Microbioessais (bactéries, algues, zooplancton, cultures cellulaires, génotoxicité et/ou biotechnologies en toxicologie aquatique);
Effets toxiques sur les organismes aquatiques (les effets et le devenir des HAP, des PCB, des métaux lourds et autres substances).

L'colloque regroupa 17 de présentations lors des séances plénières et 18 autres aux séances d'affichage. Quatre séances sont consacrées a des thèmes spéciaux avec 10 présentations.

EDITORS COMMENTS/REMARQUE DES EDITEURS

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Les commanditires suivants, spécialistes régionaux en toxicologie environnementale, ont le plaisir de vous présenter, avec Environnement Canada, Conservation et Protection, ces comptes rendus.

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The Steering Committee of the 15th Annual Aquatic Toxicity Workshop wishes to record its appreciation of the efforts of the panelists for preparing the outlines of their papers and their subsequent delivery and to the panel moderators for guiding panelists and audience during the workshop sessions.

Le comité d'organisation du 15e colloque annuel sur la toxicologie aquatique aimerait noter son appréciation auprès des conférenciers pour leur présentation ainsi qu'aux modérateurs pour leur aide lors du déroulement de l'colloque.

PROGRAM AND/PROGRAMME ET TABLE OF CONTENTS/TABLES DES MATIERES

FIRST SESSION/PREMIERE SEANCE

ECOTOXICOLOGY OF AQUATIC SEDIMENTS/
ECOTOXICOLOGIE DES SEDIMENTS AQUATIQUES

CHAIRPERSON: Dr. P.E. Ross, Illinois Natural History Survey,
Champaign, IL, USA.
MODERATOR: Dr. P. Lundahl, Lavalin Environnement, Montréal, PQ

Development of an Acute and Chronic Sediment Bioassay Protocol Using Larval Mayflies and Juvenile Fathead Minnows. Gail Krantzberg[1] and Richard Pope[2], [1]Water Resources Branch, Ontario Ministry of the Environment, Toronto, Ontario M4V 1P5, [2]Tarandus Associates Inc., 21 Greystone Crescent, Brampton, Ontario L6G 2B2.

OVERVIEW OF SEDIMENT BIOASSAY

Sediment bioassays measure the effects of contaminated sediments on the biota. Sediment elutriates have been prepared as liquid phase matrices, principly to assess the impacts of dredging activities on water column organisms (1,2). For example, one toxicity test exposes Daphnia to an elutriate (3). Pore waters have been considered as an alternate liquid phase to examine the effects of contaminated sediments on the burrowing infauna and to identify the route of exposure of different organisms to different pollutants (4).

By far the most frequently described approach is solid phase testing with either benthic or water column organisms (5,6). For the purpose of evaluating the impacts of inplace pollutants on the biota, as opposed to the consequences arising from dredging operations, the focus of this study was on the solid phase bioassay.

The principle objective of this study was to contribute to the development of a methodology for assessing the chronic and acute toxicity of sediments to biota. This included an examination of the effects of bioassay assembly and sediment manipulation techniques to the response of the test organisms, and the sensitivity of growth as a chronic endpoint.

Experiment 1: To determine the efforts of settling time, following the addition of sediments and water to the bioassay container, on toxicity to mayflies.

It is reasonable to expect that the exposure of an organism to contaminants will vary with the state to which the sediment-water system is in equilibrium. We therefore examined whether an organism's response varied with the length of settling time of the bioassay assembly

proceeding the introduction of the organism. The duration of exposure required for the response of organisms in test sediments to differ significantly from the controls was also not known. As a result, the experiment was designed so that half of the replicates could be harvested at day 10 and half could be harvested at day 21.

2L widemouth glass jars were filled to a depth of 3 cm with sediment (surface area = 100 cm^2) and water was gently added. Organisms were introduced at 3 time intervals; 5 hours settling plus 1 hour of aeration, 1 day settling plus 1 hour and 5 days settling plus 1 hour aeration. At each time interval, either 8 mayflies (c.a. 25 mg/ individual wet weight) or c.a. 1.5 gm oligochaetes wet weight (c.a. 150 individuals) were added to the chambers. Each treatment had 4 replicates. Water and sediment samples were collected as animals were added and when replicate containers were harvested (time = 10d or 21d).

Analysis of the growth response of Hexagenia suggested that biomass changes were influenced both by sediment type and by the duration of the period of equilibration (Table 1). Growth in both test sediments was greatest when the mayflies were added 5 days after chamber assembly, followed by a 1 day equilibration period. Growth was poorest when organisms were added 6 hours following assembly (1 hour after aeration). Growth inhibition more pronounced by day 21, as compared to day 10.

Experiment 2: To determine the effects of settling time on toxicity to fathead minnows at 2 different densities.

Fathead minnows weighing c.a. 0.5 gm per individual were added to each bioassay chamber at a rate of 10 or 15 individuals per replicate. Four replicates of each treatment were harvested after 10 or 21 days exposure.

In accordance with the biomass changes noted for mayflies, growth inhibition was least when the fathead minnows were added 5 days after chamber assembly. There appeared to be no notable difference between the 6 hr. (5 hr. settling plus 1 hr. aeration) and 1 day equilibration periods with respect to biomass changes, and the effects of fish density were variable. Growth inhibition was greater with 15 as compared to 10 fish in some, but not all cases, and density apparently exerted no

influence on biomass changes in the controls. This last finding is of interest, since it may indicate that the stress of possible overcrowding was exacerbated by the contaminated sediments. By day 21, all fish had decreased in weight. Minnows from the test sediments lost more weight than did the controls.

Experiment 3: To compare the toxicity of intact sediment cores to homogenized sediments for mayfly nymphs and fathead minnows.

Current methods for assembly of sediment bioassays often involve sieving and homogenizing the sediment. This effectively exposes the organisms to a uniform dose of contaminants that is in reality a mean dose of the heterogeniously distributed contaminants. In some cases, the extensive aeration of the sediment also results in a transformation of chemical species to forms that are of greater or lesser bioavailability. We examined the question of sediment homogenization by using diver-collected cores. The cores used were acrylic tubes of comparable surface area to the 2L glass jars. Organisms were introduced into the cores and into homogenized sediments from the same site as those where cores were collected. Eight Hexagenia nymphs (c.a. 40 mg/individual net weight) or 10 juvenile fathead minnows (c.a. 400 mg/individual net weight) were the test organisms. Mortality and biomass changes over three weeks were the endpoints examined. pH and dissolved oxygen were monitored in all chambers.

In Site A, intact sediments resulted in higher mortality and poorer growth than homogenized sediments for mayfly nymphs, but did not significantly influence mortality or growth in fathead minnows. Intact sediments from Site B resulted in better growth for mayfly nymphs than homogenized sediment. Mortality was <10% in both treatments. Homogenization resulted in substantial mortality for fathead minnows (87% vs 20% in intact cores). In Site C (sandy sediment), homogenization resulted in higher mortality than in the intact cores for Hexagenia. This was most likely caused by the elimination of the surface layer of fine-grained material (present in intact cores) and therefore, the elimination of suitable substrate for burial and feeding. Homogenization did not effect growth of fathead minnows, and may have ameliorated toxicity as measured by mortality.

TABLE 3: Effect of Settling Time on Growth of Hexagenia limbata
Values in parentheses are standard deviations

MEASUREMENT	SETTLING TIME 5h	24h	120h	5h	24h	120h	5h	24h
	Toronto STP			Rice Lake			Control	
Percent Biomass Change (Day 10)	-3 (0.9)	-2 (0.1)	25 (7)	17 (2)	30 (6)	25 (10)	103 (8)	113 (3)
Percent Mortality (Day 10)	4 (5)	6 (7)	12 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Percent Biomass Change (Day 21)	4 (9)	18 (10)	69 (3)	42 (9)	77 (25)	129 (50)	163 (10)	159 (15)
Percent Mortality (Day 21)	7 (9)	12 (10)	12 (10)	8 (11)	8 (11)	0 (0)	0 (0)	0 (0)

As a result of these preliminary experiments, we recommend further detailed examination of bioassay design and chronic endpoints, including bioaccumulation, in order to determine the significance of inplace pollutants.

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TOXICITY OF ST. LAWRENCE RIVER (LAKE ST. LOUIS) SEDIMENT ELUTRIATES AS A FUNCTION OF BIOASSAY RESPONSES, USING MICRO-ORGANISMS, AND WATER QUALITY GUIDELINES

Sloterdijk, Harm H.[1], L. Champoux[1,2], P. Ross[2,3], V. Jarry[2], Y. Couillard[2]

1) Environment Canada, 1001 Pierre Dupuy, Longueuil, Québec, J4K 1A1
2) Département des sciences biologiques, Université de Montréal, Montréal, Québec, M3C 3J7.

A sediment study, involving both chemical and biological analyses, was carried out in the St. Lawrence River near Montreal (Lake St. Louis). About 60 stations were sampled during 1984-85, and the sediments were analysed for support parameters, heavy metals, and organochlorinated compounds. Subsamples were elutriated using a 1 to 4 sediment/water ratio. The resulting elutriates were analysed for various chemical parameters, while toxicity was measured using the Microtox test, algal 14C assimilation, and lethality/developmental inhibition in cladocerans, rotifers and nematodes. The results showed a great variety of responses and sensitivity, and correlations between the tests were generally non-significant. In terms of positive responses (i.e. toxic effects algal and Microtox tests were the most sensitive. Toxic responses could not be explained in simple terms of contaminant concentrations. Contamination indices, calculated using desorbed contaminant concentrations and water quality guidelines, of the different elutriates did not show any correlation with toxic responses. Therefore, the chemistry of elutriates and the use of water quality guidelines are not predictive of the toxic potential of contaminated sediments. Biotests can give an insight into the hazard assessment of sediments, but no single test will be sufficient; the use of a battery of standardized biotests, representing different levels of organization/food chain, is highly recommended.

EVALUATION COMPARATIVE DE LA TOXICITE DES ELUTRIATS DE SEDIMENTS DU FLEUVE ST-LAURENT (LAC ST-LOUIS) ENTRE DES REPONSES DE BIOESSAIS SUR MICROORGANISMES ET LES CRITERES DE QUALITE DE L'EAU.

Sloterdijk, Harm H.[1], L. Champoux[1,2], P. Ross[2], V. Jarry[2], Y. Couillard[2]

1) Environment Canada, 1001 Pierre Dupuy, Longueuil, Québec J4K 1A1

2) Département des sciences biologiques, Université de Montréal, Montréal, Québec, M3C 3J7

RESUME

Une étude de sédiments, impliquant à la fois des analyses chimiques et biologiques, a été effectuée sur le fleuve St-Laurent, près de Montréal (Lac St-Louis). Environ 60 stations ont été échantillonnées en 1984-85, et les sédiments ont été analysés pour les paramètres de base, les métaux lourds et les composés organochlorés. Des sous-échantillons ont été élutriés en utilisant un rapport sédiment/eau de 1 à 4. Les élutriats ainsi obtenus ont été analysés pour les différents paramètres chimiques, tandis que la toxicité a été mesurée à l'aide du test Microtox, de l'assimilation algale du C_{14}, et de la létalité ou l'inhibition de la croissance chez les cladocères, rotifères et nématodes. Les résultats ont démontré une grande variété de réponses et de sensibilité, et les corrélations entre les tests n'étaient généralement pas significatives. En termes de réponses positives (i.e. d'effets toxiques), les tests avec algues et Microtox ont été les plus sensibles. Les réponses toxiques ne pourraient pas être expliquées simplement en termes de contaminants. Les indices de contamination des différents élutriats, calculés en utilisant les concentrations de contaminant libéré et les critères de qualité de l'eau, n'ont démontré aucune corrélation avec les réponses toxiques. Ainsi, la chimie des élutriats et l'utilisation des critères de qualité de l'eau ne permettent pas de prévoir le potentiel toxique des sédiments contaminés. Les biotests peuvent fournir un aperçu quant à l'évaluation des risques des sédiments, mais un seul test ne sera ps suffisant; l'utilisation de toute une gamme de biotests normalisés, représentant différents niveaux organisationnels ou de la chaîne alimentaire, est fortement recommandée.

SECOND SESSION/DEUXIEME SEANCE

ECOTOXICOLOGY OF ACID PRECIPITATIONS AND DISCHARGES/ ECOTOXICOLOGIE DES PRECIPITATIONS ET DEVERSEMENTS ACIDES

CHAIRPERSON: Dr. J.H. McCormick, Environmental Research Laboratory, Duluth, MN, USA
MODERATOR: H. Sloterdijk, Environnement Canada, Longueuil, PQ

BIOACCUMULATION ET EFFETS SOUS-LÉTAUX DE L'ALUMINIUM CHEZ DES OEUFS ET JUVÉNILES DE SALMONIDÉS: EXPÉRIMENTATION EN LABORATOIRE ET EN MILIEU NATUREL

par

Denis Brouard[1]
Raymond Van Coillie[2]
Yvan Vigneault[3]
Claude Thellen [4]

[1]Gilles Shooner et Associés inc.
[2]Environnement Canada
[3]Pêches et Océans Canada
[4]Environnement Québec

RÉSUMÉ

En simulant en laboratoire des conditions de pH et d'aluminium observées in situ, on peut évaluer partiellement le danger sous-létal de l'aluminium en milieu modérément acide pour des oeufs et juvéniles de saumon. L'aluminium pénètre relativement rapidement dans les oeufs jusqu'à un plateau de bioaccumulation atteint après neuf jours. La pénétration de ce métal s'avère plus prononcée chez les oeufs post-fertilisés que chez les oeufs embryonnés car le chorion de ces derniers a une perméabilité réduite à l'aluminium. Les teneurs très majoritaires du métal bioaccumulé se retrouvent dans le chorion des oeufs, ce qui semble protéger l'embryon contre l'agression toxique sous-létale de l'aluminium. Chez les juvéniles de salmonidés on observe une bioaccumulation d'aluminium surtout au niveau des branchies altérées par un pH modérément acide. Ensuite il y a une diffusion interne d'aluminium vers le foie. Face à cette agression toxique sous létale d'aluminium les juvéniles réagissent par une synthèse accrue de protéines ce qui induit une surconsommation d'oxygène.

Par ailleurs des bioessais de 200 jours, réalisés en milieu naturel avec des oeufs de saumon, révèlent d'une part que les mortalités cumulatives à l'éclosion se situent en-deçà de 5% et d'autre part que la bioaccumulation de l'aluminium est relativement faible (<8 mg/g). Vraisemblablement la présence élevée de matière organique dans les eaux naturelles contribuerait à atténuer considérablement la toxicité de l'aluminium en le rendant non biodisponible.

1. INTRODUCTION

A la fonte des neiges et lors de pluies abondantes, les précipitations acides apportent des ions H^+ dans le sol où elles provoquent des échanges cationiques (SNSF, 1980). Dans les sols qui renferment une part relativement importante d'aluminosilicates, comme c'est le cas dans la région de la Côte-Nord du fleuve Saint-Laurent, les ions H^+ déplacent, non seulement des cations comme Na^+, K^+, Ca^{+2} et Mg^{+2}, mais également Al ce qui induit un surcroît de ce métal dans les eaux de surface. La concentration en aluminium augmente avec l'acidité sous pH 7,0 dans les eaux de surface. De fait, une corrélation synergique existe entre ces deux facteurs. Soulignons que c'est l'aluminium organique qui prédomine dans les eaux naturelles parmi les différentes formes de spéciation du métal (Baker et Schofield, 1980; Campbell et coll., 1982 et 1984).

La valeur de 100 µg Al/L peut représenter une indication normative pour la toxicité létale de l'aluminium inorganique à pH 4,5-5,5 vis-à-vis du saumon (Van Coillie et coll., 1983). Cette valeur doit cependant être considérée en fonction de la spéciation de l'aluminium total dosé dont Al inorganique dissous représente 2 à 17% selon les conditions de pH, dureté et teneur organique (Campbell et coll., 1982; Van Coillie et coll., 1983). Étant donné que les concentrations d'Al total dépassent nettement cette indication normative, dans les rivières à saumons de la Côte-Nord du fleuve Saint-Laurent (Brouard, 1984; Walsh et Vigneault, 1986), nous avons tenté de préciser en laboratoire le danger toxique sous-létal de l'aluminium en deçà de 600 µg Al total/L à pH 5,5 pour les salmonidés. Par ailleurs, dans le cadre de bioessais in situ, nous avons évalué la bioaccumulation de l'aluminium dans des oeufs de Salmo salar de même que les mortalités induites.

2. MÉTHODOLOGIE

2.1 Bioessais de bioaccumulation de l'aluminium chez des oeufs et alevins vésiculés de saumon en laboratoire

Les expériences de bioaccumulation d'aluminium en laboratoire ont été effectuées en quasi-obscurité à 0,5°C dans des auges dont l'eau était renouvelée quotidiennement à 50%. Les procédures expérimentales sont décrites en détail par Brouard et Lachance (1986).

Le choix du pH 5,5 et des teneurs d'aluminium de 100 à 300 µg Al/L a été fixé en fonction des valeurs minimales observées pour ces paramètres lors d'une étude réalisée antérieurement au secteur amont de la rivière des Escoumins (Brouard et coll., 1983). De plus, afin de rapprocher le plus possible les conditions de laboratoire des conditions d'incubation prévalant en milieu naturel, une eau synthétique répondant aux principales caractéristiques des rivières à saumon de la Côte-Nord du fleuve Saint-Laurent a été préparée selon le protocole établi par l'agence américaine EPA (Peltier, 1978). Une matière humique provenant de la tourbe "Aldrich" a été préparée et ajoutée selon une procédure utilisée par Campbell et coll. (1982) pour simuler des conditions organiques naturelles.

2.2 Bioessais de toxicité sous-létale de l'aluminium chez des juvéniles de salmonidés en laboratoire

L'ajustement de l'expérimentation sous-létale a été réalisé en fonction des données acquises antérieurement par notre équipe (Brouard et coll., 1982, 1983 et 1986; Van Coillie et coll., 1983

et 1986). Ces renseignements nous ont amenés à ne pas dépasser 500-600 μg Al/L pour l'étude de la toxicité sous-létale de l'aluminium chez les salmonidés.

Dans l'ensemble les bioessais à flux continu avec des juvéniles de Salvelinus fontinalis (âge 0^+) eurent lieu dans les mêmes conditions que celles décrites précédemment pour les bioessais d'oeufs de saumons.

A la fin des bioessais, divers examens et/ou analyses ont été réalisés:

- dosage de l'aluminium dans les branchies et foies selon la méthode de Agemian et coll. (1980);

- prélèvement et fixation de branchies et foies dans la glutaraldéhyde 5%-formaldéhyde 4% en tampon cacodylate de Na 0,1 M, préparation histologique ultérieure dans résine Epon pour observation microscopique et ultrastructurale (Chevalier et coll., 1985) et microanalyse cytochimique d'Al aux rayons-X par longueur d'onde dispersive en microscopie électronique (Van Coillie et coll., 1975);

- test respirométrique mesurant la concentration résiduelle d'oxygène selon la méthode de Giles et Klapart (1979).

- détermination du taux de synthèse de protéines ou d'ARN dans les branchies et le foie selon les procédures décrites par Van Coillie (1977) et Brouard (1988).

2.3 Bioessais d'oeufs de saumon en milieu naturel

Pour analyser les facteurs mortalité et bioaccumulation chez les oeufs de Salmo salar, il s'est avéré opportun de différencier la mortalité d'origine abiotique, associée à des teneurs élevées d'ions hydrogène et de métaux tels que l'aluminium, par rapport à la mortalité d'origine biotique, principalement attribuable à la prédation et aux infestations fongiques par les saprolégniales.

Afin d'atténuer le plus possible le bruit de fond lié aux mortalités biotiques, un dispositif d'incubation semi-naturel, permettant d'éliminer complètement le facteur de prédation, a été mis au point (Brouard et Lachance, 1986). De plus, les traitements prophylactiques apportés aux oeufs ont permis de supprimer une bonne partie de la mortalité d'origine biotique attribuable aux infestations fongiques.

3. RÉSULTATS ET DISCUSSION

3.1 Pénétration de l'aluminium dans les oeufs et alevins de saumon

L'aluminium se concentre rapidement dans les oeufs post-fertilisés et, après neuf jours de bioessais, sa bioaccumulation tend vers un plateau, lequel est plus faible lorsqu'il y a 10 mg/L de matière humique dans le milieu (Figure 1a). La bioaccumulation de l'aluminium chez les oeufs embryonnés apparaît également rapide et s'avère affaiblie en présence de 10 mg/L de matière organique (Figure 1b). Elle est toutefois plus faible que celle observée pour les oeufs post-fertilisés. En présence de 300 μg Al/L, la bioaccumulation du métal ne dépasse guère 16 μg Al/g au stade embryonné alors qu'elle atteint 50 μg Al/g au stade post-fertilisé.

Ces résultats indiquent qu'il existe une différence de perméabilité du chorion entre ces deux stades vis-à-vis de l'aluminium. A ce propos, signalons que le chorion, issu de la zona radiata péricellulaire de l'ovocyte, s'avère très perméable lors de la fertilisation et pendant quelques jours après celle-ci. Ensuite, les pseudokératines qui forment près de 75% du chorion se polymérisent progressivement de telle sorte que cette enveloppe devient dure et relativement imperméable (Hurley et Fisher, 1966). Cette différence histophysiologique du chorion entre les deux stades étudiés peut-elle influencer la pénétration de l'aluminium dans les oeufs. Pour tenter de répondre à cette question, nous avons analysé et comparé les teneurs d'aluminium des chorions et des parties internes des oeufs aux deux stades considérés.

Les résultats de cet examen présentés au tableau 1 indiquent que:

- la perméabilité du chorion à l'aluminium est plus élevée à la phase post-fertilisée qu'à la phase embryonnée: de fait, le pourcentage d'aluminium bioaccumulé dans le liquide périvitellin équivaut à 69%, lors de la première phase, par rapport à 18% lors de la seconde phase;

- le chorion s'avère, par unité de poids, la composante qui capte le plus d'aluminium dans l'oeuf; on y retrouve en effet 398 et 439 μg Al/g poids frais aux deux phases étudiées, ce qui dépasse considérablement les teneurs d'Al mesurées dans les autres composantes. Le chorion aurait ainsi un rôle de protection partielle contre les excès d'aluminium dans le milieu, lequel rôle serait analogue à celui explicité par Van Coillie (1977) pour certains métaux bivalents (Cd, Cu, Zn).

- malgré cette protection, il y a diffusion partielle de l'aluminium provenant du liquide périvitellin.

Les bioessais réalisés avec des alevins vésiculés n'ont pas permis de mettre en évidence une bioaccumulation de l'aluminium. En effet, les teneurs de ce métal dans les alevins demeuraient au voisinage de 1 μg Al/g. Ceci pourrait s'expliquer comme suit: les alevins vésiculés testés venaient d'éclore et bénéficiaient encore de leur réserve vitelline qui leur permettait d'être relativement indépendants du milieu ambiant, laquelle "protection" disparaîtrait ultérieurement lorsque l'épuisement progressif de leur sac vitellin les forcerait à échanger avec le milieu. Bref, l'expérimentation faite avec de l'aluminium en concentrations sous-létales (300 μg/L), à des conditions modérément acides (pH 5,5, eau très douce), révèle que la pénétration de ce métal chez les jeunes stades de développement du saumon est assujettie à certains facteurs d'origine abiotique et biotique:

- La présence de matière organique humique à une concentration de 10 mg/L atténue la biodisponibilité et la bioaccumulation de l'aluminium de deux à trois fois;

- La bioaccumulation de l'aluminium varie avec les stades de développement: de fait, ce métal est préférentiellement accumulé comme suit: oeufs post-fertilisés > oeufs embryonnés > alevins vésiculés. Ceci correspond au gradient obtenu pour la bioaccumulation du cadmium chez Salmo salar (Rombough et Garside, 1982).

3.2 Effets sous-létaux de l'aluminium chez les salmonidés

Les résultats de bioaccumulation sous-létale d'aluminium, dans des branchies d'ombles de fontaine, traitées en laboratoire avec 300 µg Al inorganique/L à pH 5,5, révèlent que la bioaccumulation s'amplifie avec la concentration d'essai et est réduite en présence de matière organique (Tableau 2). De plus, la bioaccumulation branchiale sous-létale d'aluminium chez les salmonidés apparaît comme un phénomène relativement rapide (<10 jours), ce qui rappelle celle observée pour les oeufs de saumon.

Outre l'analogie de bioaccumulation branchiale d'Al chez des salmonidés *in vitro* et *in situ* pour 300 µg Al/l à pH 5,5, (Brouard, 1988), il y a aussi une ressemblance entre les effets histopathologiques branchiaux provoqués par cette concentration d'aluminium et ce pH chez les salmonidés *in vitro* et *in situ*. De fait, après sept jours de traitement avec 300 µg Al inorganique/L, on observait une desquamation épithéliale, une multiplication de cellules à chlorures et une hyperplasie entre les lamelles secondaires dans les branchies de *Salvelinus fontinalis* (Van Coillie et coll., 1986) comme dans celles de cette espèce séjournant dans les lacs modérément acides (pH 5,5) avec 300 µg Al/L (Chevalier et coll., 1985). Le fait que les mêmes altérations histopathologiques aient été constatées, après un traitement de sept jours avec le pH 5,5 seulement, soutient que ce dernier exerce plus d'effet histologique que 300 µg Al/L dans les branchies de salmonidés.

Pour caractériser la distribution de l'aluminium bioaccumulé dans les branchies et foies de salmonidés, des microanalyses d'Al aux rayons-X (longueur d'onde dispersive en microscopie électronique à transmission) ont été effectuées sur des coupes ultraminces (0,3 µm d'épaisseur) de branchies et de foies de truites traitées durant sept jours avec ce métal à pH 5,5. Il est connu que la

préparation histologique de telles coupes élimine, lors de leur déshydratation, les composés non absorbés dans la matrice macromoléculaire histologique, l'aluminium adsorbé étant alors "lessivé", il ne reste que l'aluminium absorbé.

Il en résulte que le seuil minimal de détection d'Al avec les méthodes histochimiques de fluorescence se situe à environ 100 g Al/g en poids sec. Compte tenu de ce seuil, nous n'avons pu déceler Al que dans des coupes ultraminces de branchies et de foies des truites qui avaient été traitées avec 600 μg Al/L à pH 5,5. Dans leurs branchies, un rapport P/B (Peak/Background) d'Al significatif de 1,3 ± 0,2, uniquement dans la membrane des cellules épithéliales d'échange, montre que l'aluminium pénètre dans les lamelles branchiales secondaires via ces cellules. En considérant que ce rapport P/B de 1,3 correspond à environ 300 μg Al/g en poids sec, que les cellules épithéliales où il a été décelé représentent approximativement 10% de arcs branchiaux prélevés et que le poids sec des branchies ne dépasse guère 10% de leur poids frais, il est possible d'estimer la concentration d'Al absorbé dans les branchies des truites traitées avec 600 μg Al/L à pH 5,5, à savoir 3μg Al/g en poids frais. Ceci équivaut à 25% de l'aluminium bioaccumulé dans leurs branchies ce qui indique que 75% de cet aluminium serait donc simplement adsorbé en surface vraisemblablement avec le mucus branchial surabondant.

Dans le foie de ces truites, Al ne fut histochimiquement détecté qu'au niveau de la chromatine diffuse des cellules hépatocytaires. A ce niveau, son rapport P/B correspondait à 1,21 ± 0,1, soit environ 100μg Al/g en poids sec. Mentionnons que leur foie contenait 0,25 ± 0,03 μg Al/g en poids frais, ce qui représentait 2% de l'aluminium bioaccumulé dans leurs branchies. En

poids sec, cette concentration équivalait à 1,25 µg Al/g. La différence entre cette dernière valeur et celle précisée par microanalyse histochimique suggère que, dans le foie, l'aluminium se lie surtout à l'euchromatine des hépatocytes. Ceci a été corroboré à partir de digestions enzymatiques faites avec de la DNAse sur des coupes ultraminces de foie, le rapport P/B d'Al diminuant alors de 1,2 à une valeur non significative. Ceci révèle que l'aluminium y était fixé dans l'ADN, comme cela a été constaté avec des cellules humaines (McDonald et Martin, 1988). Bref, l'aluminium ne pénètre que partiellement dans les cellules branchiales épithéliales d'échange des salmonidés: cette pénétration ne s'observe en effet que pour 10% de l'aluminium bioaccumulé au niveau de leurs branchies. Ensuite, il s'y répartit dans leurs organes, entre autres dans leurs foies où on retrouve 2% du métal total bioaccumulé dans les branchies.

L'agression toxique sous-létale de l'aluminium chez les salmonidés via sa bioaccumulation branchiale et sa pénétration hépatique entraîne-t-elle des réactions chez ces salmonidés? Ceci a été vérifié lors de bioessais réalisés avec Salvelinus fontinalis traités avec 100, 300 ou 600 µg Al/L à pH 5,5 pendant sept jours. De fait, les truites manifestaient alors une hyperventilation branchiale. Pour vérifier si cette amplification de consommation d'oxygène correspond à un anabolisme accru, la consommation d'oxygène et les niveaux de synthèse des ARN et des protéines des branchies et du foie de ces truites ont été mesurés à la fin des bioessais de sept jours. Ces résultats exposés aux tableaux 3, 4 et 5, révèlent:

- qu'une augmentation de la consommation d'oxygène s'observe chez les salmonidés à pH 5,5 et s'accroît avec les concentrations d'aluminium (100 à 600 μg/L) à ce pH. Cet effet synergique du métal est amoindri en présence de matière humique;

- que cette réponse physiologique synergique aux agressions du pH 5,5 et de l'aluminium s'accompagne d'une augmentation concomitante de la synthèse protéique dans les branchies et, à un plus faible degré, dans le foie;

- que cette amplification anabolique protéique branchiale et ensuite hépatique est vraisemblablement "une synthèse sur demande" vu qu'une amplification analogue se constate aussi au niveau des ARN nécessaires pour une telle synthèse.

3.3 Bioessais d'oeufs de saumon in situ

Les expériences réalisées en milieu naturel, font ressortir que la mortalité cumulative, principalement d'origine abiotique étant donné les traitements prophylactiques apportés aux oeufs, ne dépasse guère 3% à l'éclosion pour la période expérimentale d'incubation de 200 jours. La mortalité globale, attribuable en grande partie aux infestations fongiques, se situe quant à elle à près de 8% lorsque les oeufs n'étaient pas traités au vert de malachite. Ces taux de mortalité sont du même ordre de grandeur que ceux rapportés par Warner (1963), dans l'évaluation de la survie des oeufs de Salmo salar ouananiche en milieu naturel au nord du Maine, soit 6,8% peu avant l'éclosion.

Par ailleurs les concentrations d'aluminium dans les oeufs de saumon restèrent relativement constantes lors de l'incubation de ceux-ci et ne montrèrent qu'une faible tendance à la bioaccumula-

tion. Les valeurs moyennes se situent à 5,2 µg/g (poids humide) dans les oeufs alors que les valeurs extrêmes sont de 2 et 8 µg/g.

En résumé, les effets prédécrits pour l'aluminium sont toutefois considérablement réduits en présence de 10 mg/L de matière organique, laquelle teneur se révèle généralement minimale pour les rivières de la Côte-Nord. Dans ces conditions, la spéciation du métal indique qu'il est labile et biodisponible à moins de 10% à pH modérément acide (Campbell et coll., 1984). L'aluminium représente dès lors un danger écotoxique amoindri vis-à-vis duquel existent divers processus de défenses biochimiques et histochimiques.

4. CONCLUSION

En simulant en laboratoire des conditions de pH et d'aluminium observées in situ le danger sous-létal de l'aluminium a été évalué partiellement, en milieu modérément acide, vis-à-vis des salmonidés.

Les oeufs de ceux-ci bioaccumulent l'aluminium relativement rapidement mais de façon limitée car une tendance à un plateau de bioaccumulation se constate après neuf jours. La pénétration du métal s'avère plus prononcée chez les oeufs fertilisés que chez les oeufs embryonnés car le chorion de ces derniers a une perméabilité réduite à l'aluminium. Les teneurs très majoritaires du métal bioaccumulé se retrouvent dans le chorion des oeufs, ce qui protège l'embryon contre l'agression toxique sous-létale de l'aluminium.

Chez les salmonidés juvéniles, ce métal se bioaccumule également de façon assez rapide et limitée à pH 5,5 dans les branchies. De fait, en sept jours, une présence de 300-600 μg Al/L dans le milieu occasionne des teneurs bioaccumulées de 9-12 μg Al/g. Ceci soutient que la bioaccumulation branchiale de l'aluminium plafonne relativement rapidement chez les salmonidés. Ce métal est alors principalement adsorbé en surface des branchies vue que sa concentration absorbée dans les membranes de leurs cellules épithéliales d'échange correspond à seulement 10% de la bioaccumulation constatée. Il traverse alors, en petite quantité, les branchies puisqu'on le détecte dans le foie, où sa teneur ne représente que 2% de sa bioaccumulation branchiale.

Au niveau hépatique l'aluminium se fixe surtout à l'ADN. Malgré sa pénétration limitée, il induit une synthèse protéique sur demande qui nécessite une élaboration d'ARN. Cette réponse anabolique à l'agression toxique sous-létale de l'aluminium s'observe dans les branchies et, à un moindre degré, dans le foie.

La plupart des phénomènes décrits précédemment sont considérablement atténués lorsqu'on ajoute 10 mg/L de matière humique dans le milieu. Ceci souligne que la matière organique des eaux naturelles, pour laquelle l'aluminium a une affinité, exerce un rôle protecteur diminuant l'écotoxicité du métal vis-à-vis des salmonidés. En considérant les points suivants:

- l'effet protecteur de la matière organique aquatique;
- la spéciation de l'aluminium à pH 5,5 auquel la forme biodisponible du métal est relativement atténuée;
- la protection qu'offre le chorion des oeufs de salmonidés;
- la faible pénétration du métal au-delà de la surface branchiale;

- la réponse anabolique de protection contre l'agression toxique sous-létale d'aluminium;
- les faibles taux de mortalité observés lors des bioessais de 200 jours en milieu naturel;

Il appert qu'une présence de 300 µg Al/L à pH 5,5 ne représente pas un danger écotoxique sous-létal à court terme pour les salmonidés dans les rivièrs à saumons de la Côte-Nord du fleuve Saint-Laurent. Ceci ne tient toutefois pas compte du coût énergétique des réponses anaboliques de protection, lequel pourrait affecter à long terme d'autres fonctions telles que la reproduction.

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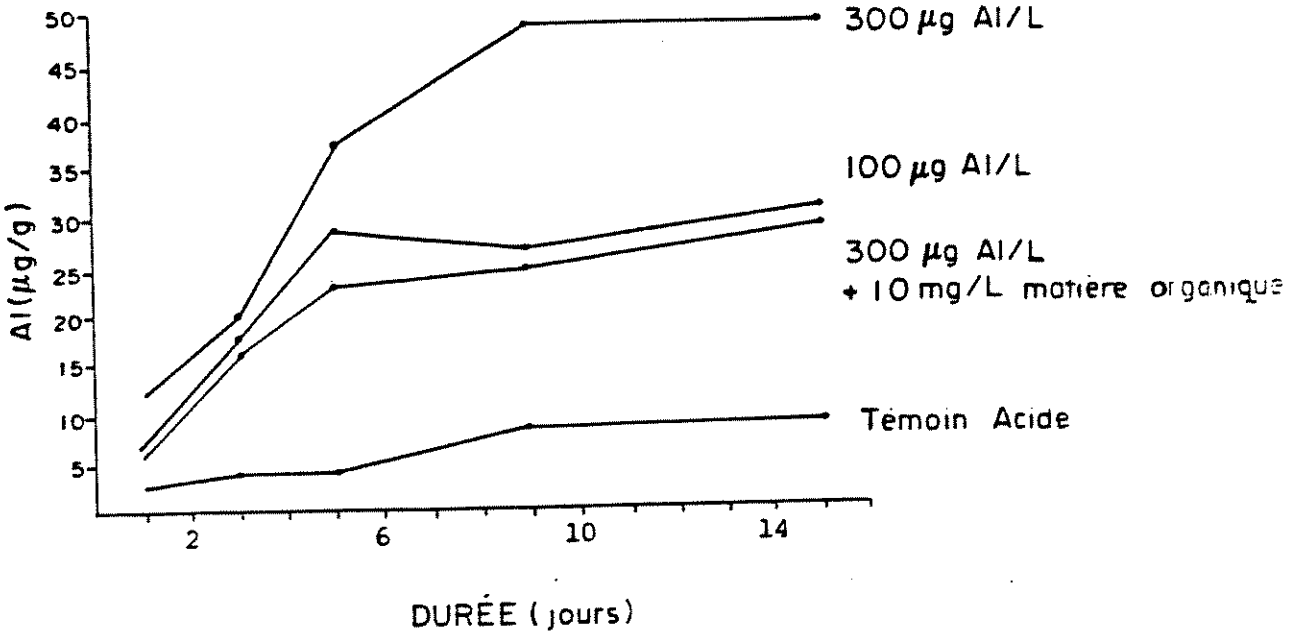
A) OEUFS POST-FERTILISÉS



B) OEUFS EMBRYONNÉS

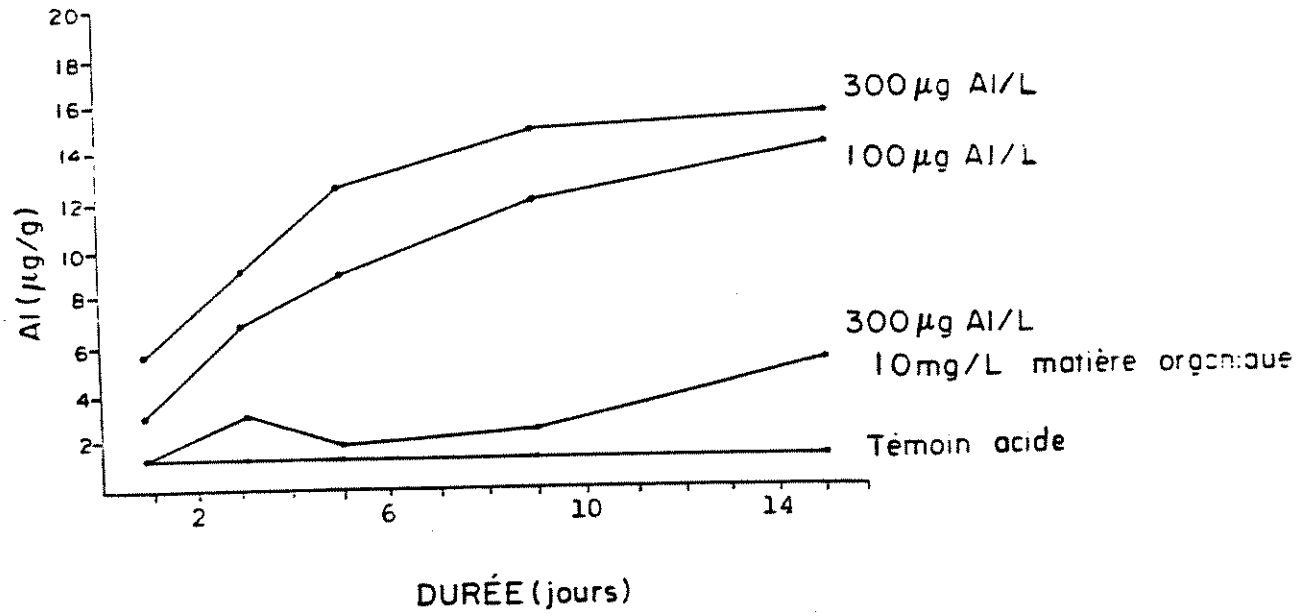


FIGURE 1. Bioaccumulation d'aluminium (µg/g, poids humide) chez des oeufs post-fertilisés et embryonnés de saumon (Salmo salar) durant 15 jours de bioessais sous différentes conditions à pH 5,5.

TABLEAU 1. Répartition de l'aluminium bioaccumulé dans des oeufs de saumon (Salmo salar) après quinze jours d'incubation expérimentale avec ce métal.

PHASES	PARTIES DES OEUFS				
	CHORION	LIQUIDE PÉRIVITELLIN	VITELLUS	EMBRYON	OEUF TOTAL
I OEUFS POST-FERTILISÉS TRAITÉS AVEC 300 ppb Al à pH 5,5					
Poids frais* pour 10 oeufs ($\mu g \times 10^3$)	5,2	19,4	118,1	17,7	160,4
Teneur en Al (μg/g poids frais)	398,1	282,3	3,1	1,9	49,6
Quantité d'Al (μg pour 10 oeufs)	2,07	5,48	0,37	0,03	7,95
% d'Al	26%	69%	5%	0,4%	100%
II OEUFS EMBRYONNÉS TRAITÉS AVEC 300 ppb Al à pH 5,5					
Poids frais* pour 10 oeufs ($\mu g \times 10^3$)	5,6	57,9	81,4	71,0	215,9
Teneur en Al (μg/g poids frais)	439,3	10,5	2,6	1,6	15,7
Quantité d'Al (μg pour 10 oeufs)	2,46	0,61	0,21	0,11	3,39
% d'Al	73%	18%	6%	3%	100%

* Poids moyen frais: les intervalles de confiance n'excédaient pas 12% de la valeur de la moyenne.

Tiré de Brouard (1988).

TABLEAU 2. Bioaccumulation d'aluminium dans les branchies d'ombles de fontaine (Salvelinus fontinalis) juvéniles lors de bioessais en laboratoire.

TRAITEMENTS durant 7 jours	TENEURS D'Al dans les BRANCHIES en µg poids frais
pH 5,5	1
pH 5,5 + 100 µg/L Al*	1,8 (0,4)**
pH 5,5 + 300 µg/L Al*	8,7 (2,8)
pH 5,5 + 600 µg/L Al*	12,5 (3,1)
pH 5,5 + 600 µg/L Al* + matière humique (10 mg/L)	5,7 (1,9)

* Les teneurs mesurées d'Al dans les aquaria furent généralement inférieures de 15% aux concentrations nominales de ce métal.

** ( ) = écart type

Tiré de Brouard (1988).

TABLEAU 3. Consommation d'oxygène chez des salmonidés exposés à l'aluminium à pH 5,5.

Traitement de 7 jours avec Salvelinus fontinalis*	Consommation d'oxygène ± écart-type (mg O_2/h/g poids frais)	
pH 6,7 (témoins)	0,33 ± 0,05	
pH 5,5	0,41 ± 0,07	(+ 24%)
pH 5,5 + 100 µg Al/L**	0,63 ± 0,08	(+ 91%)
pH 5,5 + 300 µg Al/L**	0,85 ± 0,14	(+ 158%)
pH 5,5 + 600 µg Al/L**	1,08 ± 0,22	(+ 227%)
pH 5,5 + 600 µg Al/L** (avec 10 mg/L matière humique)	0,49 ± 0,09	(+ 49%)

* Truites juvéniles (âge 0^+)

** Al inorganique: les écarts entre les teneurs nominales et mesurées d'aluminium dans les aquaria ne dépassaient pas 15%.

Tiré de Brouard (1988).

TABLEAU 4. Synthèse de protéine branchiales et hépatiques chez des salmonidés exposés à l'aluminium à pH 5,5.

Traitement de 7 jours avec Salvelinus fontinalis*	Synthèse des protéines branchiales*** ± écart-type	Synthèse des protéines hépatiques*** ± écart-type
pH 6,7 (témoins)	3050 ± 215	4200 ± 451
pH 5,5	4971 ± 514(+63%)	4662 ± 428(+11%)
pH 5,5 + 100 µg Al/L**	5612 ± 398(+84%)	5207 ± 559(+24%)
pH 5,5 + 300 µg Al/L**	7473 ± 644(+145%)	5758 ± 678(+37%)
pH 5,5 + 600 µg Al/L**	8751 ± 901(+187%)	6465 ± 595(+54%)
pH 5,5 + 600 µg Al/L** (avec 10 mg/L matière humique)	5156 ± 617(+69%)	5078 ± 715(+21%)

* Truites juvéniles (âge 0+)

** Al inorganique: les écarts entre les teneurs nominales et mesurées d'aluminium dans les aquaria ne dépassaient pas 15%.

*** dpm (désintégrations par minute) dues au 14C/heure d'incorporation de L leucine 14 C/mg de protéine/g en poids frais de branchies ou de foie.

Tiré de Brouard (1988).

TABLEAU 5. Synthèse d'acides ribonucléiques (ARN) branchiaux et hépatiques chez les salmonidés exposés à l'aluminium à pH 5,5.

Traitement de 7 jours avec Salvelinus fontinalis*	Synthèse d'ARN*** branchiaux ± écart-type	Synthèse d'ARN*** hépatiques ± écart-type
pH 6,7 (témoins)	760 ± 68	950 ± 84
pH 5,5	1034 ± 119(36%)	1036 ± 98(+9%)
pH 5,5 + 100 µg Al/L**	1274 ± 156(+68%)	1055 ± 109(+11%)
pH 5,5 + 300 µg Al/L**	1452 ± 147(+91%)	1102 ± 120(+16%)
pH 5,5 + 600 µg Al/L**	1865 ± 201(+145%)	1321 ± 91(+39%)
pH 5,5 + 600 µg Al/L** (avec 10 mg/L matière humique)	1205 ± 134(+59%)	1084 ± 88(+14%)

* Truites juvéniles (âge 0^+)

** Al inorganique: les écarts entre les teneurs nominales et mesurées d'aluminium dans les aquaria ne dépassaient pas 15%.

*** dpm (désintégrations par minute) dues au 14C/heure d'incorporation de L leucine 14 C/mg de protéine/g en poids frais de branchies ou de foie.

Tiré de Brouard (1988).

TOXICOLOGIE ENDOCRINIENNE DES POISSONS.

Alice Hontela , Yves Roy et Gaston Chevalier

Laboratoire de Toxicologie de l'Environnement (TOXEN), Université du Québec à Montréal, Montréal, P.Q., H3C 3P8

INTRODUCTION

L'acidification des eaux douces de surface par les précipitations acides répresente un danger de plus en plus réel pour la survie de la faune aquatique, y compris les poissons. Le Québec est parmi les régions le plus sévèrement affecté par les pluies acides.

Les effets nocifs des milieux acidifiés aggravés par l'aluminium rendu soluble, sur la santé des poissons commençent à être bien définis grâce à des recherches intenses de plusieurs équipes des spécialistes. Ainsi, il a été établi à la fois par des travaux au laboratoire et sur le terrain que les poissons exposés à l'eau acide manifestent, entre autres, des problèmes fonctionels au niveau de:

-l'osmo-régulation[1] (perte des ions, désequilibre acido-basique)

-la réproduction[2] (développement des oeufs et survie des jeunes, déformation des embryons)

-la réspiration (échanges gazeux[1], déformation des branchies[3,4])

-la croissance (croissance des os[5], métabolisme).

En dépit de ces données sur les changements pathologiques qui sont reliés à l'acidité chez les poissons, il est difficile de

1.démontrer des rélations de cause à effet entre l'acidité et des problèmes spécifiques de santé dans le milieu naturel où plusieurs facteurs environnementaux agissent simultanément

2.détecter ces problèmes dans un stade précoce, avant que les dommages grâves ou même irréversibles ne se développent.

Donc, il y a un intérêt à établir des indicateurs de stress causé par l'acidité qui soient spécifiques, sensibles et utilisables en le milieu naturel.

Hypothèse de travail.

Nous avons formulé une hypothèse qui pourrait permettre de développer ces indicateurs de stress. Il est bien établi qu'une exposition à un milieu acide résulte en un déséquilibre ionique interne, dû surtout à une perte de Na et Cl au niveau des branchies. Le système endocrinien qui produit les hormones osmorégulatrices fait partie

intégrante des mécanismes homéostasiques qui s'activent dans la réponse de l'organisme pour maintenir constants les paramètres osmotiques internes du poisson façe aux agressions du milieu externe,comme l'acidité. Cette activation du système endocrinien osmorégulateur pourrait être mise en évidence à des niveaux sous-létales d'exposition à l'acidité,soit au stade même de l'ajustement physiologique au cours lequel le milieu interne résiste efficacement aux perturbations , grâçe aux efforts homéostasiques intenses. Donc, l'on peut conçevoir que les changements de l'activité endocrine du système osmo-régulateur puisse être utilisés comme indicateurs de stress causé par l'acidité.

Les deux hormones osmorégulatrices que nous avons étudiées à ce jour sont la vasotocine, hormone neurohypophysaire, et les urotensines I et II, des hormones du système caudal neurosécreteur (l'urophyse). Les deux hormones ont des effets reconnus sur la régulation de transport ionique à travers des membranes osmoeffectrices, l'équilibre hydrique et aussi,sur la vasoconstriction au niveau des branchies.

METHODES

Nous avons étudié des ombles de fontaine soumis à des conditions contrôlées de pH et des teneurs d'aluminium total, au laboratoire. Aussi, nous avons analysé des poissons des lacs naturels acidifiés aux caractéristiques physico-chimiques définies. Les organes endocriniens ainsi que le sang, les branchies et plusiuers autres organes ont été prelévés. L'activité des systèmes endocriniens producteurs de la vasotocine et des urotensines a été évaluée par des dosages radioimmunologiques[6,7] ou la histologie morphométrique[8]. Les concentrations des ions plasmatiques a été mésuré par absorption atomique et la morphologie des branchies étudiée au microscope optique et éléctronique.

RESULTATS

Dans une étude sur le terrain, les poissons provenant de lacs à pH acide et de lacs à pH neutre ont été comparés. Il a été montré que la synthèse et la libération des urotensines dans le système caudal neurosecréteur sont stimulés chez les poissons des eaux acidifées. Ces poissons démontraient aussi des changements histopathologiques dans les branchies. Dans une étude au laboratoire, les poissons ont été soumis aux conditions contrôlées de pH et d'aluminium et les niveaux d'urotensines ont été mesurés. Nous avons détecté une corrélation entre les niveaux d'urotensine I et le pH,mais pas les niveaux d'aluminium (Figure 1). Par contre, une corrélation entre l'urotensine II et l'aluminium mais, non le pH, a été détecté . Donc les urotensines semblent être prometeur comme indicateurs de stress causé par l'acidité et peut-être même pour differençier l'effet du pH et celui d'aluminium. Des expériences parallèles au laboratoire ont permis de déterminer des niveaux de vasotocine dans l'hypophyse et l'hypothalame (Figure 2), la partie du cerveau où cette hormone est synthétisée. Une activation de la sécrétion de la vasotocine a été observée, en corrélation avec des faibles diminutions des niveaux de Na plasmatique chez les poissons soumis aux conditions sous-létales d'acidité.

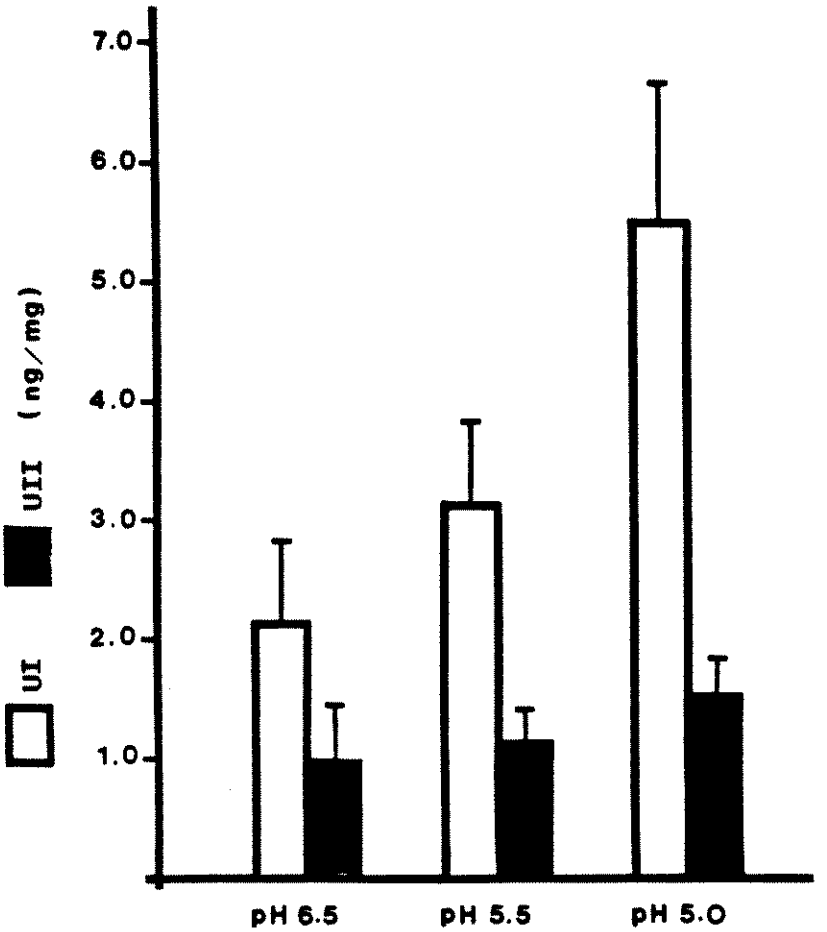


Figure 1. Concentrations d'urotensine I (UI) et d'urotensine II (UII) dans le système caudal neurosecréteur de S.fontinalis exposés à des milieux de pH 6.5, 5.5 ou 5.0. Une corrélation négative entre UI et le pH est notée ($t=2.45, p<0.05$), alors que UII et le pH ne sont pas corrélés d'une façon significative.

CONCLUSION

Nos résultats indiquent que les variations de l'activité du système endocrinien osmo-régulateur du poisson pourraient être utilisés pour la détection du stress acide à des niveaux sous-létales dans le milieu naturel et pour l'évaluation de l'intensité de ce stress. Considérés avec d'autres paramètres physiologiques, comme les concentrations ioniques sanguines, l'hématocrite et la morphologie des branchies, les paramètres endocriniens pourraient devenir des indicateurs diagnostiques d'une valeur significative.

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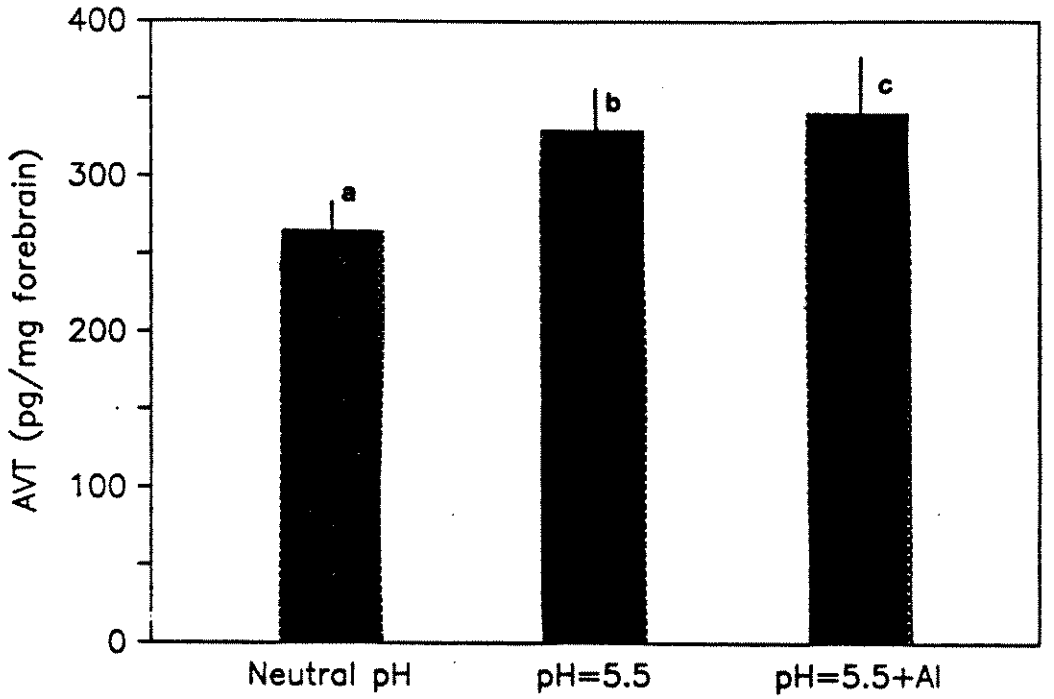

Figure 2. Concentrations de la vasotocine (AVT) dans l'hypothalame de S.fontinalis dans le milieu à pH 6.5 ou 5.5 avec 0 ou 500 ug Al/L ajouté. a<b,c ($p<0.05$).

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TOXICITY OF FLUORO-AL COMPLEXES (ALF_x) TO JUVENILE ATLANTIC SALMON (Salmo salar)

K. Wilkinson, P.G.C. Campbell and P. Couture
INRS-Eau, Université du Québec
C.P. 7500, Ste-Foy (QC) G1V 4C7.

ABSTRACT

The effect of fluoride complexation on aluminum toxicity towards juvenile Atlantic salmon (Salmo salar; age 1+) was evaluated in a series of soft water experiments. By selectively varying the concentrations of either aluminum (0-12 µM), fluoride (0-20 µM) or both ions, we were able to determine the effect of aluminum-fluoride complexation at pH 4.5 and 4.9. Using lethal indicators (LLC, cumulative mortality), we could demonstrate the attenuating effect of aluminum-fluoride complexation on the toxicity of inorganic Al toward Salmo salar. Despite this attenuation, residual toxicity in the presence of fluoride ion is greater than can be explained by the concentrations of the free Al^{3+} ion and its hydroxo-complexes. Possible explanations for these observations are discussed.

RÉSUMÉ

Réalisées dans des conditions similaires à celles rencontrées dans les eaux courantes sur le Bouclier canadien au printemps, nos expériences ont pour but d'expliquer l'effet de la complexation de l'aluminium par des fluorures sur sa toxicité envers le saumon atlantique (Salmo salar). Nous utilisons des tacons (âge 1+) dans une série d'expériences en système confiné avec renouvellement de l'eau deux fois par jour; les concentrations des ions majeurs sont maintenues constantes de même que le pH (4,5 ou 4,9) et la température (10°C) et ce, tout en variant les concentrations d'aluminium (0-12 µM) et/ou de fluorures (0-20 µM). En suivant des paramètres léthaux (CL-minimum; mortalité cumulative), on peut démontrer que la complexation de l'aluminium inorganique par les fluorures réduit sa toxicité. Cependant, cette atténuation s'avère moins importante que celle prédite; en effet, la toxicité résiduelle de l'aluminium en présence de fluorures dépasse celle qui aurait été anticipée en fonction des concentrations de l'ion libre, $[Al^{3+}]$, et de ses hydroxo-complexes, $[Al(OH)_n]$. Des explications possibles de ces observations sont discutées.

INTRODUCTION

One of the most frequently documented consequences of acid precipitation is certainly the geochemical mobilization of aluminum. The soft waters of the Canadian Shield with their weak buffering capacity are particularly susceptible to any fluctuation in acidic inputs. A drop in pH (especially during snowmelt) leads not only to an important increase in the dissolved aluminum concentrations but also to a modification in the speciation of this element. In their studies of a small New England stream, Driscoll et al. (1980) noted an increased contribution of AlF_x complexes during this critical period of low pH. For several salmon rivers on the North Shore of the Gulf of Saint-Lawrence, Hansen and Campbell (1987), also using chemical equilibrium calculations, reported that AlF_x species could represent up to 60%

of the dissolved inorganic aluminum during the snowmelt period.

Despite the obvious geochemical importance of aluminum-fluoride complexes, there is very little literature available with regard to their toxicity to aquatic biota. Among the available data, there is no consensus concerning the bioavailability of AlF_x (Driscoll et al., 1980; Clark and LaZerte, 1985; Cameron et al., 1986). Our experiments were designed to elucidate the effects of AlF_x on juvenile Atlantic salmon (Salmo salar; age 1+). By choosing a soft water medium ([Ca], [Mg], [Na] constant) and by selectively varying the concentrations of either aluminum, fluoride, or both ions, we were able to determine the effect of aluminium fluoride complexation at pH 4.5 and 4.9, while maintaining physicochemical conditions that closely ressemble those of Canadian Shield waters.

Using lethal indicators (cumulative mortality; least lethal concentration, LLC), we examine the effect of aluminum fluoride complexation on the toxicity of inorganic Al toward Salmo salar and test the hypothesis that toxicity of Al in the presence of fluoride ions can be explained by the free-ion model of metal toxicity (Morel, 1983; Pagenkopf, 1983).

METHODS AND MATERIALS

Juvenile Atlantic salmon (Salmo salar; age 1+) were exposed to various aluminum, fluoride, and hydrogen-ion concentrations (Table 1) in a series of semi-static experiments (water renewal every 12 hours). Fish (8-10 per basin) were exposed to synthetic Shield water (Van Coillie et al., 1983) during a two-day progressive acidification-acclimation period, after which Al-containing test waters (with or without fluoride) were employed. The bassins were kept at 9-11°C in a enclosed cold room with a fluorescent 16-hour photoperiod.

The synthetic media were allowed to age 24 hours prior to fish exposure. Nitric acid (Aristar grade) and sodium hydroxide were used to adjust the pH. An aluminum sulfate stock solution was added to the 64 L bassins to obtain the desired Al levels in the exposure media. To avoid organic complexation of the aluminum, the fish were not fed after transfer to the acclimation bassins.

Water aluminum concentrations were determined by graphite furnace atomic absorption spectrophotometry (total Al) or by an automated pyrocatechol-violet method (total dissolved Al, dissolved inorganic and dissolved organic bound Al; Rogeberg and Henriksen, 1985). The distribution of Al among the different inorganic monomeric forms was calculated from the results for dissolved inorganic monomeric Al, pH, and total fluoride, with the aid of the MINEQL chemical equilibrium model (Westall et al., 1976).

Major ion concentrations in the synthetic media were determined by atomic emission spectrophotometry. Fluoride was determined potentiometrically with a fluoride selective electrode.

Table 1: Nominal experimental conditions.

EXPERIMENT	$[Al]_t$	$[F]_t$	pH	COMMENTS
A	CONSTANT (9 µM)	VARIABLE (0-12 µM)	4.5	
B	VARIABLE (0-11 µM)	NIL	4.5	
C	VARIABLE (0-11 µM)	CONSTANT (18 µM)	4.5	
D	VARIABLE (0-12 µM)	VARIABLE (0-11 µM)	4.5	Σ $Al(OH)_n$ + Al^{3+} = CTE = 2.8 µM
E	VARIABLE (0-12 µM)	VARIABLE (0-11 µM)	4.9	Σ $Al(OH)_n$ + Al^{3+} = CTE = 2.8 µM

RESULTS

Experimental conditions (pH, $[Al]_t$, $[F]_t$: Table 1) were chosen to avoid exceeding the solubility limit for aluminum hydroxide ($Al(OH)_3(s) = Al^{3+} + 3\ OH^-$; $\log K_{sp} = -32.65$) and to minimize the problems of slow Al precipitation that have plagued earlier experiments on Al toxicity (Burrows, 1977). Indeed, 85-100 % of the Al added to the exposure media could be accounted for as dissolved inorganic monomeric Al, as determined by the ion-exchange/catechol violet procedure of Rogeberg and Henriksen (1985).

Results from experiment A show that fluoride complexation attenuates Al toxicity to juvenile Atlantic salmon; as the fluoride concentration is increased (0 to 12 µM) for a fixed aluminum concentration (9 µM), salmon mortality decreases (Fig. 1). Just such a qualitative response, i.e. diminished toxicity as the concentration of Al^{3+} is reduced, would be predicted from the free-ion model for metal toxicity. Indeed, speciation calculations on the test solutions indicate that the free-ion concentration decreases from 6.1 µM (68 % of the total inorganic species) to 0.4 µM (5% of the total) as the fluoride concentration increases. However, when the lowest lethal concentrations of Al^{3+} are compared among experiments A, C (with F^-) and B (without F^-), we note that the LLC values are much lower in the presence of fluoride (Table 2), suggesting that fluoro-Al species must have some residual toxicity.

Table 2: Lowest lethal concentrations of aluminum in the presence and absence of fluoride complexation (at pH 4.5).

EXPERIMENT	$[F]_t$ (μM)	LEAST LETHAL CONCENTRATION (expressed as μmol $[Al^{3+}] \cdot L$)
B	0	2.3
A	0-12	1.2
C	18	0.03

Experiments D and E were designed to test this idea. Here, using equilibrium calculations, we were able to keep the concentration of the free Al^{3+} ion and its hydroxy-complexes constant while increasing both the aluminum and fluoride concentrations (i.e., $[AlF_x]$ increases, $[Al^{3+}]$ and $[Al(OH)_n]$ remain constant). Implicit in this approach is the assumption that equilibrium conditions exist in the exposure media and that the concentration of aluminum in solution remains constant. The equilibrium assumption is acceptable provided the solubility limit for $Al(OH)_3(s)$ is not exceeded; equilibrium among the various monomeric aluminum species (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, AlF^{2+}, ...) is known to be attained rapidly (Baes and Mesmer, 1976; Plankey et al., 1986). As well, the frequent water renewal (twice daily) assured a reasonably stable total aluminum concentration. We can thus assume that the fish in experiments D and E were exposed to constant Al^{3+} and $Al(OH)_n$ concentrations but increasing levels of $[AlF_x]$. If fluoro-Al species do not contribute to aluminum toxicity, the cumulative mortality curves should be independent of the fluoride concentrations. As can be seen in Figures 2A (pH 4.9) and 2B (pH 4.5), this is clearly not the case. The increase in mortality as AlF_x concentrations increase is substantial proof of the toxicity of AlF_x species. Note also that there is an apparent pH effect; for comparable concentrations of Al^{3+}, $Al(OH)_n$ and AlF_x, mortality would appear to occur earlier at the higher pH.

DISCUSSION

To elicit a biological response from the test fish, aluminum must obviously interact with a biological (gill) membrane. For other metals such as Cd, Cu or Zn, this interaction with the gill surface has been represented in terms of the formation of M-L-gill surface complexes (Pagenkopf, 1983), where L-gill = a cellular ligand

$$M^{z+} + \text{L-gill} = M^{z+}\text{-L-gill}$$

and the biological response is proportional to the concentration of surface complexes, $[M^{z+}\text{-L-gill}]$.

$$\text{biological response} \propto [M^{z+}\text{-L-gill}] = K\,[\text{L-gill}]\,[M^{z+}]$$

By analogy with this approach, which has proved successful in explaining the toxicity of metals both to fish (Pagenkopf, 1983) and to other aquatic organisms (Morel, 1983), one would expect $[Al^{3+}]$ to be the best predictor of aluminum toxicity.

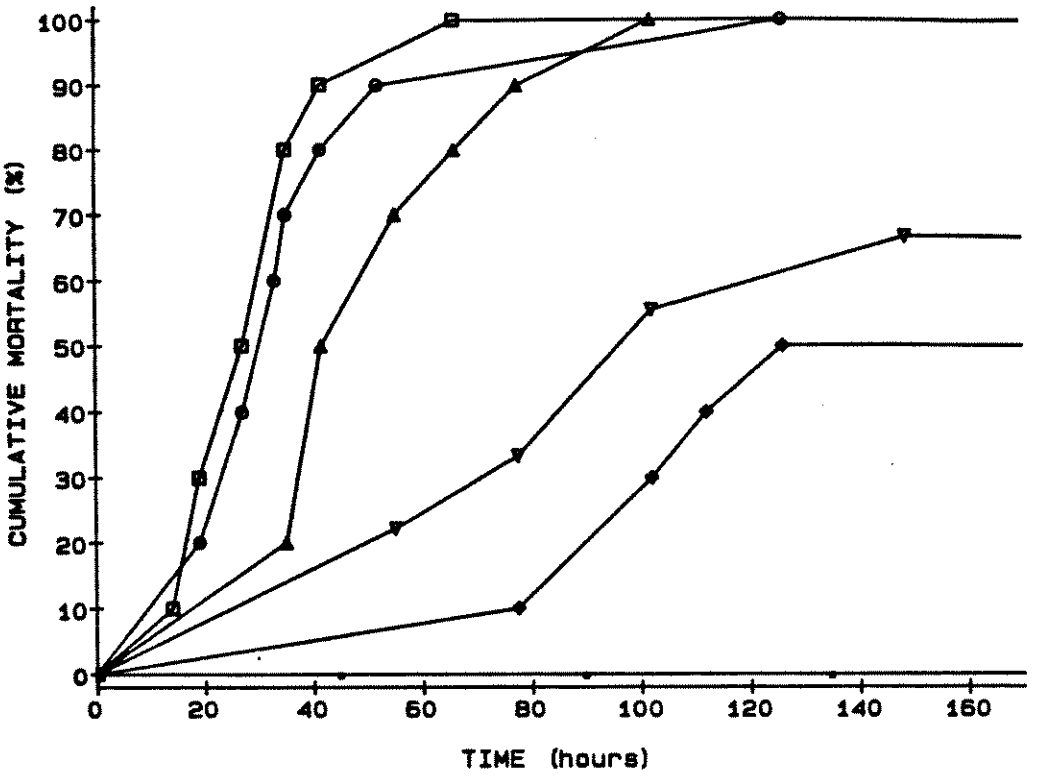

Figure 1: Cumulative mortality of Salmo salar at pH = 4.5 and [Al] = 9 µM: (○) 0.7 µM F; (□) 2.7 µM F; (Δ) 5.3 µM F; (◇) 7.7 µM F; (▽) 9 µM F; (•) 12.1 µM F.

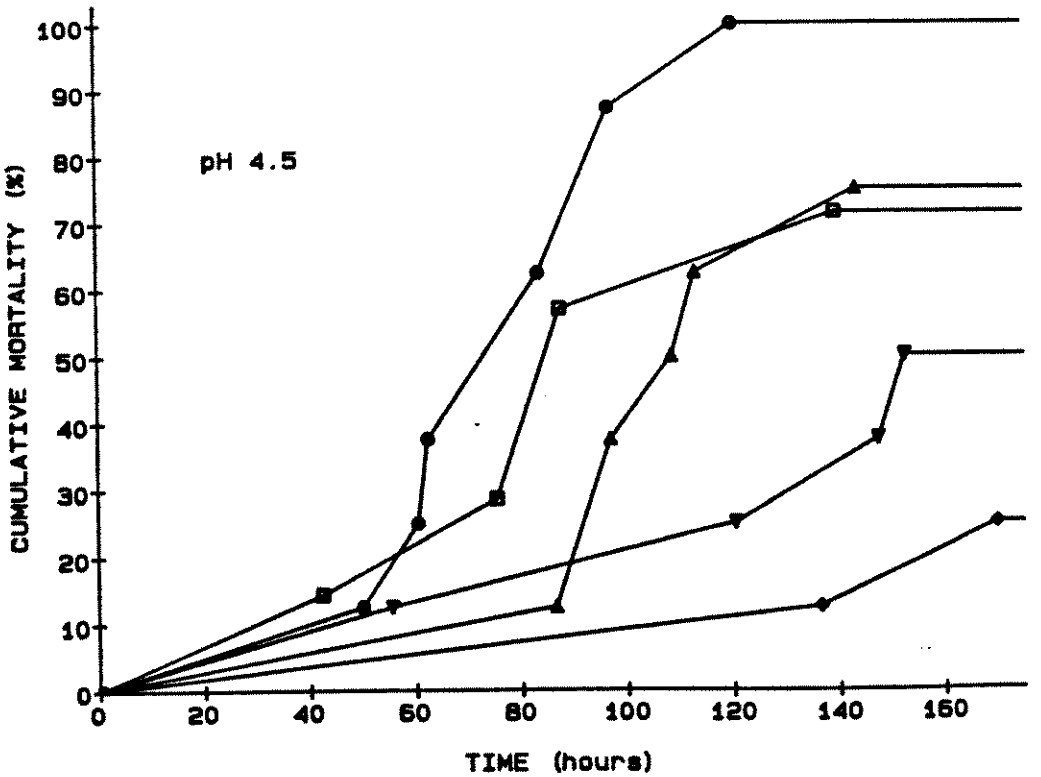

Figure 2A: Cumulative mortality of Salmo salar at pH = 4.5 and Σ $[Al^{3+}]$ + $[Al(OH)_n]$ = 2.8 µM: (○) 9.0 µM AlF_x; (□) 6.9 µM AlF_x; (Δ) 5.0 µM AlF_x; (▽) 4.0 µM AlF_x; (◇) 3.0 µM AlF_x.

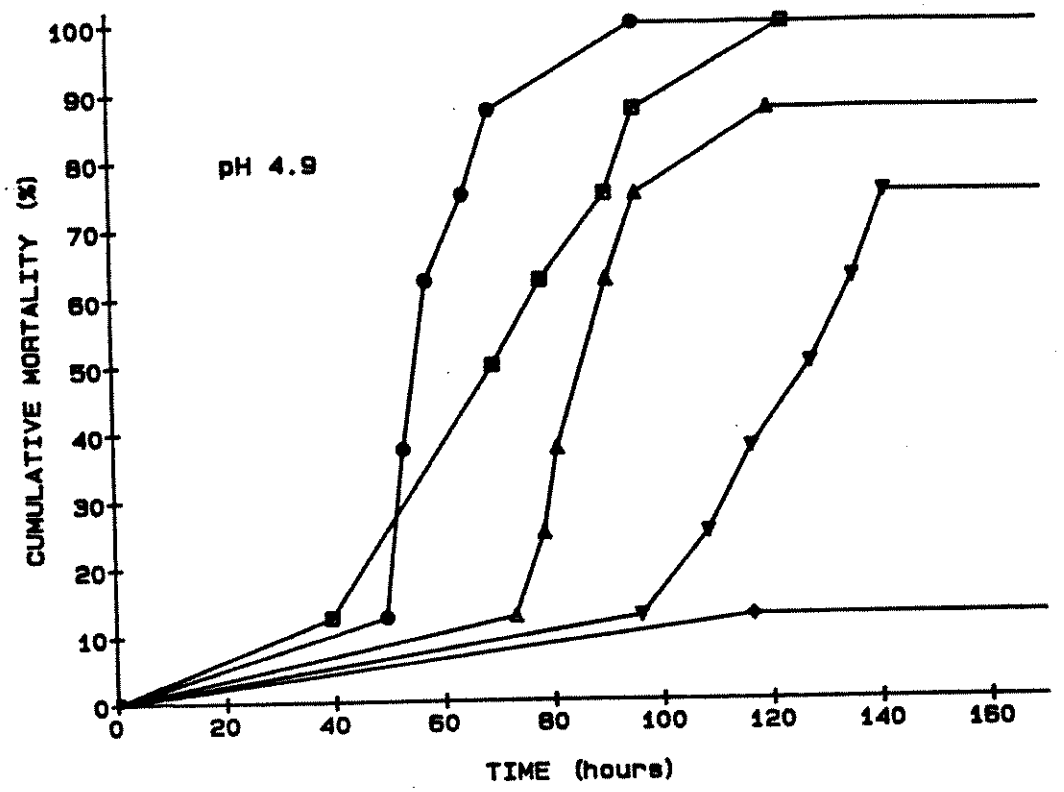

Figure 2B: Cumulative mortality of Salmo salar at pH = 4.9 and $\Sigma[Al^{3+}]$ + $[Al(O)_n]$ = 2.8 µM: (○) 9.0 µM AlF_x; (□) 7.0 µM AlF_x; (Δ) 4.9 µm AlF_x; (▽) 3.0 µM AlF_x; (◇) 1.0 µM AlF_x.

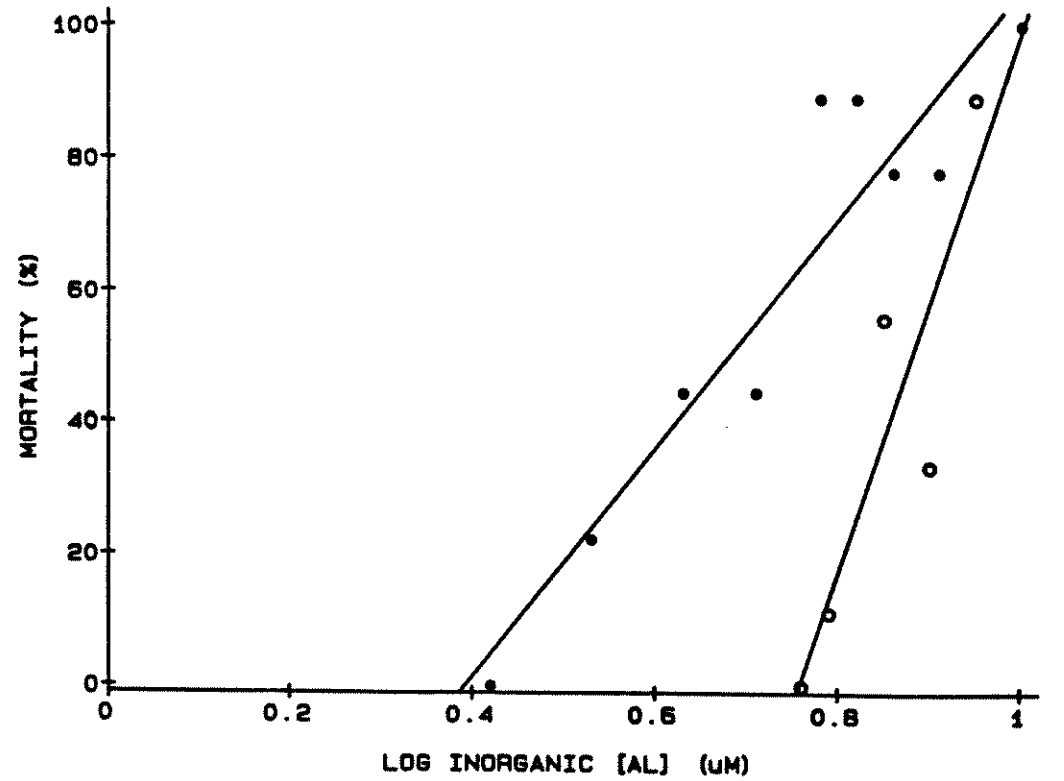

Figure 3: Toxicity curves - mortality as a function of inorganic aluminum (log) for experiment B (•: no fluoride) and experiment C (O: 18 µM fluoride).

The results of the present research indicate that the simple free-ion model of metal toxicity is not sufficient to explain aluminum toxicity towards juvenile Atlantic salmon. When the results from different experiments are pooled and compared, fish mortality is not proportional to the Al^{3+} concentration (Figs. 2A and 2B), nor is it a unique function of the total concentration of inorganic aluminum (Fig.3). It appears that the best predictor of fish mortality will be the concentration of several (but not all) Al species in solution (e.g., $\Sigma\ AlOH^{2+} + AlF^{2+}$).

There is a natural (but regretable!) tendency to interpret this type of result as an indication that these species are the "toxic species" or "bioavailable forms" of aluminum in solution (e.g., Helliwell et al., 1983). Such an interpretation is clearly incompatible with current equilibrium models of metal ion toxicity (Morel, 1983). Since equilibrium among the various monomeric aluminum species (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, AlF^{2+}, ...) is rapid (Baes and Mesmer, 1976; Plankey et al., 1986), and since they are all charged, hydrophilic species unlikely to pass biological membranes by simple diffusion, no one form can be considered more or less available for equilibration with binding sites on the membrane (Neville and Campbell, 1988). A more realistic approach to the present results is to focus on the Al species that exist not in solution, but rather at the gill membrane. If mixed ligand complexes form at the membrane surface

$$Al^{3+} + \text{L-gill} = Al^{3+}\text{-L-gill}$$

$$Al^{3+}\text{-L-gill} + Y = Y\text{-}Al^{X+}\text{-L-gill}$$

(where $Y = HO^-$ or F^-), and if these surface complexes are responsible for the toxic response, then one would observe an apparent dependence of mortality on the concentrations of AlY^{2+} in solution. The existence of similar mixed hydroxo species, HO-Al-L_2, has been postulated for in vitro systems containing aluminum and such ligands as salicylate, pyrocatechol and 1,2-dihydroxynaphthalene-4-sulfonate (Marklund et al., 1986), and it seems reasonable to consider their possible existence at biological interfaces. Further experiments will be needed to test this mechanism of aluminum toxicity.

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THIRD SESSION/TROISIEME SEANCE

MICRO-BIOASSAY IN AQUATIC TOXICOLOGY/ MICROBIOESSAIS EN TOXICOLOGIE AQUATIQUE

CHAIRPERSON: Dr. J. de la Noüe, GREREBA, Université Laval
Ste-Foy, PQ
MODERATOR: Dr. B. Pinel-Alloul, Département des sciences
biologiques, Université de Montréal, Montréal, PQ

EFFETS STIMULATEURS ET INHIBITEURS DE *PSEUDOMONAS* SUR LA CROISSANCE DE MICRO-ALGUES

A. DAKHAMA, J. DE LA NOÜE et M.C. LAVOIE, Groupe de Recherche en Recyclage Biologique et Aquiculture (GREREBA), Faculté des Sciences et de Génie, Université Laval, Ste Foy (Qc) G1K 7P4

RESUME

Les effets stimulateurs ou inhibiteurs de *Pseudomonas* sur la croissance de micro-algues ont été étudiés au laboratoire sous conditions contrôlées. Trois souches de *Pseudomonas* ont été isolées à partir de cultures d'algues au laboratoire. *P. diminuta* et *P.vesicularis* sont capables de stimuler la croissance de certaines micro-algues. En présence de *P. diminuta*, et en l'absence de tout apport de carbone organique exogène, la phase de croissance de *Scenedesmus ecornis* se prolonge nettement par rapport au témoin axénique. *P. aeruginosa* exerce une forte inhibition, liée à la toxicité de ses produits d'excrétion pigmentés. Le niveau d'inhibition semble varier selon l'espèce d'algue et montre une corrélation avec la concentration des inocula d'algues et de bactéries.

L'existence de telles interactions suggère que la croissance et la composition des populations phytoplanctoniques peuvent être contrôlées par la qualité et la quantité de la flore bactérienne associée.

INTRODUCTION

Dans leur habitat, les micro-algues sont toujours accompagnées de bactéries. Une telle association implique nécessairement des interactions entre ces deux types de micro-organismes(1-3). Les micro-algues sont une importante source de carbone organique (4,5) profitable aux bactéries hétérotrophes(6-9). En retour, ces dernières sont souvent responsables de la prolifération des populations phytoplanctoniques dans les milieux chargés en matière organique(10-12). La qualité de la flore bactérienne peut être considérée comme un facteur de contrôle de la croissance des micro-algues (13).

Cependant, peu d'informations sont disponibles sur la nature et la spécificité de telles interactions. Les objectifs de ce travail sont donc de rechercher des interactions spécifiques entre ces micro-organismes, d'en établir la nature, grâce à des méthodes qualitatives et quantitatives, d'examiner l'influence des bactéries hétérotrophes sur la croissance des micro-algues et d'évaluer leur rôle possible dans le maintien et la diversité des populations phytoplanctoniques.

MATERIELS ET METHODES

Les micro-algues utilisées ont été maintenues au laboratoire en cultures monospécifiques, sur milieu artificiel stérile, renouvelé périodiquement. *Scenedesmus ecornis* a été rendue axénique par repiquages successifs sur milieu gélosé. *Pseudomonas aeruginosa* a été isolée à partir d'une culture de *Anabaena sp.*, alors

que *P. vesicularis* et *P. diminuta* ont été isolées des cultures de *S. ecornis* et de *S. obliquus* .

La figure 1 résume les méthodes développées pour étudier l'interaction entre les deux groupes de micro-organismes utilisés.

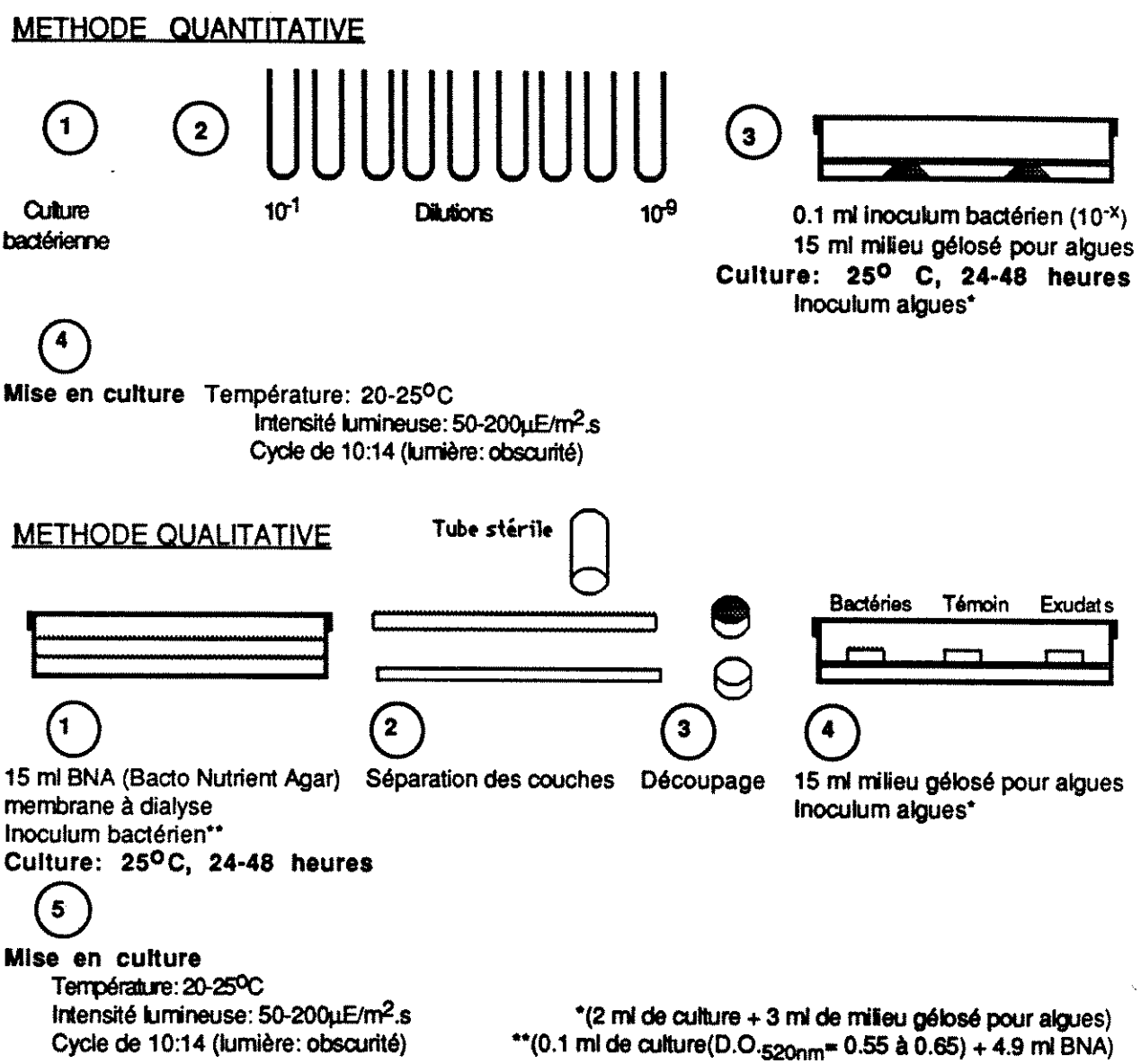

Figure 1: Méthodes quantitative et qualitative employées pour étudier l'interaction entre micro-algues et bactéries.

La méthode quantitative permet d'estimer le degré de stimulation ou d'inhibition en fonction des quantités d'algues et de bactéries utilisées. La méthode qualitative sert à déterminer certaines caractéristiques, physiques (thermosensibilité et taille moléculaire) et chimiques (sensibilité aux enzymes) de l'interaction.

Une stimulation se manifeste par le développement d'une zone de croissance

intense au contact des bactéries ou leurs exudats avec le tapis d'algues. Inversement, une inhibition se traduit par l'apparition d'une zone claire à ce niveau.

La thermosensibilité des substances inhibitrices a été évaluée après traitement à la température (95°C durant 15, 30 et 60 minutes).

La nature biochimique de ces substances a été recherchée par l'étude de la sensibilité aux enzymes. Des solutions enzymatiques, préparées dans du tampon phosphate 0.01M à pH 7.0, ont été activées pendant une heure à 37°C et incorporées dans une gélose molle, qui est ensuite coulée sur un tapis d'algues. Les mêmes solutions enzymatiques, inactivées à la chaleur, ont servi de témoins.

Enfin, une culture mixte de *S. ecornis* et de *P. diminuta* a été examinée en milieu liquide. La croissance a été évaluée par dénombrement d'algues et de bactéries viables.

RESULTATS

Comme l'indique le tableau 1, les espèces d'algues utilisées varient dans leur réponse aux trois types de bactéries. *Pseudomonas aeruginosa* inhibe la croissance de la plupart des algues, mais ne montre aucun effet sur la croissance de *Chlorella vulgaris*, *Anabaena sp.* et *Cylindrospermum sp.* . Les deux autres souches de bactéries stimulent la croissance de toutes les algues à l'exception de *Cylindrospermum sp.* et de *Phormidium bohneri* .

Tableau 1: Effet de *Pseudomonas* sur la croissance de micro-algues. (---;--;-)=inhibition forte; moyenne; faible. 0= aucun effet. (++;+)=stimulation moyenne; faible.

Micro-algues	*P. aeruginosa*	*P. diminuta*	*P. vesicularis*
Ankistrodesmus falcatus	---	+	+
Chlorella sp.	---	+	+
Chlorella vulgaris	0	+	+
Gloeocystys sp.	--	+	++
Hormidium flaccidum	--	+	++
Scenedesmus ecornis	---	+	+
Scenedesmus obliquus	---	+	+
Scenedesmus obliquus CCAP	---	+	++
Anabaena sp.	0	+	+
Cylindrospermum sp.	0	0	0
Oscillatoria agardhii	--	++	++
Phormidium bohneri	-	0	0
Spirulina maxima	---	++	+

Le niveau d'interaction semble varier en fonction de la densité à la fois des algues et des bactéries (figure 2.).

En présence de *P. diminuta* et en l'absence de tout apport de carbone organique exogène, le taux de division de *Scenedesmus ecornis* se maintient et la phase de croissance se prolonge nettement par rapport au témoin axénique (figure 3). Cette extension de la période de croissance est obtenue après la phase de déclin de la

population bactérienne. Ces observations semblent indiquer que les bactéries hétérotrophes peuvent contribuer au maintien de la population phytoplanctonique.

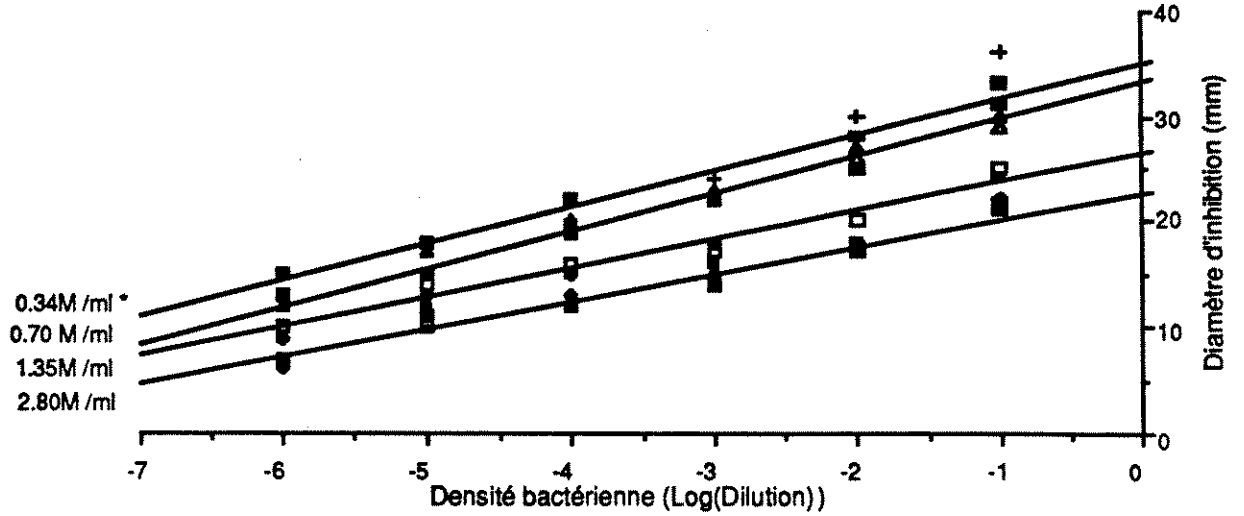

Figure 2: Variation du diamètre d'inhibition en fonction de la densité bactérienne (*P.aeruginosa*) pour différents inoculas d'algues (*S.obliquus* CCAP).
* : densité d'algues en millions/ml.

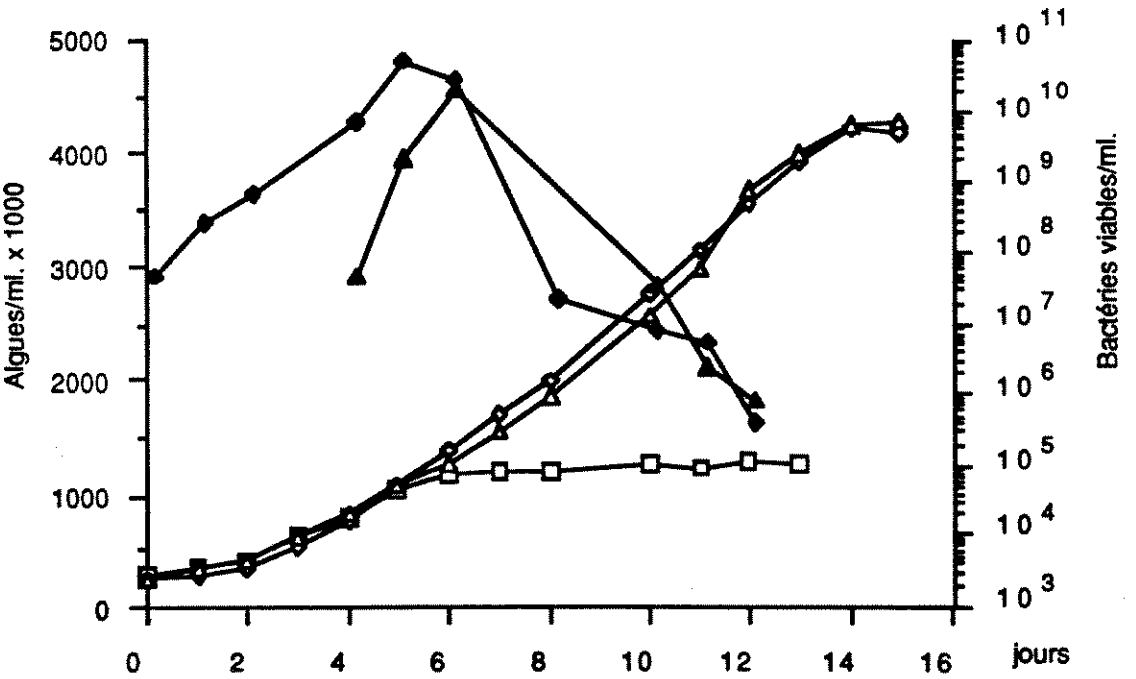

Figure 3: Effet de *Pseudomonas diminuta* sur la croissance de *Scenedesmus ecornis*.
□ *S. ecornis* en culture axénique.
◇ △ *S. ecornis* en culture mixte.
◆ ▲ *P. diminuta* en culture mixte.

Les premières données sur la nature des substances inhibitrices produites par *Pseudomonas aeruginosa* montrent que celles-ci ont un poids moléculaire inférieur à

1000, sont thermostables, sont extractibles à l'éthanol et au chloroforme et résistent à toutes les enzymes utilisées (protéase, protéinase K, carboxypeptidase, trypsine, ∝-chymotrypsine, pepsine, pectinase, ∝-amylase, lipase, phospholipase, ribonucléase A, désoxyribonucléase 1, catalase).

Une chromatographie,sur papier Whatman 3MM, de l'extrait ethanolique a été réalisée avec le mélange solvant (25%), isopropanol : eau : ammoniaque (80:15:5.). Après migration, des bandes découpées dans le chromatogramme ont été reportées sur une culture de *Scenedesmus obliquus* en milieu solide.

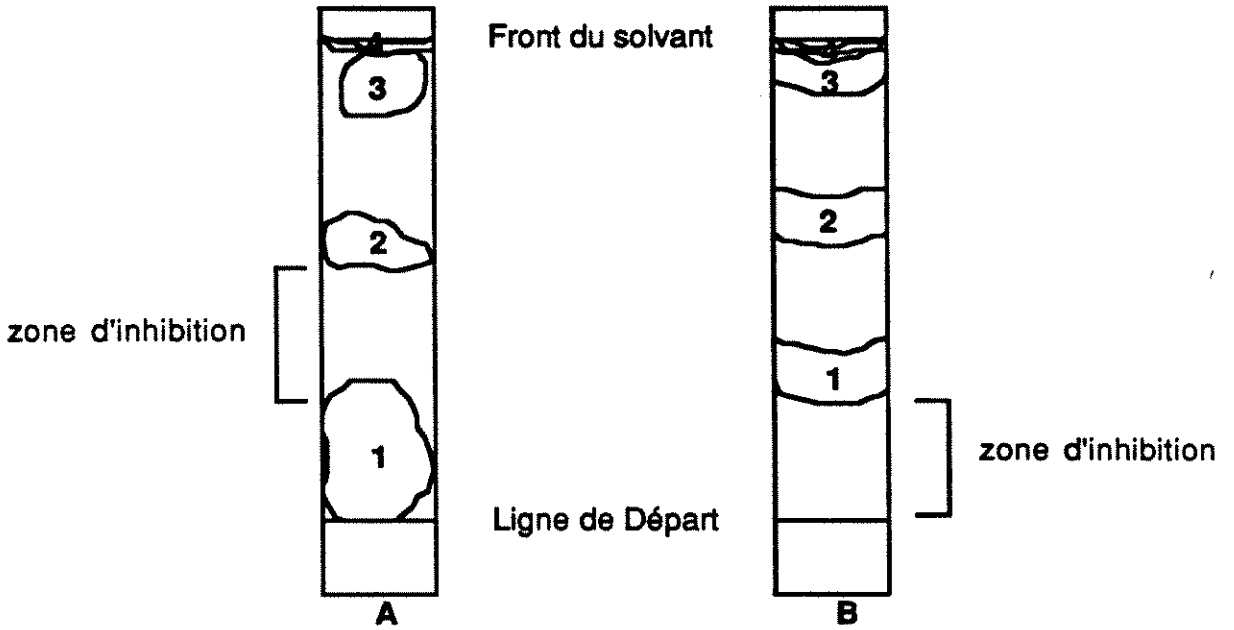

Figure 4: Chromatographie sur papier Whatman 3MM de l'extrait éthanolique des exudats pigmentés de *P. aeruginosa* (P.M. inférieur à 1000) lyophilisé et repris dans de l'éthanol 95% (A) ou du chloroforme (B).Solvant (25%) : isopropanol:eau:ammoniaque (80:15:5).

Les zones d'inhibition reportées sur le chromatogramme (figure 4) indiquent la présence d'au moins deux substances inhibitrices. Des analyses complémentaires sont actuellement entreprises dans le but de déterminer la nature exacte de ces substances, qui pourraient trouver une utilisation pratique dans l'élimination des algues indésirables dans les bassins d'eau.

DISCUSSION

Il est clairement admis que la croissance des micro-algues peut être influencée aussi bien par les facteurs physico-chimiques que par les facteurs biotiques. L'étude des relations biotiques est d'un grand intérêt pour la compréhension de la dynamique des populations phytoplanctoniques. Les observations effectuées dans ce travail montrent que la croissance des micro-algues peut être modifiée par la qualité et la quantité de la flore bactérienne associée. La variation de la réponse des algues à l'action des bactéries hétérotrophes pourrait avoir une influence sur la succession et la dominance des populations phytoplanctoniques. Il est parfaitement évident que, dans le milieu naturel, les interactions entre ces deux types de micro-organismes sont d'une grande complexité, car elles font intervenir plusieurs espèces simultanément et dépendent de

nombreux facteurs. Une étude expérimentale, au laboratoire, est donc nécessaire pour expliquer les mécanismes de ces interactions et leur nature. La stimulation de la croissance des algues par les bactéries hétérotrophes pourrait s'expliquer par la libération de facteurs de croissance(14,15), de substrats azotés issus de la décomposition de la matière organique du milieu (16), par apport de CO_2 (17,18) ou encore par la dégradation d'auto-inhibiteurs(19) ou de métabolites autotoxiques produits par les algues(20). Les résultats de la figure 3 indiquent que *P. diminuta* est probablement capable de métaboliser les produits extracellulaires de *S. ecornis* et de contribuer à sa croissance, possiblement par production de CO_2, de facteurs de croissance ou par décomposition d'autoinhibiteurs.

Les bactéries sont également capables d'inhiber la croissance des algues par compétition pour des nutriments inorganiques(21) ou par libération de substances antialgales(22). L'inhibition exercée par *P. aeruginosa* apparaît liée à la toxicité de ses produits d'excrétion pigmentés (figure 4) et ne résulte pas de phénomènes de compétition.

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A MULTIPARAMETER TOXICITY TEST USING SALMONELLA TYPHIMURIUM

by

Gregory E. Kupillas and Robert G. Arnold

ABSTRACT

A battery of measurements based on the physiological responses of S. typhimurium to chemical toxicants provided both a measure of relative toxicity and an indication of the biochemical source of chemical effects. Prior to its application to a series of chlorinated phenols, the test battery was calibrated using chemicals (CN^-; 2,4-DNP; chloramphenicol) for which a toxic mechanism has been clearly established. Parameters devised to serve as measures of relative toxicity among the chlorinated phenols correlate well with acute fish toxicity and chemical octanol/water partition data. At relatively low chemical concentrations, chlorinated phenols appear to inhibit energy generation in the test species. At high concentrations, inhibition of biosynthetic functions seems to overshadow catabolic effects. The microbial test battery may be an economically attractive alternative to toxicity tests involving marine animals, particularly if a more sensitive test microorganism is selected.

INTRODUCTION

Fish bioassay procedures exhibit great sensitivity to a broad range of contaminants (4, 5, 8) and have been relied upon for the development of waste-discharge regulations. Unfortunately aquatic fish-toxicity experiments suffer from a number of impracticalities including (i) associated expense, (7, 6); (ii) 24- or 96-hour exposure period; and (iii) requirements for large volumes of test water.

A number of alternative bioassay procedures have been developed which can be divided into three main categories: those based on bacterial luminescence, measurement of viability among specific bacteria or groups of bacteria, and assays based on specific ecological effects (3, 6, 10, 11, 24). Because none of these procedures is without merit or disadvantage as an indicator of chemical toxicity, the utility of a battery of toxicity screening procedures has frequently been stressed.

Described here is a battery of biochemical measurements (growth and respiration rates, intracellular ATP concentrations) which, when performed simultaneously on a pure culture of Salmonella typhimurium, provide both an estimate of relative chemical toxicity and a broad indication of the mechanism for the toxic response. The utility of the test battery was established for chemicals whose inhibitory mechanisms are known (cyanide; 2,4-dinitrophenol; chloramphenicol). Test procedures were extended to establish the relative toxicities of a series of chlorinated phenols.

MATERIALS AND METHODS

S. typhimurium (TA98), one of the several mutant strains described by Maron and Ames (18), was used as tester organism. The strain has several mutations in addition to its histidine auxotrophy. The rfa mutation results in a defective lipopolysaccharide coat and sensitivity to toxicants which are otherwise denied access to internal inhibitory sites (1). The tester strain was grown in an undefined medium consisting of 8g/ℓ Difco-Bacto nutrient broth, 5 g/ℓ NaCl, pH_0 6.6, 37°C. Plates were prepared by adding 2% agar (Difco, Bacto) to the liquid medium.

Experiments were initiated by inoculating 200 mls of the autoclaved medium from a culture of S. typhimurium grown overnight at 37°C. Growth was monitored spectrophotometrically (λ=600; 1-cm pathlength) using a Perkin-Elmer Hitachi 200 spectrophotometer. Temperature and dissolved oxygen were maintained via agitation at 200 rpms in a Labline orbit shaker bath. When the culture reached early log growth (A_{600} = 0.05, cm^{-1}), 50-ml aliquots were transferred from the original culture into four 125-ml Erlenmeyer flasks. Three of the flasks received precalculated concentrations of the model inhibitors or phenolic compounds (Table 1). A chemical-free control was maintained in the fourth flask for measurement of the uninhibited growth rate and intracellular

ATP concentration or respiration rate. Toxicants which are insoluble in H_2O were added in an appropriate solvent. Non-toxicity of the solvent was established via separate experiment. Following the culture split, measurements of culture optical density were continued at approximately 15-minute intervals in all four flasks.

Dissolved oxygen concentration was continuously measured in 3-ml culture samples over a period of sufficient length to establish the steady rate of dissolved oxygen demand. Temperature was maintained at 37°C during measurement of dissolved oxygen demand. The rate of O_2 consumption by S. typhimurium was calculated from the slope of the time-dependent dissolved O_2 record.

Samples were quenched within 1-2 seconds of withdrawal in hot (>80°C) 0.025M hydroxymethylaminomethane (tris) buffer (J.T. Baker Chemicals) with 2 x 10^{-3} M EDTA (Allied Chem.) pH 7.75. Following cell lysis, the cell/buffer mixture was brought to room temperature, and ATP concentration was measured in a Turner luminometer, Model TD-20C, using a luciferase/luciferin kit (Sigma, FLE50).

RESULTS

Specific inhibitors

Growth curves developed in experiments involving specific inhibitors (cyanide; 2,4-DNP; and chloramphenicol) are provided as Figure 1(a-c). All the specific inhibitors caused a decrease in culture specific growth rate (k) at concentrations from 10^{-5} to 10^{-4} M. The effects of inhibitors on specific growth rate are summarized in Figure 2. Specific growth rate values indicated were extracted from the Figure 1 slopes, 30 minutes following chemical addition.

Oxygen-demand data were developed from continuous records of solution dissolved oxygen concentration are summarized in Figure 3(a-c). Reported rates were normalized for growth using contemporary measurements of culture optical density (values extracted from Figure 1). Following the introduction of cyanide or chloramphenicol, reduction of respiration rate was directly related to inhibitor concentration. However, when 2,4-DNP was added, S. typhimurium initially responded with an increase in respiration rate. At the highest concentration added ($5x10^{-4}$M), a modest decline in oxygen demand was apparent although the respiration rate still exceeded that of the control.

Normalized culture ATP levels are reported as a function of inhibitor dosage and identity in Figure 4(a-c). Values shown represent intracellular ATP levels 30 minutes following inhibitor addition. S. typhimurium responded to the introduction of cyanide with a reduction in intracellular ATP which was directly related to the total cyanide concentration. The intermediate concentration of 2,4-DNP (10^{-4}M) caused a severe, time-dependent drop in intracellular ATP level. No further drop in ATP was observed at the $5x10^{-4}$M 2,4-DNP concentration, however. Additions of chloramphenicol produced successive, time- and concentration-dependent increases in ATP concentration.

Phenolic compounds

Figures 5 through 8 illustrate the results of corresponding chemical toxicity studies which were identical in form to those involving the model chemical inhibitors. Growth inhibition experiments indicated that each of four compounds in the phenolic series was capable of reducing the specific growth rate in exponentially growing cultures of S. typhimurium (TA98). The sensitivity of growth rate to compounds in the series was directly related to their respective degree of halogenation. The response of culture respiration rate to increasing concentrations of inhibitor was similar for all compounds in the series -- inhibition of culture respiration was directly related to the concentration of halogenated phenolic compounds tested.

Results of intracellular ATP measurements were not as consistent. 2-chlorophenol produced a straightforward dose-response curve in which intracellular ATP concentration decreased with successive compound additions. The other three chlorinated phenols produced significant reduction of intracellular ATP at low concentrations, followed by staged recovery of cellular ATP at

successively higher chemical doses.

DISCUSSION

General

Cellular metabolic activities in aerobically growing S. typhimurium may be broadly classified as catabolic or biosynthetic in nature. Processes so divided are linked via intracellular pools of metabolites which are produced via catabolic activities and consumed, for the most part, during the synthesis of macromolecules required for growth. Hydrolysis of adenosine triphosphate (ATP), whose production is biochemically linked to exergonic reactions on the catabolic side, is the primary source of energy for biosynthesis; NAD(P)H, also produced by catabolism, serves as reductant for the stabilization of reduced, intracellular conditions in the midst of oxidizing, extracellular surroundings. Nicotinamide adenine dinucleotide (NADH) also plays an essential role as link between the soluble and membrane-dependent processes of catabolism.

For our purposes, it is necessary to separate the processes of proton retranslocation and oxidative phosphorylation from the remainder of the catabolic reactions, including electron transport. Accepting the tenets of Mitchell's chemiosmotic hypothesis (20), these catabolic subsets are linked by the cell's ability to maintain a transmembrane, protonmotive force. Cellular damage and/or solution chemistry which renders the cytoplasmic membrane permeable to protons or other cations will uncouple electron transport (and catabolic processes which precede it) from oxidative phosphorylation.

Based on the foregoing, cellular metabolism has been simplified to include three sets of reactions: (i) catabolism, excluding proton retranslocation for oxidative phosphorylation; (ii) oxidative phosphorylation; and (iii) biosynthetic reactions.

Specific (model) inhibitors

The activities of the model inhibitors used in this investigation are well established. Cyanide interferes with cellular respiration by binding to the cytochrome oxidase(s) of many aerobic bacteria, and blocks electron transfer to molecular oxygen (16, 12). There should follow a corresponding loss of ATP production, reduction of the intracellular ATP pool, and loss of available energy for biosynthesis. The anticipated effect of cyanide addition to an exponential-phase culture of S. typhimurium is reduction of respiration rate, intracellular ATP level, and specific growth rate.

2,4-DNP uncouples electron transport and oxidative phosphorylation by increasing the conductance of the cytoplasmic membrane to protons (12, 13 19), in effect, removing the catabolic engine from the driveshaft. Consequently, decreases in growth and ATP level would be expected in the presence of 2,4-DNP. However, apart from secondary metabolic effects arising from its addition, respiration rate should be unaffected or (given that oxidative phosphorylation or intracellular energy status controls the overall rate of bacterial catabolism) even increase following addition of the drug.

Chloramphenicol is a broad-spectrum bacteriostatic agent which binds to the 50S subunit of bacterial ribosomes to block protein synthesis, resulting in interference with general biosynthetic activity (21, 22). Because protein synthesis represents approximately 60 percent of the overall drain on cellular chemical energy (9), the intracellular ATP pool should increase in the presence of chloramphenicol. Since coupled electron transport must work against the intracellular ATP concentration and because general catabolic activity is regulated by cellular energy status (23), a decrease in oxygen demand should be expected.

The generalized bacterial responses to model inhibitors are summarized in Table 1. From the table, it is evident that the metabolic responses of S. typhimurium to model inhibitors paralleled predictions based on theory. The mixed bacterial reaction to 2,4-DNP indicates that uncoupling, which is apparent at low chemical doses, is overshadowed by secondary effects at a chemical concentration of $5x10^{-4}$M. The nature of the text battery response, i.e. monotonic decrease in specific growth rate, stabilization or partial recovery of ATP pool, and drop in the normalized respiration rate, suggests that concentrations of 2,4-DNP as high as $5x10^{-4}$M interfere with

biosynthetic activity as much or more than energy generation.

On the basis of experiments involving model inhibitors, it is apparent that the battery of microbial tests employed here is capable of descriminating among compounds whose primary mechanism of toxicity affects (i) catabolic processes ahead of oxidative phosphorylation, (ii) linkage between electron transport and oxidative phosphorylation (primarily membrane permeability to ions), and (iii) biosynthetic activity. By applying the test battery to additional compounds, it should be possible to determine both their relative strengths as toxicants and the nature of their primary inhibitory mechanism.

Phenolic series

The responses of S. typhimurium to the series of chlorinated phenols are summarized in Figure 5 through 8. Again, plots of the normalized rate of oxygen demand and intracellular ATP represent interpolated values after a 30-minute exposure period. The metabolic response of S. typhimurium was similar for each of the four compounds tested. Rates of specific growth and normalized O_2 utilization decreased monotonically with increased toxicant concentration in each case. For the majority of compounds tested, (2,4-dichlorophenol, 2,4,6-trichlorophenol, PCP), reductions in intracellular ATP levels were observed at low contaminant concentrations. Higher toxicant levels, however, reversed the situation, leading to ATP concentrations which were as high or higher than that of the control culture. Only 2-dichlorophenol failed to exhibit that trend; it is possible that a greater contaminant dose would have produced such a pattern. The observed metabolic response to the test battery suggests that at low concentrations the chlorinated phenols interfere primarily with energy generation but do not act as uncoupling agents. At higher concentrations, their primary toxic effects appear to be on the biosynthetic side of cellular metabolism. Because no clearing of these suspensions was noted at high toxicant concentrations, it is unlikely that the chlorinated phenols caused cell lysis.

It is widely held that the mechanism of phenolic disinfectants is related to their lipophilic properties. Such disinfectants are strongly associated with microbial lipids; their biological activities are associated with disruption of cell membrane functions (14). In the context of this study, membrane-dependent aspects of cellular energy generation provide a plausible explanation for observed disruption of catabolic function at low concentrations. However, biosynthetic roles for the cell membrane which might be sensitive to chemical interference are less obvious. Interference with transmembrane transport activities could be responsible for such secondary effects. Unfortunately, these experiments do not support meaningful speculation in this area.

The assumed relationship between phenolic hydrophobicity and toxic function suggests that the octanol/water partition coefficient of these chemicals (or other physical measures of their lipophilic nature) should be correlated with measures of their relative toxicity. Such correlations were explored by Kwasniewska and Kaiser (15) for yeast and by Liu et al. (17) for a Bacillus sp. isolated from activated sludge. Estimates of toxicant concentrations which produce a 50 percent decrease in specific growth rate are plotted as a function of the octanol/water partition coefficient in Figure 9. Because the ionized form of phenolic compounds has considerably lower affinity for cellular material than the unionized form (14), the estimated concentrations yielding a 50 percent loss of specific growth rate (ECk50) were adjusted to their respective unionized concentrations using K_a data and equation (1).

$$\frac{[HA]}{[H^+][A^-]} = K_a \quad \text{(definition)}$$

$$\frac{[A^-]}{[HA]} = \frac{1}{K_a\,[H^+]}$$

$$\frac{[HA] + [A^-]}{[HA]} = \frac{1 + K_a\,[H^+]}{K_a\,[H^+]}$$

$$\frac{[HA]}{[HA] + [A^-]} = \frac{K_a [H^+]}{1 + K_a [H^+]} \quad (1)$$

Concentrations so adjusted are also plotted as a function if K_{ow} in Figure 9. Model improvements evident in the figure support previously developed concepts of regarding the relative toxicities of phenolic species. Model statements and goodness-of-fit statistics are provided in the figure captions. A similar exercise involving respiration data (not shown) produced similar results.

In order to serve as an adequate surrogate for less convenient assays of aquatic tosicity microbial-based assays must (i) respond to changes in chemical composition in the same manner and (ii) provide a degree of sensitivity to toxicants which is comparable to established bioassay systems. Figure 10 illustrates the relative merit of the Salmonella-based assay in terms of both of these criteria. Where the pH of fish toxicity data were available, concentrations of phenolic compounds which formed the basis of the comparison were corrected to the concentration of their protonated forms using equation 1. The figure indicates that chemical-dependent changes in the 24-hr LC_{50} for guppies (26) is parallelled by changes in the S. typhimurium ECk_{50}. Parallelism between the relative toxicity line and the 1:1 line suggests that percentage changes in guppy LC_{50}s are roughly equal to percentage changes measured via the bacterial toxicity test.

The position of the Figure 10 data points relative to the 1:1 line also indicates that the S. typhimurium test is considerably less sensitive -- by a factor of approximately 40 -- than the guppy bioassay. Taken together, results suggest that widespread application of a similar, microbial-based bioassay will depend upon utilization of a microorganism which is more sensitive to chemical toxicity. In other respects, the test battery offers promise.

SUMMARY AND CONCLUSIONS

The following conclusions are supported by experimental evidence:

(i) The responses of S. typhimurium to inhibitors with established mechanisms of toxicity paralleled responses predicted on theoretical grounds.

(ii) The test battery selected clearly differentiates among toxicants whose primary activity involves disruption of (a) energy generation, ahead of establishment of a transmembrane protonmotive force; (b) the integrity of membrane function; and (c) biosynthesis.

(iii) The use of individual metabolic indicators in lieu of the test battery could yield confusing results; the effect of uncouplers, which tend to promote futile catabolism (i.e., without ATP generation), could not be observed by measuring respiratory rate, and ATP concentration alone would provide a poor indication of toxic effects on the biosynthetic side of metabolism.

(iv) Chlorinated phenols appear to induce a mixed metabolic response in that intracellular ATP levels are depressed at low chemical levels but recover at higher concentrations of inhibitor. Growth and respiration are lower at all inhibitory concentrations of the compounds tested.

(v) The response of S. typhimurium to the chlorinated phenols tested was highly correlated with their octanol/water partition coefficients, suggesting that phenolic toxicity is associated with bacterial membrane function.

(vi) The relative sensitivities of S. typhimuirum (ECk_{50}) and guppies (24-hour LC_{50}) to the series of phenolic compounds tested were similar; however, the guppies responded to these chemicals at concentrations more than an order of magnitude lower than the concentrations which produced a 50 percent reduction in S. typhimuirum specific growth rate.

(vii) Development of the test battery described here about a microorganism which exhibits greater sensitivity to chemical toxicants might yield a bioassay procedure of immense practical value.

ACKNOWLEDGMENTS

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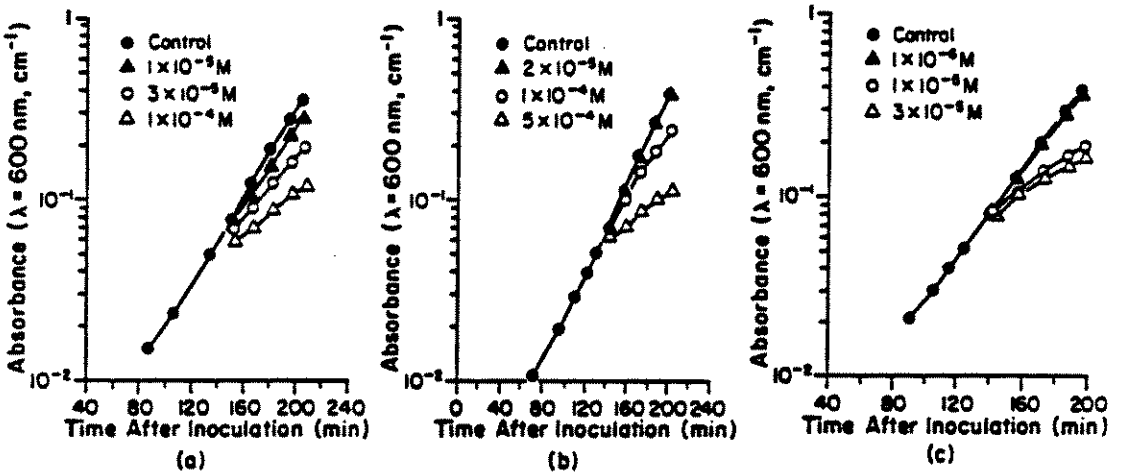

Figure 1. Growth of S. typhimurium as a function of time and concentration of specific inhibitors: (a) total cyanide; (b) 2,4-DNP; (c) chloramphenicol.

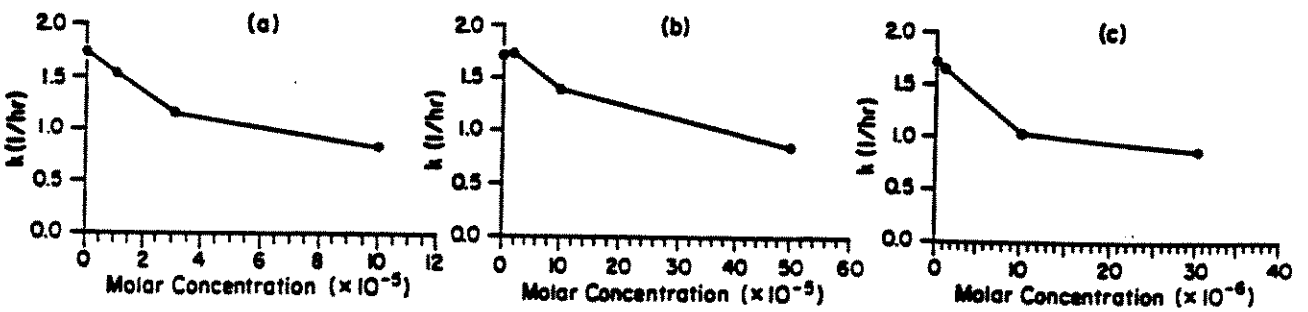

Figure 2. Summary of growth-rate data. Specific growth rate of S. typhimurium as a function of inhibitor concentration: (a) total cyanide; (b) 2,4-DNP; (c) chloramphenicol.

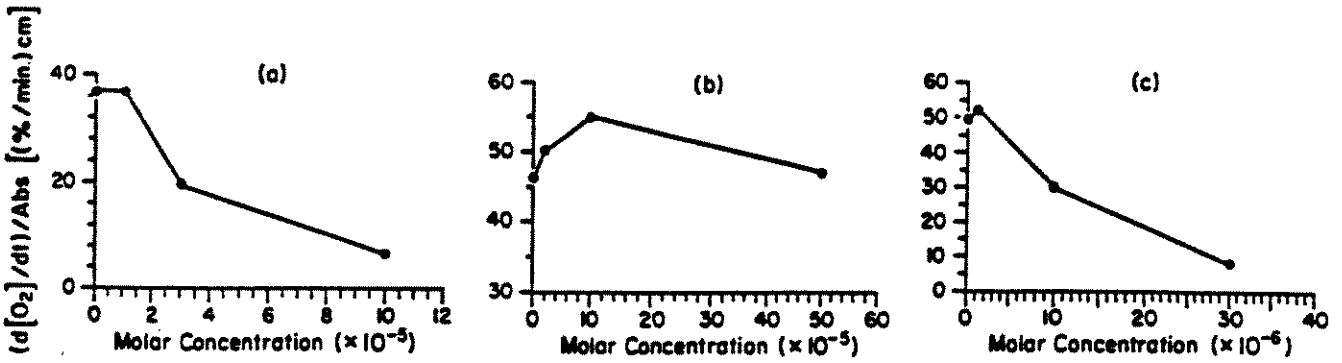

Figure 3. O_2 utilization rate in cultures of S. typhimurium (normalized for cell density) as a function of inhibitor concentration: (a) total cyanide; (b) 2,4-DNP; (c) chloramphenicol. Figure represents O_2 utilization rate measurements 30 minutes after inhibitor addition.

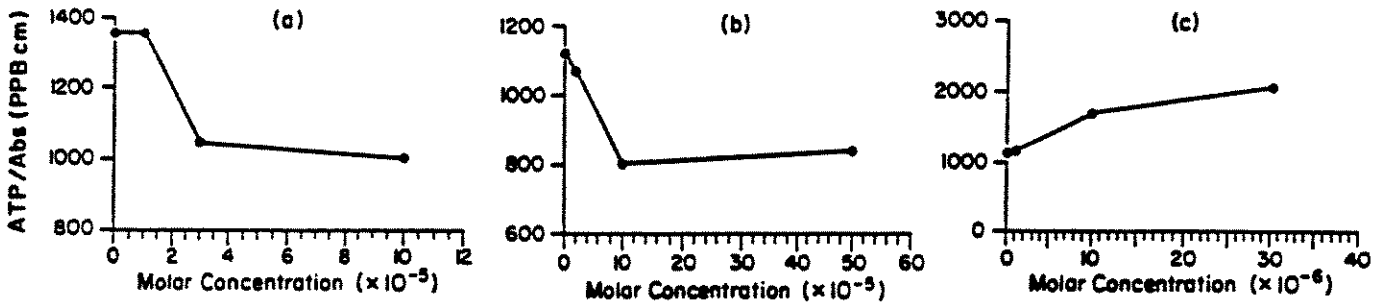

Figure 4. Intracellular concentration of ATP in cultures of S. typhimurium (normalized for cell density) as a function of inhibitor concentration: (a) total cyanide; (b) 2,4-DNP; (c) chloramphenicol. Figures represent ATP measurements 30 minutes following inhibitor addition.

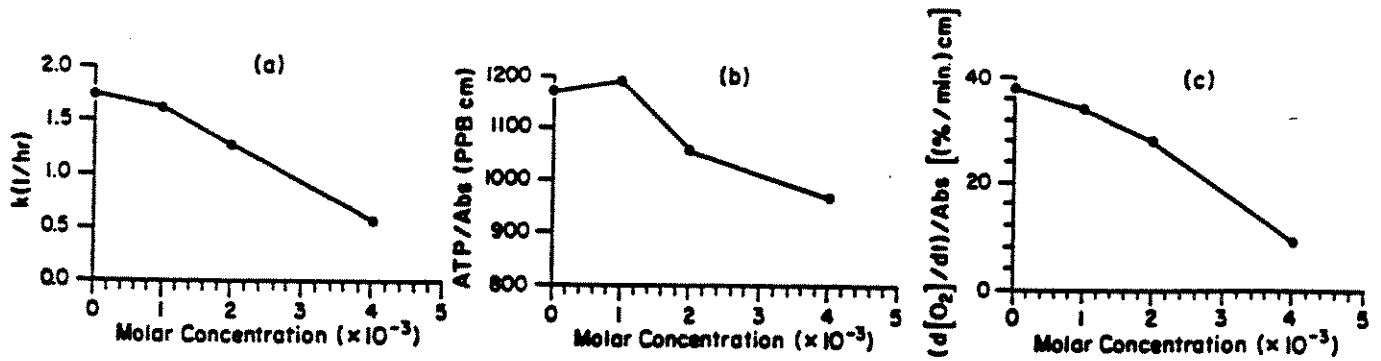

Figure 5. Concentration-dependent inhibition of S. typhimurium by 2-chlorophenol, as measured by three test parameters: (a) specific growth rate; (b) intracellular ATP concentration; and (c) O_2 utilization rate (normalized for cell density).

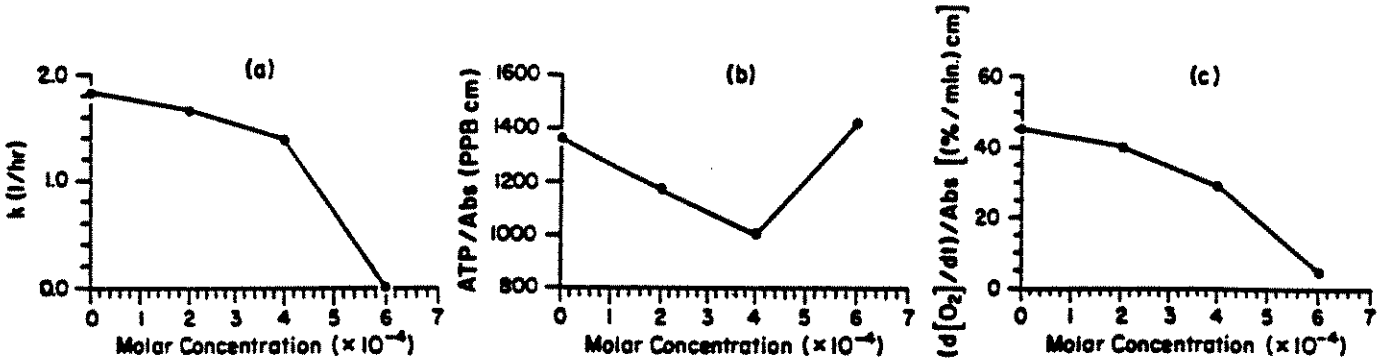

Figure 6. Concentration-dependent inhibition of S. typhimurium by 2,4-dichlorophenol, as measured by three test parameters: (a) specific growth rate; (b) intracellular ATP concentration; and (c) O_2 utilization rate (normalized for cell density).

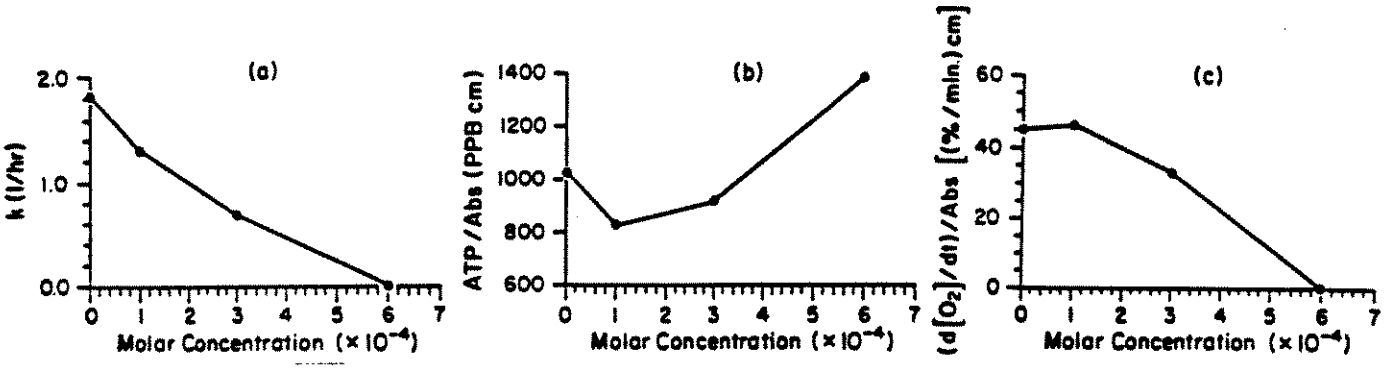

Figure 7. Concentration-dependent inhibition of S. typhimurium by 2,4,6-trichlorophenol, as measured by three test parameters: (a) specific growth rate; (b) intracellular ATP concentration; and (c) O_2 utilization rate (normalized for cell density).

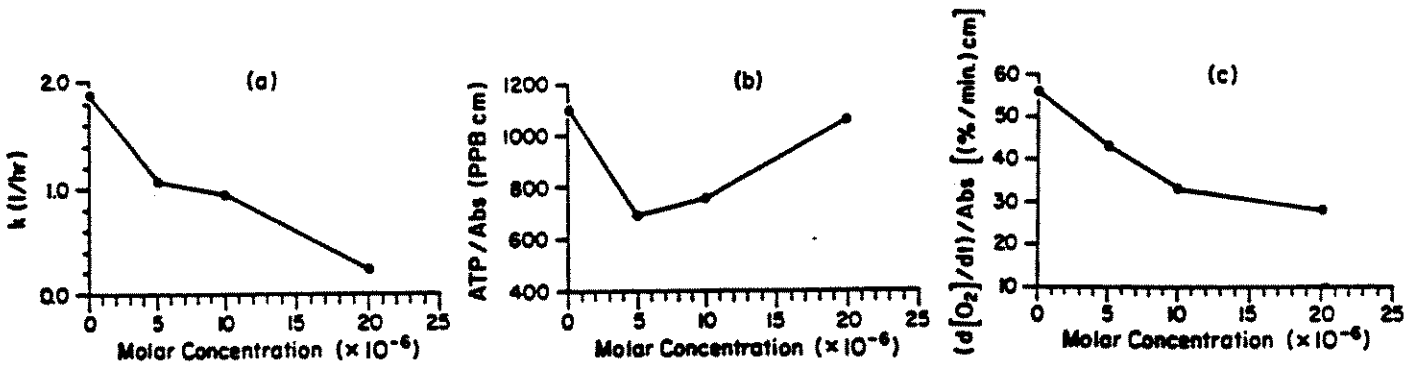

Figure 8. Concentration-dependent inhibition of S. typhimurium by pentachlorophenol, as measured by three test parameters; (a) specific growth rate; (b) intracellular ATP concentration; and (c) O_2 utilization rate (normalized for cell density).

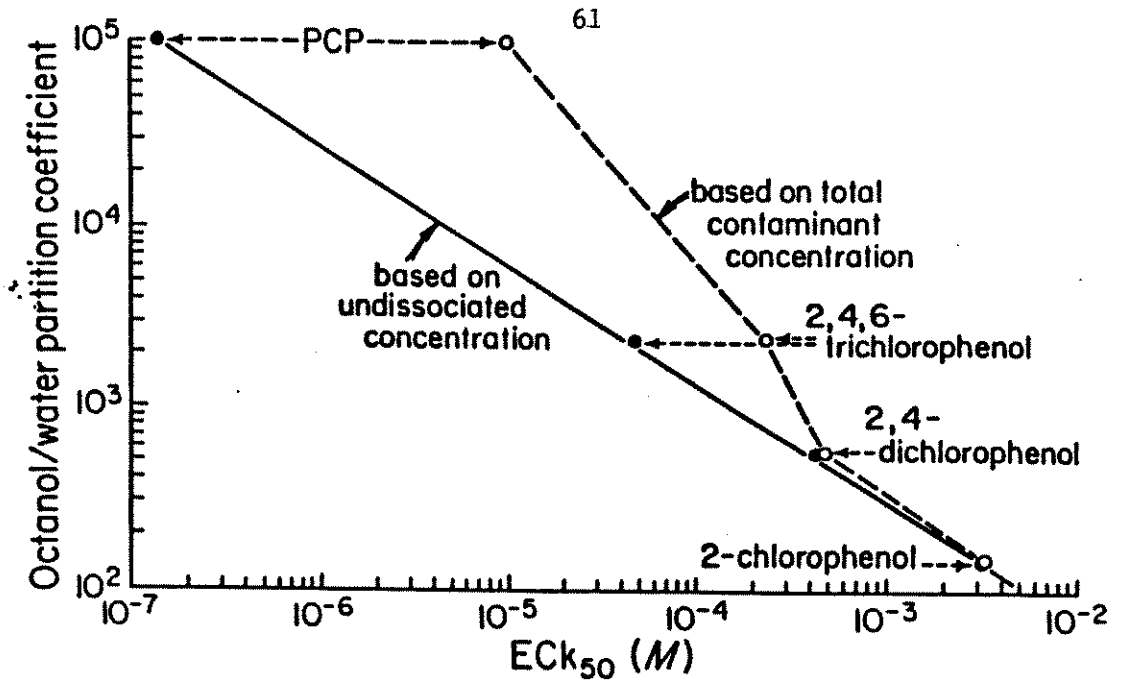

Figure 9. Compound toxicity (ECk_{50}), based on (i) total and (ii) undissociated concentrations of selected chlorinated phenols, versus compound octanol-water partition coefficient.

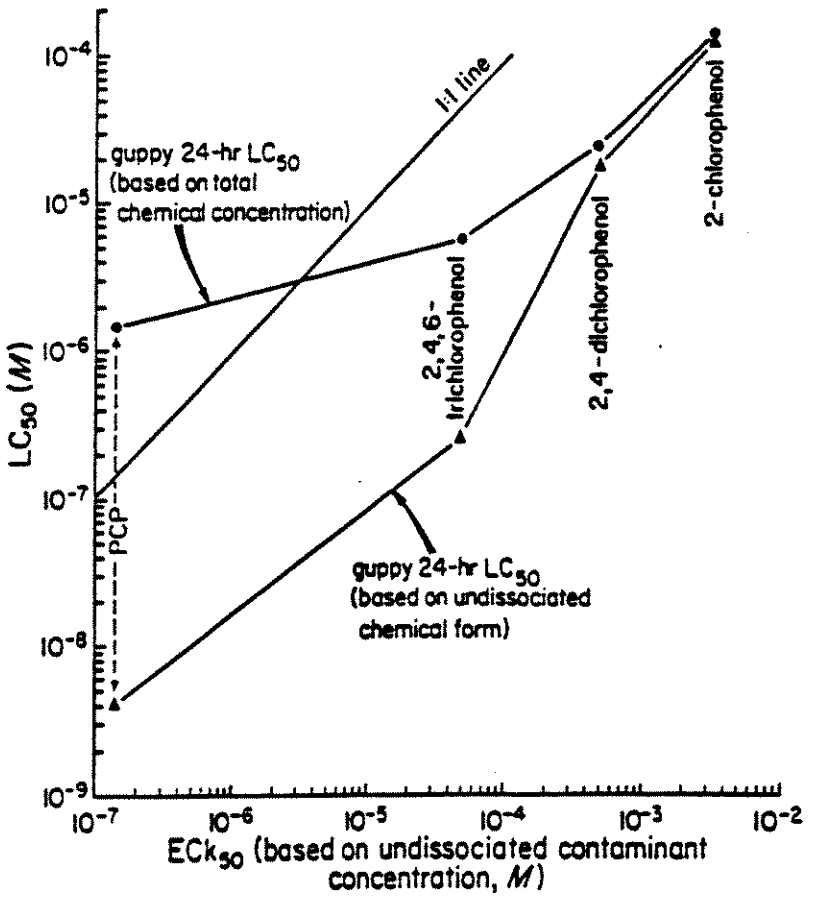

Figure 10. Guppy LC_{50} versus S. typhimurium ECk_{50} for the series of chlorinated phenols tested. Guppy LC_{50} data are presented as both uncorrected (total) concentrations and corrected (undissociated) concentrations of the chemicals tested.

UN TEST DE TOXICITE MULTIPARAMETRIQUE UTILISANT SALMONELLA THYPHIMURIUM.

Par

Gregory E. Kupillas et Robert G. Arnold

RESUME

Une série de mesures basées sur les réactions physiologiques de S. typhimurium à des produits chimiques toxiques a donné en même temps une mesure de la toxicité relative et une indication de la source biochimiques des effets. Avant d'être appliqué à une série de phénols chlorés, la batterie de tests a été calibrée à l'aide de produits chimiques (CN^-; 2,4-DNP; chloramphenicol) dont un mécanisme toxique a été clairement établi. Les paramètres choisis pour servir de mesures à la toxicité relative des phénols chlorés démontrent une corrélation valable avec la toxicité aigue du poisson et des données de partition chimique octanol/eau. A des concentrations chimiques relativement basses, les phénols chlorés semblent inhiber la production d'énergie chez les organismes expérimentaux. A des concentrations élevées, l'inhibition des fonctions biosynthétiques semble voiler les effets cataboliques. La batterie de tests microbiens peut être une possibilité économique intéressante pour les tests de toxicité impliquant des organismes marins, particulièrement si un test avec un microorganisme plus sensible est choisi.

A RAPID BIOASSAY PROCEDURE FOR SEDIMENTS BASED ON EXOENZYME ACTIVITY OF INDIGENOUS MICROORGANISMS

K. Lee[1], C.N. Ewing[1], K.-L. Tay[2] and E.M. Levy[3]

Kenneth Lee Research Limited[1], 30 Forest Road, Dartmouth, Nova Scotia, Canada, B3A 2M3

Environmental Protection[2], Environment Canada, 45 Alderney Drive, Dartmouth, Nova Scotia, Canada, B2Y 2N6

Department of Fisheries and Oceans[3], Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada, B2Y 4A2.

ABSTRACT: Procedures to measure exoenzyme activity of indigenous bacteria in suspended sediments using inexpensive, commercially available, substrate analogues conjugated with the fluorescent compound 4-methylumbelliferone are described. Experiments with prohibited and restricted substances (Cd, Cu, Hg, Ni, Pb, Zn), as defined by the Ocean Dumping Control Act (ODCA), demonstrated that bioassays based on microbial exoenzyme activity are sensitive to a wide range of toxicants, and are rapid to conduct.

INTRODUCTION: Coastal marine sediments are recognized as important sites for the regeneration of nutrients, transformation of trace metals and degradation of organic compounds. Since these processes are primarily mediated by microorganisms (Bauer et al., 1981; Haines et al., 1982; Lee et al., 1985), procedures have been developed to assess the environmental impact of pollutants on microbial activity in sediments. However, existing toxicity tests using bacteria, such as the Microtox (Photobacterium sp.) bioluminescence test (Bulich, 1979), the bacterial (Spirillum volutans) mobility test (Dutka et al., 1983), the E. coli K-12 SOS Chromotest (Quillardet et al., 1982), and the Ames (Samonella typhimurium) test (Ames et al., 1975), cannot accurately predict toxicant effects in the natural environment since the test organisms are not representative of the indigenous biota (Chapman & Long, 1983). Furthermore, laboratory cultures of microorganisms, generally contain strains with great resistance to stress. The design of new methods to predict pollutant impact should incorporate interactions with higher levels of organization and natural diversity (Ausmus, 1984; Neuhold, 1986). In order to obtain accurate predictions of toxicant effects on the environment, it is essential to use samples collected from the area of concern which contains natural assemblages of the biota (Barnhart and Vestal, 1983).

The toxicity of most pollutants can be attributed to enzyme inhibition (Buikema et al., 1980; Vives-Rego et al., 1986). Significant correlations between enzyme tests and bioassays with higher organisms have been recorded (Buikema et al., 1980). Numerous studies have also shown that bioassays based on enzyme activity are generally more sensitive than population level (LD_{50}) tests (Liu, 1981), and are faster to conduct, and more reliable than measurements of whole organism activity (Wieser and Zech, 1976).

By excreting extracellular enzymes, bacteria cleave high molecular weight compounds outside the cell before uptake. In terms of ecological

significance, such extracellular hydrolysis of large macromolecules is the first and often rate limiting step in organic matter degradation (Hoppe, 1986; King, 1986). The activity bacterial exoenzymes including phosphatase (Perry, 1972; Petterson & Jansson, 1978), glucosidase (Hoppe, 1983; Somville, 1984), protease (Somville & Billen, 1983) and chitinase (O'Brien & Colwell, 1987), in the environment, has recently been measured fluorometrically using commercially available fluorescent substrate analogues.

In the present study, the sensitivity of sediment bioassay test procedures based on fluorometric measurement of microbial exoenzyme activity was evaluated.

MATERIALS AND METHODS: Cores of coastal marine sediments from Long Cove (44° 44.43'N, 62° 44.62'W) and Lawrencetown Beach (44° 38.58'N, 63° 20.44' W), Nova Scotia were collected in polycarbonate tubes (8 cm i.d.). The cores were maintained in seawater, at ambient temperature, until analysis within six hours.

Stock solutions (5mM) of 4-Methylumbelliferyl-B-D-glucoside, 4-Methylumbelliferyl-phosphate (Sigma Chemical Co.) or L-Leucine-4-methylcoumarinyl-7-amide (Fluka Chemical Co.), were prepared in 1:10 methanol:synthetic ocean water (SOW; Morel et al., 1979). 1µM analytical standards of 4-methylumbelliferone (Sigma Chemical Co.) in SOW were prepared immediately prior to use from 2.5mM stock solutions of MUF dissolved in methanol (stored at -20°C). Six metals (Hg, Cd, Cu, Pb, Zn, and Ni) classified as prohibited (Schedule I) and restricted (Schedule II) substances under the Ocean Dumping Control Act (1975) were used in the assessment of the bioassay procedures. With the exception of a $PbCl_2$ (14.5mM) stock solution made with distilled water to prevent precipitation, the stock solutions of $HgCL_2$ (1.5mM), $CuCL_2.2H_2O$ (4.7mM), $CdSO_4$ (26.7mM), $ZnSO_4.7H_2O$ (45.9mM), and $NiCl_2.6H_2O$ (51.1mM), were prepared with SOW. A NaOH/Borax buffer (pH 10.8) was prepared by bringing 50mL of a 0.05M sodium tetraborate solution and 67mL of 0.2M NaOH, to a final volume of 200mL with distilled water.

A 0.5g sample of homogeneously mixed sediment from the upper 2cm of the cores was added to triplicate 10mL test tubes containing 3.0mL of SOW with known concentrations of toxicant. After incubation for 30 minutes at ambient temperature, enzyme specific MUF-substrates (100µl of a 1:32 dilution of stock solutions in SOW) were added to each tube (final substrate concentration of 5µM) and the samples incubated for another 60 minutes, with agitation ever 15 minutes, after which enzymatic activity in the tubes was terminated by freezing in dry ice. Experimental blanks consisting of identical samples of sediment in SOW spiked with a 1.0µM methylumbelliferone standard were prepared concurrently.

2.5mL aliquots of supernatant from frozen sediment samples which had been thawed in an ice bath and centrifuged at 4750 rpm for 5 minutes, were decanted into a cuvettes containing 0.25mL of NaOH/Borax buffer (added to maximize the fluorescence of liberated MUF). Fluorescence of the samples at 455nm emission with 365nm excitation was measured using a Perkin-Elmer MPF-44B fluorescence spectrophotometer. Standard curves were prepared using known dilutions of the methylumbelliferone stock solution.

Exoenzyme activity (as µM product/g sediment/h) was plotted against toxicant concentration (Fig. 1) and EC_{50} value for each metal (the effective concentration resulting in a 50% depression of enzyme activity), calculated from regressions of % depression to Log toxicant concentration (Fig. 2). 95% confidence intervals were calculated for all EC_{50} estimates using the method of Sokal and Rohlf (1969).

RESULTS: The addition of metals at toxic concentrations typically caused a significant depression of exoenzyme activity in slurries of sandy sediment from Long Cove, with increasing concentration (example for nickel: Fig. 1, 2). Of the sediment samples tested, phosphatase enzyme activity appeared to show the highest activity and variation between replicate samples. While in slurries of sandy sediment, phosphatase, glucosidase and protease yielded similar coefficients of variation (9.8, 9.5 and 8.0%, respectively), in muddy sediments, the mean coefficient of variation (c.v.) for phosphatase activity was 17.8% while glucosidase and protease yielded values of 9.3 and 6.7%, respectively. Fairly precise EC_{50} values could be determined since the regressions of toxicant effect on Log toxicant concentration (Fig. 2) yielded a high degree correlation ($r \geq 0.874$ in sandy sediments, $r \geq 0.807$ in muddy sediments). In sandy sediments, glucosidase activity displayed the following toxicity order, or sensitivity pattern, to the metals tested, Hg=Zn>Cu>Cd>Pb=Ni (Table 1). These EC_{50} values are quite similar to those reported by Barnhart and Vestal (1983) in freshwater sediments, as determined by the use of chromogenic substrates. With the exception of copper, phosphatase activity showed a similar toxicity order (Cu>Zn>Hg>Ni>Pb>Cd) to that of glucosidase but with considerably higher EC_{50} values. A different toxicity order (Cu>Pb>Zn>Hg=Ni>Cd) was observed for protease activity (Table 1), since the induction of the enzyme appeared to be extremely sensitive to lead.

EC_{50} values in fine sediment (mud collected from the same region), were generally much higher than those observed in the sandy sediments (Table 1). The toxicity orders obtained with glucosidase and phosphatase enzyme activity, Cu=Hg>Zn>Ni=Cd>Pb and Cu>Hg>Zn>Cd>Ni>Pb respectively, closely resembled those observed in sandy sediment. Protease activity in the fine sediment was also very sensitive to lead and displayed a similar toxicity order (Cu>Zn>Pb>Hg>Ni>Cd) to that observed in the sandy sediment.

DISCUSSION: At present, the major factor in the development of toxicity assessment procedures, concerns their ecological validity. There is no conclusive evidence supporting the premise that the environmental impact of toxicants can be predicted from tests based on the responses of a single species. Laboratory toxicity assays must incorporate ecological principles into their design. Bulk chemical analyses and studies of community structure are insufficient to accurately predict the impact of wastes in the environment. In order to gain complete understanding of the environmental effects of contaminants, one must examine their effects at the ecosystem level, not just the population and community level, and focus on primary metabolic processes since a disturbance of these processes will have ramifications throughout the ecosystem. Thus for a more accurate extrapolation of laboratory results to nature, we propose that microbial bioassays should be conducted with actual field samples containing natural assemblages of the biota.

In the bioassay procedure described, the high fluorescence of methylumbelliferyl, enables the measurement of enzyme activity with substrate concentrations at or below their ambient concentration to minimize alterations to the physical and/or chemical nature of the sediments. The sensitivity of the fluorometric procedures to toxic contaminants in sediments is similar to that reported in other assays based on the use of chromogenic enzyme substrates and ^{14}C-acetate incorporation (Barnhart & Vestal, 1983). However, in comparison to chromogenic and radioisotope assays, fluorogenic tests can be conducted more rapidly and at much less expense. By using natural sediments

Figure 1: The effect of nickel on exoenzyme activity in slurries of sandy sediment. Activity in control tubes: Glucosidase, 1.34±0.06 nM glucose/g/h; Phosphatase, 5.3±0.3 nM PO_4/g/h; Protease, 3.1±0.2 nM leucine/g/h.

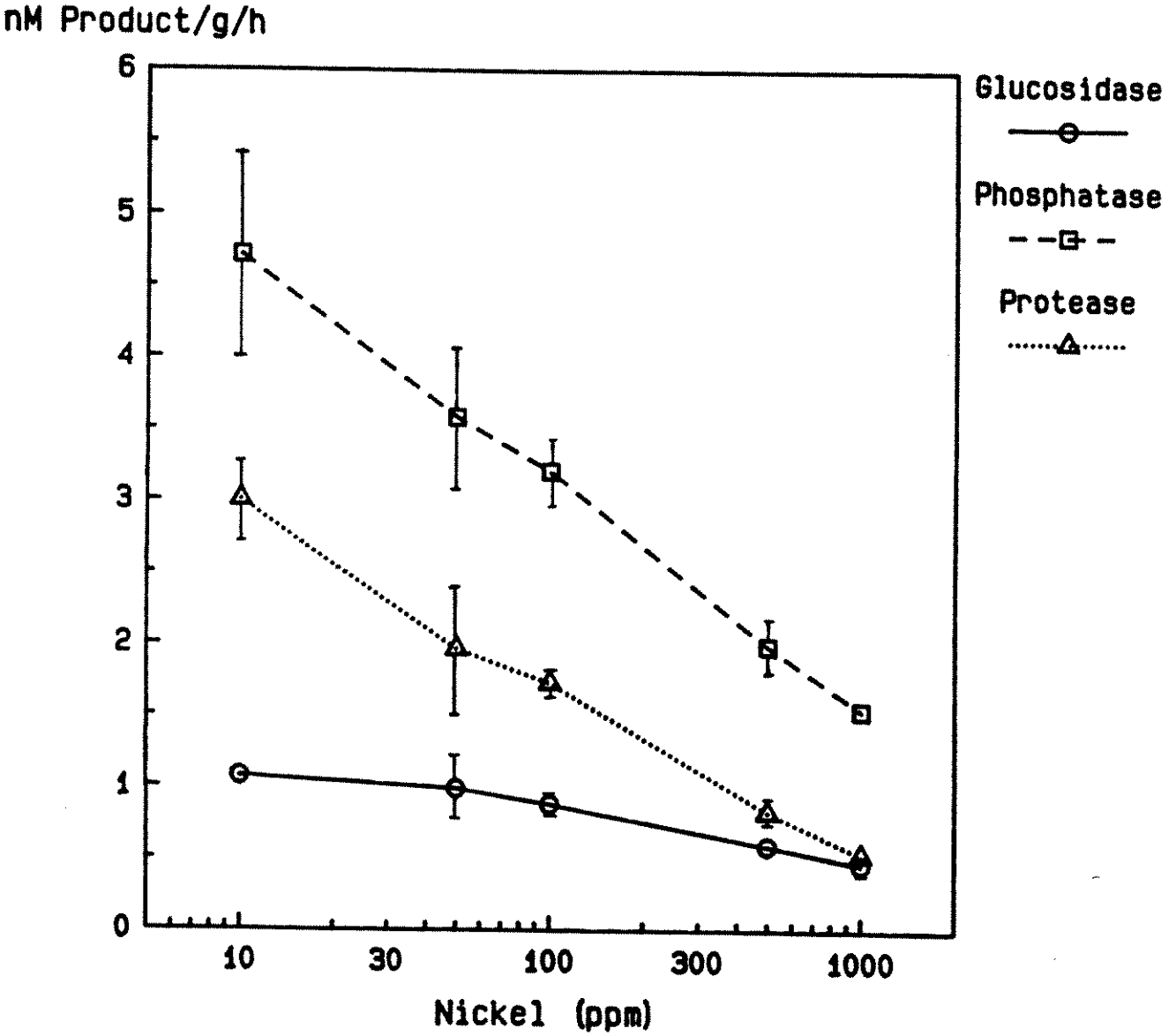

Figure 2: The effect of nickel on exoenzyme activity in slurries of sandy sediment.

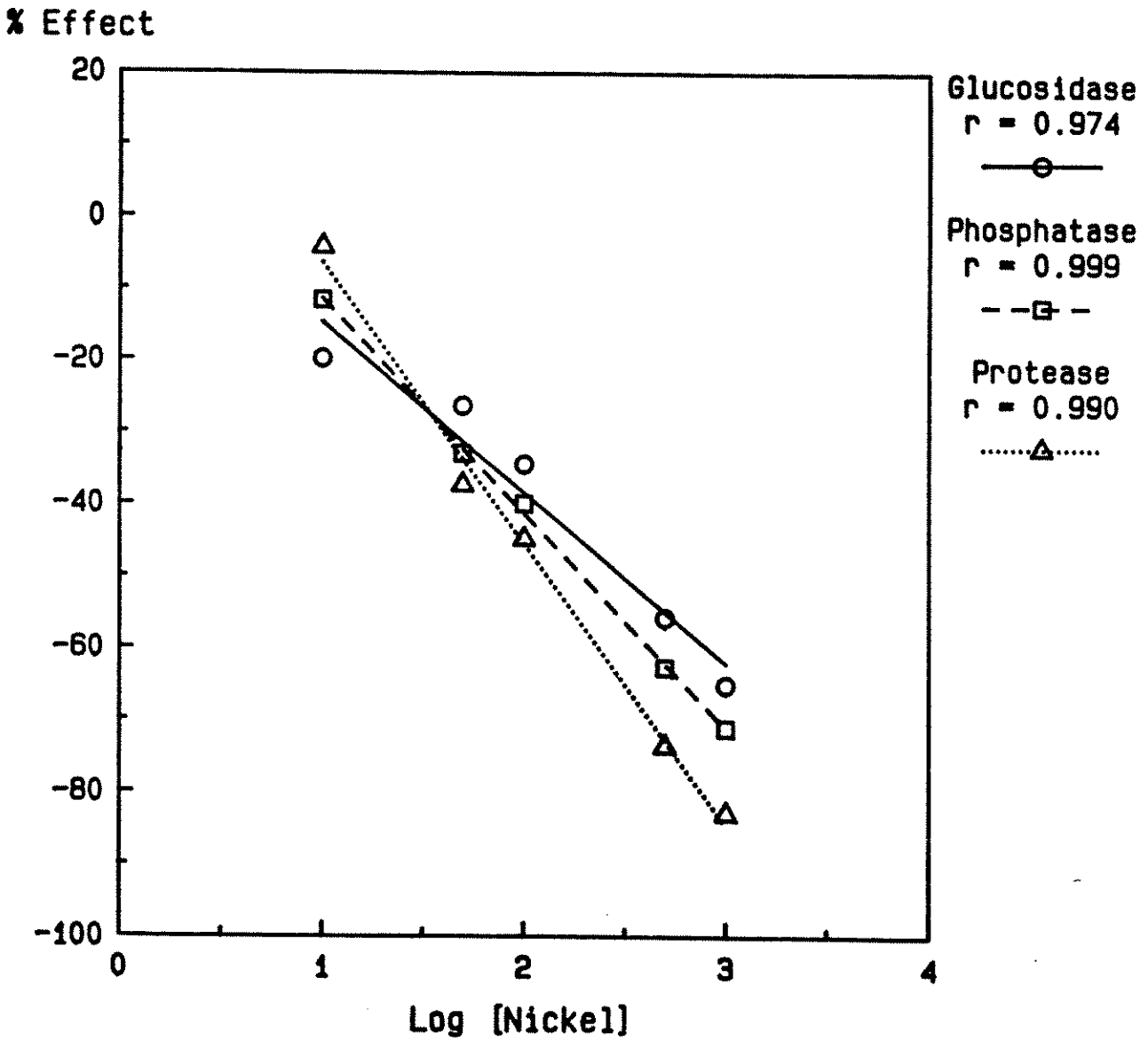

Table 1: The toxicity of six metals to glucosidase, phosphatase, and protease in slurries of sandy and muddy sediment. All values are mg/L.

	SAND SITE		MUD SITE	
Glucosidase Metal	EC50	95% Confidence Interval	EC50	95% Confidence Interval
Cu	41	16 -> 107	35	<1 -> 2982
Hg	27	3 -> 242	35	10 -> 121
Ni	130	42 -> 407	310	236 -> 406
Cd	72	63 -> 81	316	32 -> 3150
Pb	129	51 -> 326	>1000	-
Zn	29	5 -> 164	76	28 -> 207
Phosphatase Metal	EC50	95% Confidence Interval	EC50	95% Confidence Interval
Cu	9	1 -> 63	5	<1 -> 193
Hg	>100	-	28	4 -> 229
Ni	173	44 -> 323	232	39 -> 1375
Cd	439	92 -> 2112	95	31 -> 234
Pb	395	112 -> 1396	769	133 -> 4446
Zn	87	37 -> 204	58	32 -> 106
Protease Metal	EC50	95% Confidence Interval	EC50	95% Confidence Interval
Cu	<1	-	10	1 -> 107
Hg	>100	-	70	12 -> 406
Ni	112	25 -> 493	120	86 -> 168
Cd	288	72 -> 1146	198	9 -> 4447
Pb	33	12 -> 92	51	17 -> 153
Zn	40	13 -> 118	25	12 -> 51

in the test procedure, the response of the entire bacterial community to a toxicant is measured. Furthermore, methylumbelliferyl enzyme substrate analogues are available for a wide range of exoenzyme systems and thus, specific substrates can be chosen for basic processes of interest such as nutrient cycling, and carbohydrate or protein degradation.

In addition to the simplicity of the analytical method, results of sediment slurry assays with the fluorogenic procedure can be obtained within a 2.5 hour period. Of the three enzyme substrates assessed in the present study, the similarity in toxicity order for the metals suggest that any one of the three can be used to test toxicant impacts. However, we advocate the use of multiple substrates, for a more accurate estimates of pollutant impact on the biota. Observed differences in toxicity order and EC_{50} values for the three MUF-substrates evaluated are most likely attributed to differences in sensitivity to toxicants by the various individual enzyme systems. The comparatively higher EC_{50} values obtained in the slurries of muddy sediment over sandy sediment are probably attributed to the higher concentrations of organic carbon in the mud which would bind with the free metal ions. While such organic chelation tends to increase the solubility of metals in aqueous solutions, it tends to reduce toxicity by lowering the concentration of toxic free metal ions that are available to the biota (Allen et al., 1980).

Open water disposal of solid or liquid wastes has become a principal method of waste disposal world-wide, since it is cheaper than landfilling or confined aquatic disposal. As a result, there exists a great need for simple and cost effective tests of toxicity for waste materials to be disposed of in the sea (Buikema et al., 1980; Busch, 1982). Bacterial activity measurements can be used to pre-screen wastes to determine suitability for oceanic disposal and/or to assess the impact of disposal activities. Since, the precise effects of pollutants in an ecosystem depend on the exact conditions experienced, as differences in parameters such as organic carbon content can greatly affect pollutant toxicity, biological impact assessment of any disposal event must be conducted under conditions as close as possible to those at the proposed dump site. Sediments are effective sinks of contaminants and therefore chemical analysis of them are useful in determining the degree and nature of contamination of a given area. However, chemical data provide little or no evidence of biological consequences of the contamination. Our sediment bioassay procedure demonstrates that the induction of microbial exoenzyme activity is sensitive to pollutants. Recent studies suggest that similar enzyme assay procedures can be employed as a tool to monitor long-term effects of contaminants on marine environmental quality (Lee et al., 1988).

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UNE METHODE RAPIDE DE BIOESSAIS POUR LES SEDIMENTS, BASEE SUR L'ACTIVITE DE L'EXOENZYME DE LA FLORE BACTERIENNE.

K. Lee[1], C.N. Ewing[1], K.-L. Tay[2] and E.M. Levy[3]

Kenneth Lee Research Limited[1], 30 Forest Road, Dartmouth, Nova Scotia, Canada, B3A 2M3

Protection de l'Environnement[2], Environnement Canada, 45 Alderney Drive, Dartmouth, Nova Scotia, Canada, B2Y 2N6

Ministère des Pêches et Océans[3], Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada, B2Y 4A2

RESUME

Ces méthodes décrivent les mesures de l'activité de l'exoenzyme de la flore indigène dans des sédiments en suspension, par l'utilisation des substrats semblables, peu coûteux et commercialement disponibles, conjugées avec le composé fluorescent 4-méthylumbelliferone. Les expériences utilisant des substances prohibées et à utilisation restreinte (Cd, Cu, Hg, Ni, Pb, Zn), tel que défini par l'ODCA (Ocean Dumping Control Act) ont démontré que les bioessais basés sur l'activité de l'exoenzyme microbien sont sensibles à une grande variété de toxiques et sont réalisables rapidement.

A SUMMARY OF THE EFFECTS OF TWO RECONSTITUTED WATERS ON ASPECTS OF THE DEMOGRAPHICS OF *Ceriodaphnia dubia*

Guy E. Melville and Dan Richert
Aquatic Biology Program, Environment Division
Saskatchewan Research Council, 15 Innovation Blvd.
Saskatoon, SK S7K 2X8

Toxicity tests are used to determine the effects of effluents and other compounds on the environment, monitor effluents, establish water quality criteria and ultimately to protect aquatic systems from the introduction of toxic substances (Cowgill, 1987). Chronic tests are especially informative since they offer reproductive as well as survival data (DeGraeve and Cooney, 1987). Cladoceran zooplankton have been used extensively as test organisms in recent years (Buikema *et al.*, 1980; Geiger *et al.*, 1980; Winner, 1981; Nebeker, 1982; Dunbar *et al.* 1983), for three reasons. Cladocerans occupy an important position in freshwater food webs (Mount and Norberg, 1984; Keating, 1985; Knight and Waller, 1987), they are sensitive to toxicants (Leeuwangh, 1978; Kenaga, 1978; Canton *et al.*, 1975; Mount and Norberg, 1984; Cowgill, 1987; Cowgill *et al.*, 1985; Takahashi *et al.*, 1987) and they can provide the data in a relatively short period of time (Geiger *et al.*, 1980; Mount and Norberg, 1984; Knight and Waller, 1987). A host of additional practical reasons also contribute to their attractiveness as test organisms (e.g., Mount and Norberg, 1984). A survey of recent literature shows that *Ceriodaphnia dubia* has quickly become the most widely used cladoceran in freshwater chronic toxicity tests.

While *C. dubia* is now the favorite chronic toxicity test cladoceran, many investigators using this animal have encountered problems in maintaining healthy cultures and in conducting valid toxicity tests (DeGraeve and Cooney, 1987; Cowgill, 1987). The foods used to sustain *C. dubia* have been the subject of much discussion with respect to such problems (Cowgill *et al.*, 1985; DeGraeve and Cooney,1987; Cowgill, 1987). However, many different foods have been successfully utilized to culture and test *C. dubia* (DeGraeve and Cooney, 1987). This suggests that food related aspects have not caused most of the major failures in culturing and testing *C. dubia*. Serious problems which have arisen have usually been attributed to the waters used for culturing and toxicant dilution, particularly synthetic or reconstituted waters (DeGraeve and Cooney, 1987). As Cowgill (1987) concludes, "It should be noted that as of this writing, no adequate and practical defined medium has been published."

In this paper we summarize the results of experiments to examine the effects of two commonly used synthetic or reconstituted waters on aspects of the demographics of *C. dubia*. The first water is synthetic, following the Marking and Dawson formula of the United States Environmental Protection Agency (EPA)(1985). It contains balanced major ions but no trace elements. The other consists of diluted Perrier mineral (ground) water, which is also used by some EPA laboratories. The Perrier water contains many trace elements but unbalanced major ions. The results of reconstituted water treatments are compared with the results of a treatment with slightly-diluted natural pond water. We hypothesize that both reconstituted waters are chronically toxic to *C. dubia*.

METHODS

Experimental treatments and the rationale for each treatment are detailed in Table 1. Treatments were done as modified (6 day) life-cycle toxicity tests (Mount and Norberg, 1984). The treatments are ranked from, hypothetically, worst quality to best quality water in Table 1. Treatments to compare potential effects of plastic vessels with those of glass vessels were not done for all types of water, for logistical reasons. Treatments were done over several experiments within a three week period, also for logistical reasons.

Reagent concentrations used in making the synthetic EPA water, following the basic Marking and Dawson formula (US EPA, 1985), are listed in Table 2. Reagents were added to Milli-Q® water, as was the Perrier water for the Perrier treatments. Pond water was filtered through Whatman® glass microfibre filters and autoclaved before being diluted with Milli-Q® water. All waters were allowed to sit under aeration at least 48 hours before being used in cultures and experiments. The plastic vessels used in some of the treatments were one-ounce cups made by Anchor Hocking Plastics Inc. of St. Paul, MN 55102.

Conductivity and pH measurements were made with commercial electronic meters. Hardness and alkalinity determinations were carried out titrimetrically according to standard methods (APHA, 1976). The Saskatchewan Research Council analytical laboratory analyzed diluted Perrier and pond water samples for a large suite of trace elements using inductively coupled plasma-atomic emissions spectroscopy.

Other culture and experimental variables are presented in Table 3. The *C. dubia* were from cultures originally started with animals obtained from the EPA in Duluth, MN. The experimental animals were cultured in the same types of water as those to be tested, from stocks that had been raised in the waters for at least two generations.

Feeding rates (Table 3) which would provide food satiation conditions were calculated using data in the literature. Nutritionally, the daily diet consisted of approximately 50% *Ankistrodesmus falcatus*, 25% *Selenastrum capricornutum* and 25% trout chow-yeast-Cerophyl® (TYC). *Ankistrodesmus falcatus* (Goulden and Henry, 1988) was chosen as the baseline food, and the feeding rate of *Ankistrodesmus* was based on data in Cowgill *et al.* (1985), who used *A. falcatus* as a sole food source for *C. dubia*. The feeding rate we used was higher than that of Cowgill *et al.* (1985), because the rate of reproduction in *C. dubia* is potentially lower when *A. falcatus*, rather than equal numbers of *A. convolutus*, are used as food (Cowgill *et al.*, 1985). This feeding rate was then multiplied by 50% in order to establish a feeding rate that would, in terms of nutrition, contribute 50% *A. falcatus* to the daily diet.

We also used feeding rates of *Selenastrum* and TYC which were higher than the rates for these foods in the literature (Goulden and Henry, 1988; US EPA, 1985). Both foods are potentially less nutritious than *Ankistrodesmus falcatus*; *Selenastrum* can produce a lower rate of reproduction (Cowgill *et al.*, 1985) while TYC can produce lower EC_{50}'s to various toxicants (Goulden and Henry, 1988). The feeding rate of each food was then adjusted according to its proportion in the daily diet.

Algal species used as food were cultured under the same general conditions as *Ceriodaphnia* (Table 3). *Selenastrum capricornutum* was grown in an ASTM (unpublished manusript) medium, while *Ankistrodesmus* was grown in an aquarium periodically replenished with aged tap water. Algal

Table 1. Experimental treatments and rationales. Parentheses indicate aspects of secondary importance in treatments. Expt. = experiment. O = set of observations.

Expt.	Treatment	Rationale
1	1. 10% Perrier, plastic vessels	soft EPA Duluth water, in disposable but potentially toxic vessels
1	2. 10% Perrier, glass vessels	soft EPA Duluth water, in non-toxic vessels
1	3. EPA soft (glass vessels)	soft EPA water
0	4. 20% Perrier (plastic vessels)	(moderately) soft EPA Duluth water, with the content of some major ions increased
1	5. EPA moderately hard + 10% Perrier, plastic vessels	(moderately hard) water with trace elements and balanced major ions, but potentially toxic vessels
1	6. = 5, but with animals cultured in 10% Perrier (plastic vessels)	to examine the rate at which potentially improved conditions might bring about increases in demographic variables
2	7. EPA moderately hard + 10% Perrier, glass vessels	(moderately hard) water with trace elements and balanced major ions, but non-toxic vessels.
2	8. Pond + 20% Milli-Q (plastic vessels)	(moderately hard) natural water with trace elements and balanced major ions.

Table 2. Reagent concentrations used in making synthetic water, after the Marking and Dawson formula of the US EPA (1985).

Water type	Reagents (mgl^{-1})			
	$NaHCO_3$	$CaSO_4.2H_2O$	$MgSO_4$	KCl
soft	40	25	25	1.7
moderately hard	120	75	75	5.0

Table 3. Culture and experimental variables.

Variable	Units	Value
Temperature	°C	22.5 - 23.0
Light intensity	$\mu Em^{-2}s^{-1}$	= 9.8
Photoperiod	h light/h dark	16/8
Dissolved O_2	mgl^{-1}	7.3 + 1.0
Feeding rate		
algae	cells $ml^{-1}d^{-1}$	
Ankistrodesmus falcatus		7.5×10^3
Selenastrum capricornutum		67.5×10^3
trout chow-yeast-Cerophyl® (TYC)	mld^{-1}	0.05
Feeding frequency	d^{-1}	2
Water volume	ml	
experiment		15
culture, glass beakers		500
Water change	wk^{-1}	3
Ceriodaphnia dubia densities	$vessel^{-1}$	
experiment		1
culture		15
C. dubia starting ages	h	< 5

Table 4. Major water quality characteristics of the waters tested. P = Perrier. EPA = EPA soft. EPA + 10% P = EPA moderately hard plus 10% Perrier. MQ = Milli-Q® water.

Characteristic	Water type				
	10% P	20% P	EPA	EPA + 10% P	Pond + 20% MQ
Hardness as $CaCO_3$ mgl^{-1}	35.0	67.0	28.0	114.	150.
Total alkalinity mg $CaCO_3$ l^{-1}	34.0	72.0	27.0	84.0	94.0
Conductivity $\mu mhos\ cm^{-1}$	90.0	160.	160.	380.	525.
pH	7.8	8.0	7.8	7.9	8.0

cultures were intentionally xenic, but monitoring by way of the Gram stain technique, using BBL™ reagents, indicated bacterial numbers were relatively low.

Cox (1976) and Sokal and Rohlf (1969) were used as statistical references.

RESULTS

The data indicate that C. dubia survival and reproduction values vary largely according to the major sources of chemical constituents, i.e., pond, EPA (including EPA water with Perrier water added) and Perrier waters. This occurs irrespective of hardness, alkalinity and the other water quality variables measured (Table 4, Appendix 1). The pattern also occurs whether the experimental chambers were glass or plastic. As a consequence, treatments are grouped according to the three main sources of chemical constituents for more formal analyses.

There was less survival in the reconstituted waters than in the pond water (Fig. 1), although only the (6d) value for the EPA water was significantly lower (X^2 = 5.603, $P < 0.025$). The pond water provided virtually 100% survivial. Survival in the EPA water was less than in Perrier (Fig. 1).

Reproduction on the other hand was lower in Perrier water than in the EPA and pond waters, in terms of percent bearing broods (Fig. 2), young per brood (pond, $P < 0.005$, Wilcoxon two-sample test, Fig. 3) and young per female per day (both $P < 0.005$, Wilcoxon two-sample tests, Fig. 4). Measures of reproduction in EPA water were lower, albeit not significantly, than those for pond water (Fig. 2-4).

Brood sizes increased significantly with increases in body size in the Perrier and EPA waters ($P < 0.05$, $P = 0.05$ respectively, Kendall rank correlations, Fig. 5). Body sizes, hence brood sizes, were larger in the EPA water than in the Perrier for animals of the same age (Fig. 5). Brood sizes were also generally larger in EPA water than in Perrier when body sizes were the same (Fig. 5). This relationship occurs over much of the overlap in the ranges in body size for the two types of water. Body size data were not collected for the pond water treatment.

Transferring C. dubia from Perrier culture water to EPA water (containing some Perrier also, Table 1) resulted in survival and reproduction values which were similar to those for animals tested in Perrier.

DISCUSSION

The results of our experiments indicate that the EPA (1985) and Perrier types of reconstituted water, as made up in this study, are chronically toxic to Ceriodaphnia dubia. Our criteria for non-toxicity are twofold. They are at least 80% survival through the test period (US EPA, 1985), and the production of ten or more young per female through the first two broods (e.g., Cowgill et al., 1985). We did not continue our experiments beyond six days because of the low survival values in the reconstituted water treatments. The EPA waters were chronically toxic in terms of survival, the Perrier in terms of growth, reproduction and, to a lesser extent, survival.

We do not know at this point exactly why different reconstituted waters are chronically toxic. It is noteworthy that the EPA-Perrier

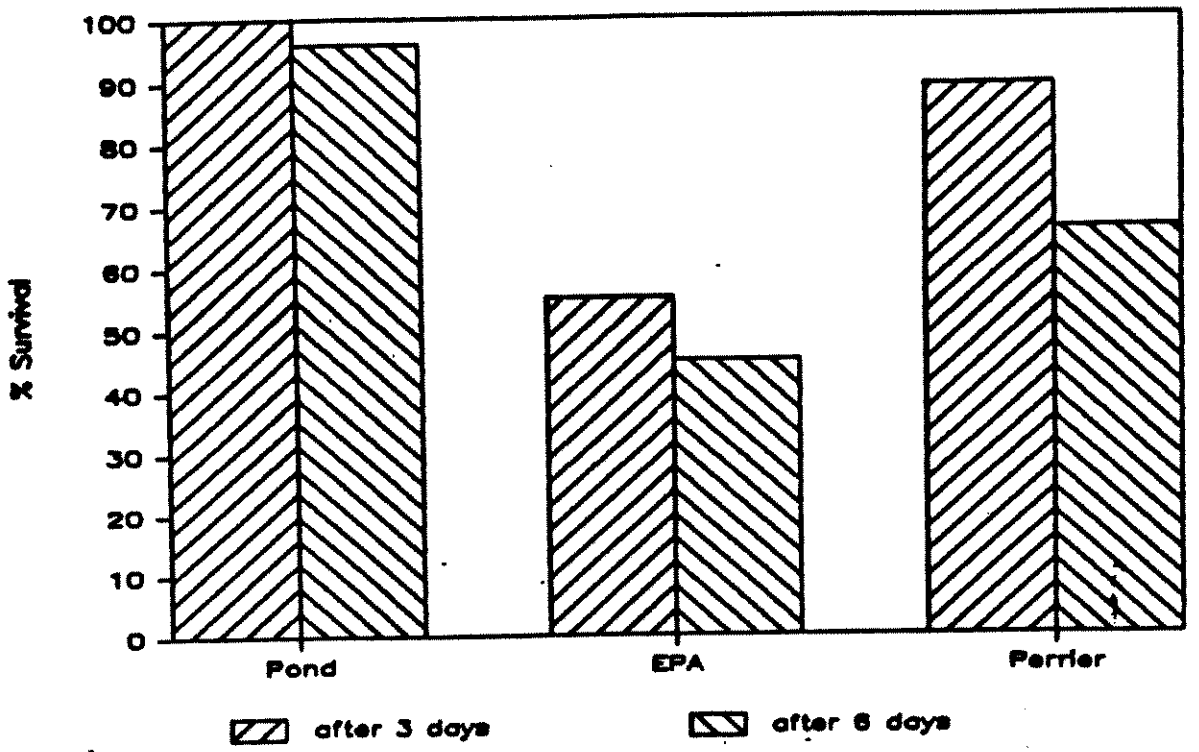

Figure 1. Survival of Ceriodaphnia dubia in three types of water. Initial n: Pond = 11, EPA = 38, Perrier = 44.

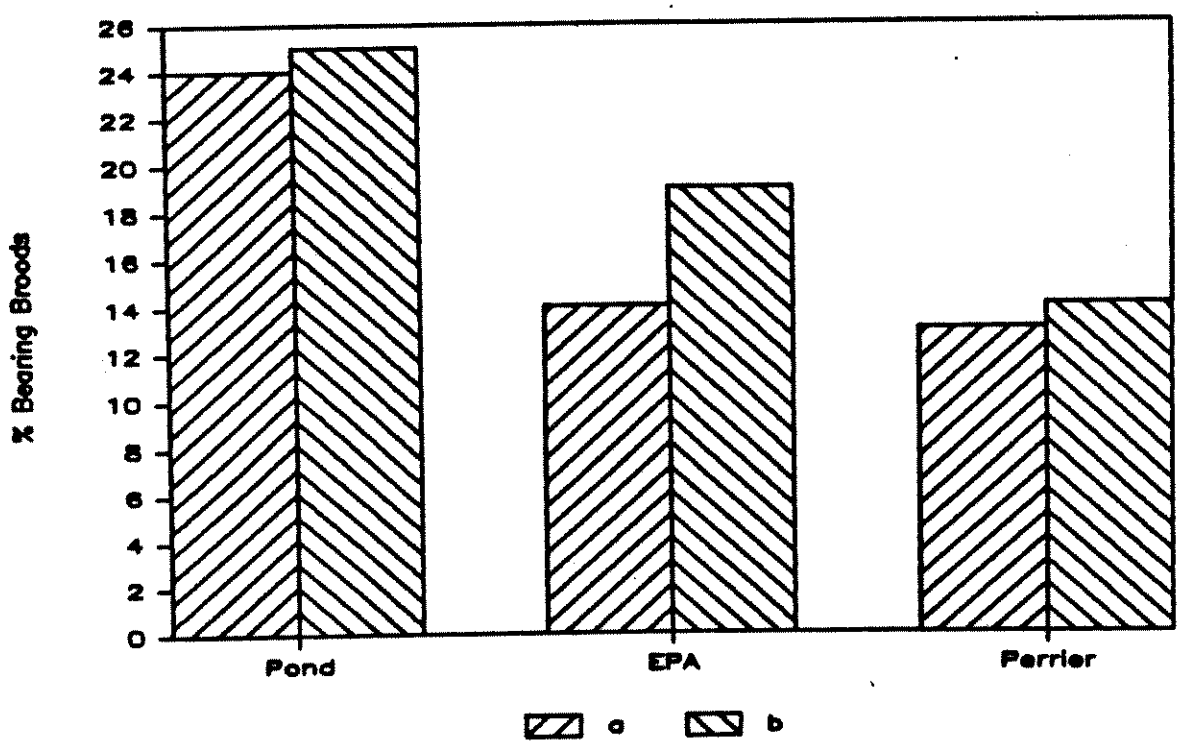

Figure 2. Ceriodaphnia dubia bearing broods in three types of water. Initial n = Fig. 1. a = total number of broods per treatment, divided by the initial number of animals in the treatment multiplied by the number of days in the experiment. b = total number of broods per treatment, divided by the sum of all days each animal in the treatment was alive.

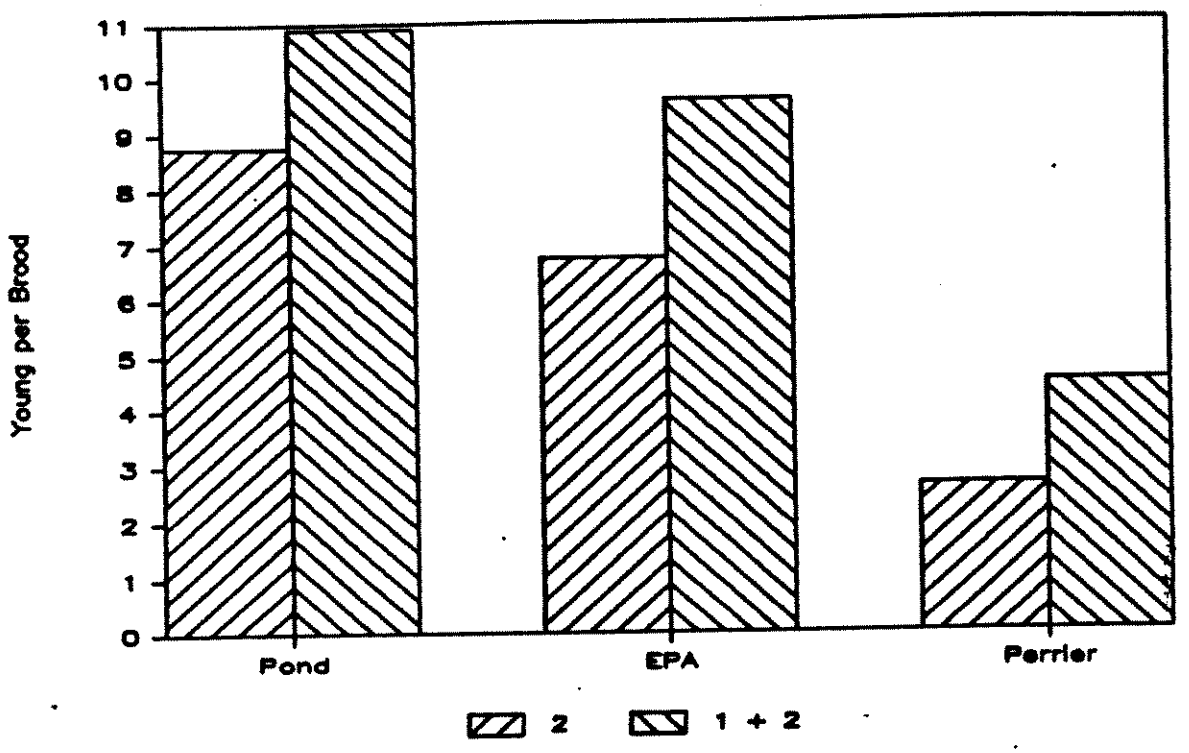

Figure 3. Number of young per brood and per two broods in Ceriodaphnia dubia in three types of water. 2 = brood 2. 1 + 2 = broods 1 + 2. Second brood n and standard deviations: Pond = 8, 1.98; EPA = 15, 2.29; Perrier = 8, 1.27.

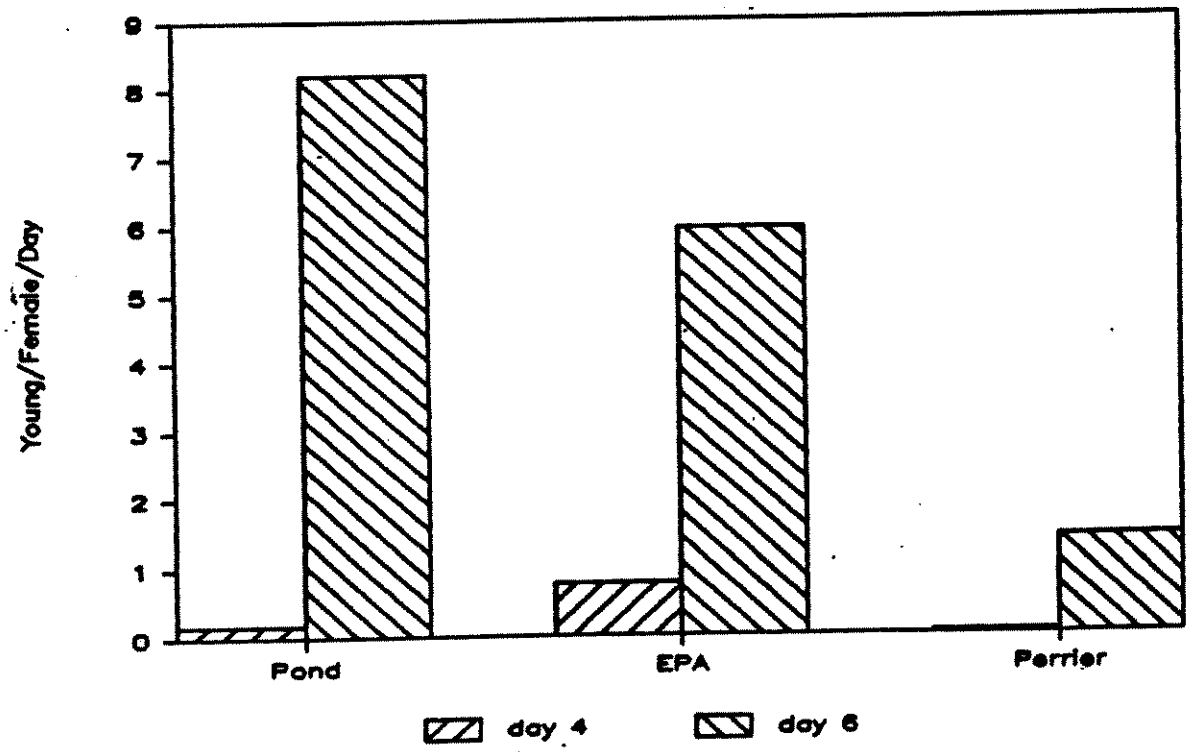

Figure 4. Number of young per female per day in Ceriodaphnia dubia in three types of water. Day 6 n and standard deviations: Pond = 18, 2.89; EPA = 17, 3.11; Perrier = 28, 1.48.

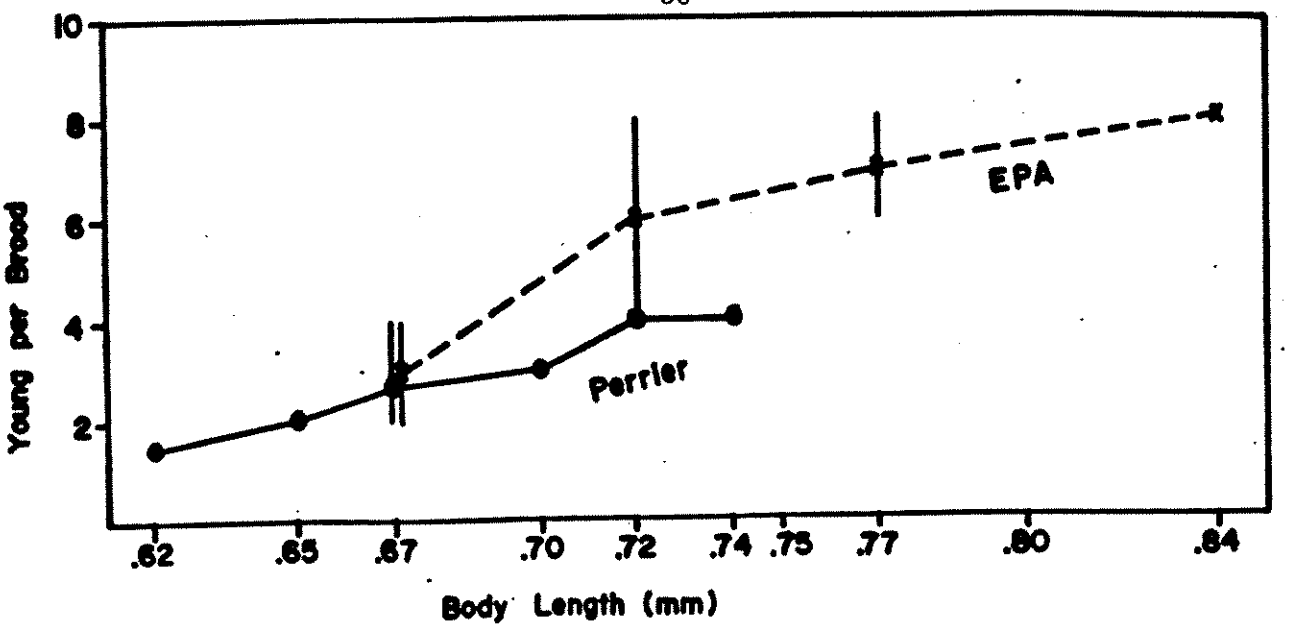

Figure 5. Number of young per brood in Ceriodaphnia dubia plotted against Ceriodaphnia body length for two types of reconstituted water. Data taken after six days of treatment. Vertical bars represent ranges of young per brood.

mixture, in which the Perrier provides trace elements, produced responses similar to those obtained in EPA water without Perrier. It is possible that the composition of the EPA water is essentially correct with respect to the major chemical elements, but that the compounds they form are toxic when the elements are added to water according to the EPA recipe (US EPA, 1985). The Perrier water may be less toxic than the EPA water, requiring only increased levels of some major ions in order to bring about reproduction in Ceriodaphnia. Both types of reconstituted water are low in the important elements phosphorus and potassium, relative to concentrations in the pond water. Future work will include studies of the effects of different concentrations of P and K, as well as the role of organic substances, on the demographics of Ceriodaphnia.

Major water quality characteristics such as hardness and alkalinity probably had no effects in our experiments. Hardness and alkalinity increased with increases in the hypothesized quality of the waters across treatments, but the total ranges of hardness and alkalinity were relatively small. Using water from Lake Huron, Cowgill et al. (1985) obtained C. dubia survival and reproduction data similar to the data we obtained from the pond water treatment. The lake Huron water was moderately soft, 68% as hard our diluted pond water and approximately equal in hardness to the middle of the range in our treatments.

Other factors such as spreading treatments over several experiments had no effect. The assumption involved in spreading treatments, i.e., that day-to-day variability within a relatively short period of time would not be a factor (e.g., Sokal and Rohlf, 1969), was correct.

The EPA and Perrier reconstituted waters are clearly unacceptable choices for culturing and testing at present. Surface waters which are well defined chemically provide one alternative, at least until we have arrived at a suitable formula for reconstituted water. In order to arrive a such a formula, we must understand more about the effects of reconstituted waters on test organisms.

The paucity of published data suggests that the scientific community still knows little of the biology of C. dubia. Thus, there is not much of a scientific basis for using it as a toxicity test organism. Ceriodaphnia dubia has become the freshwater chronic toxicity test zooplankter continent-wide, simply because investigators found it in cultures with another zooplankter, and it exhibited a few biological characteristics which they deemed desirable for culturing and testing. In this case, scientific investigation of the organism, which should be a major intermediate step in the process of test organism selection, has been with few exceptions (e.g., Cowgill et al., 1985) almost totally bypassed. The EPA water represents a similar situation with respect to reconstituted culture and dilution waters. Ceriodaphnia dubia may well prove to be a suitable, even sophisticated, 'coal mine canary' for many types of toxicity testing. However, the biology of this and other species should be well characterized scientifically before they are universally accepted as standard test organisms.

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APPENDIX 1. Some trace elements occurring in diluted (Milli-Q®) pond and Perrier waters, as indicated by spectroscopy analyses. The units are mgl^{-1}.

Element	Pond	Perrier
Ag	<0.001	<0.001
Al	0.063	0.015
B	<0.05	<0.05
Ba	0.043	0.003
Be	<0.001	<0.001
Ca	48.	14.
Cd	<0.001	<0.001
Co	<0.001	<0.001
Cr	<0.001	0.003
Cu	0.004	0.002
Fe	0.017	0.015
K	4.4	<0.02
Mg	20.	0.33
Mn	<0.001	<0.001
Mo	<0.005	<0.005
Na	42.	0.8
Ni	0.002	<0.001
P	0.52	0.05
Pb	<0.005	<0.005
Se	<0.001	<0.001
Si (soluable)	<0.2	<0.2
Ti	<0.001	<0.001
V	<0.01	<0.01
W	<0.005	<0.005
Zn	0.017	0.002

APPLICATION DES LIMITES DE DETECTION ET QUANTIFICATION POUR LE CONTROLE DE QUALITE DE BIOTESTS CONVENTIONNELS (Microtox, Algues et Daphnies).

THELLEN C. & G. JOUBERT

ENVIRONNEMENT QUEBEC
DIRECTION DES LABORATOIRES
STE-FOY, QUEBEC, G1P 3W8

Afin d'augmenter la fiabilité des biotests conventionnels utilisés dans la gestion de l'environnement, un contrôle de qualité intralaboratoire (QA/QC) doit être mis en place. A cet égard, nous avons cherché à déterminer les limites de détection et de quantification pour les biotests avec Microtox, Algues et Daphnies et ce, à partir des principes appliqués aux méthodes d'analyses chimiques. Les différentes étapes menant à la détermination de ces limites seront présentées. Essentiellement, le calcul doit tenir compte de la réponse biologique des témoins provenant d'une même population et des mêmes conditions expérimentales (même manipulateur; même jour; même modalité d'incubation). Les données compilées sur une base annuelle situent les limites de détection à 12,5 ± 4,2%, 11,4 ± 4,2% et 9,5 ± 3,1% pour les biotests Microtox, Algues et Daphnies respectivement. Les limites de quantification ont aussi été compilées et intégrées aux déterminations de toxicité. Des critères d'acceptabilité pour chaque méthode sont donc proposés en fonction des méthodes statistiques utilisées. Un contrôle de qualité à partir de toxiques de référence sera enfin présenté sous forme de chartes de contrôle. L'ensemble de ces données seront discutées dans un contexte d'assurance de qualité des méthodes bioanalytiques.

SYSTEMES CELLULLAIRES DANS L'EVALUATION TOXICOLOGIQUE: LE PRESENT ET LE FUTUR

FRANCINE DENIZEAU

DEPARTEMENT DE CHIMIE
UNIVERSITE DU QUEBEC A MONTREAL
QUEBEC, CANADA

INTRODUCTION

Il y a une vingtaine d'années, s'amorçaient d'importants efforts de développement de modèles cellulaires destinés à l'évaluation toxicologique. Des objectifs ambitieux étaient alors visés. Il était anticipé que la mise au point de nouveaux outils, simples et peu coûteux, rendrait possible l'évaluation efficace de la nocivité de milliers de substances chimiques. Dans cette démarche, une attention toute spéciale était portée à la détection rapide du potentiel cancérogène. Le bilan qui peut être dressé aujourd'hui quant à la performance des essais *in vitro* est dans l'ensemble positif, bien qu'il faille reconnaître que certaines attentes initiales n'aient pas été pleinement satisfaites. Cependant, à en juger par le nombre croissant de publications dans le domaine, il ne fait pas de doute que l'intérêt manifesté à l'égard de celui-ci ne cesse de s'affirmer. Récemment, le lancement de nouveaux périodiques, dont le champ est précisément la recherche en toxicologie sur les modèles cellulaires venait confirmer ce phénomène (1,2).

L'ampleur des sommes consacrées à la recherche et au développement dans ce secteur constituent également une marque de l'importance qui lui est accordée. A titre d'exemple, citons le cas du NTP (National Toxicology Program) américain. Il s'agit d'un programme qui coordonne les activités des grands organismes qui sont, à l'échelle nationale, les intervenants majeurs en matière de toxicologie. Les données contenues dans les plans annuels du NTP rendent possible l'appréciation des efforts globaux qui sont consentis. Recemment, en 1985 (3), ce programme allouait 52 millions de dollars à la réalisation de tests, 15 millions au développement de méthodes et 5.9 millions à la validation de méthodes déjà développées. Pour chacun de ces aspects, on note que la mutagénicité, qui porte presqu'exclusivement sur les systèmes *in vitro*

(systèmes microbiens et cellules de mammifères), reçoit une large proportion des fonds.

A ce jour, nous sommes donc à même de constater un degré d'avancement fermement établi dans le domaine de l'évaluation toxicologique pratiquée sur des cultures de tissus et de cellules. La puissance des systèmes *in vitro* repose sur un ensemble d'atouts uniques qui ont grandement contribué à leur pouvoir d'attraction:

- simplicité
- rapidité
- économie
- compatibilité avec échantillons environnementaux complexes
- pertinence à l'égard de cibles spécifiques
- possibilité de réduire l'utilisation d'animaux
- possibilité d'étudier les interactions moléculaires et les mécanismes d'action des toxiques.

Cette conjoncture favorable ne doit cependant pas nous empêcher de procéder à un examen objectif des forces et des limitations des systèmes en cause, et ainsi, de dégager les orientations futures les plus prometteuses.

En relation avec le type d'évaluation réalisée, les modèles *in vitro* peuvent être regroupés en principales catégories (4): *la première*, qui concerne la cytotoxicité générale; *la seconde*, qui s'adresse aux effets à portée spécifique sur des organes ou systèmes cibles; *la troisième* qui vise la détection du potentiel cancérigène. La présente discussion portera brièvement sur les essais de cytotoxicité et ceux relatifs au potentiel cancérigène.

TESTS DE CYTOTOXICITE

La cytotoxité peut être définie comme étant l'induction de lésions cellulaires par les agresseurs chimiques (5). Elle s'applique tant à l'examen des perturbations des fonctions de base des cellules (cytotoxicité générale) qu'à la recherche d'effets sur des fonctions spécialisées. Ces deux aspects sont cependant reliés, puisqu'une altération des premières fonctions est la plupart du temps susceptible d'avoir une influence sur les secondes.

En ce qui a trait aux fonctions de base, les paramètres les plus souvent retenus estiment soit la croissance cellulaire, l'activité de synthèse des macromolécules (protéines, acides nucléiques) ou la perte d'intégrité de la membrane plasmique. Puisqu'il s'agit là de phénomènes non spécifiques, les essais les impliquant sont en général réalisés sur des cellules moins différenciées. Habituellement, des lignées cellulaires sont mises à profit, parce que leur culture à long terme est plus aisée et qu'elles fournissent un matériel assez uniforme d'un test à l'autre. Le Tableau I présente un éventail de types cellulaires sur lesquels des indicateurs de cytotoxicité ont été mesurés.

En ce qui concerne plus spécifiquement l'évaluation à caractère écotoxicologique, les types de cellules fréquemment employés ont été dérivés d'organismes aquatiques, notamment de la truite arc-en-ciel. Des lignées cellulaires obtenues à partir des gonades (RTG-2) (6) ainsi que les hépatocytes isolés (7) ont servi à développer des systèmes performants.

Les essais de cytotoxicité trouvent leur fondement principal dans le concept de répercussions en chaîne: il est tout à fait raisonnable de croire qu'une atteinte grave et immédiate au niveau de cellules aura comme conséquence une atteinte au niveau de l'organe et éventuellement de l'organisme entier et même des populations d'organismes.

L'évaluation de la cytotoxicité *in vitro* trouve son parallèle *in vivo* dans les études de toxicité aiguë pour laquelle la notion du

LD_{50} est centrale. On se demande alors, en rapport avec la correspondance *in vivo-in vitro*, si la concentration cytotoxique pour une substance chimique peut être mise en correlation avec la valeur numérique du LD_{50}. Au cours des dernières années, des stratégies ont été élaborées dans le but d'apporter de la lumière sur cette question. Par exemple, Ekwall et ses collaborateurs (8) ont travaillé sur un indice spécifique de dosage létal, le SI_{id}. Celui-ci est obtenu en faisant le rapport de la concentration inhibitrice médiane déterminée sur des cellules HeLa après 7 jours d'incubation (IC_{50}), au LD_{50} estimé à partir d'injections intraveineuses chez la souris. Selon ce modèle, la présence d'une toxicité similaire *in vitro* et *in vivo*, c'est-à-dire d'une toxicité basale ne s'adressant pas à un organe spécifique, se traduirait par un SI_{id} de faible valeur (dosage *in vitro* relativement plus faible) alors qu'en comparaison un SI_{id} de valeur élevée suggèrerait une action sur un organe cible. Une telle approche de validation a été appliquée à plus d'une centaine d'agents, dont un bon nombre de médicaments ou drogues. Les valeurs de SIId pour 3 substances sont présentées ici à titre d'illustration. (Tableau II) (8). La valeur basse de l'indice pour la quinidine indiquerait une action sur plusieurs organes en plus du cerveau, alors que celle des deux autres composés serait plus spécifique à ce dernier. La conclusion générale des résultats de cette validation propose que les cultures cellulaires puissent être utilisées pour tester la toxicité "basale" des substances chimiques.

Bien que les stratégies du type de celle qui vient d'être exposée fassent, avec grand mérite, figure d'entreprise de pionnier, elles ne répondent certes pas à toutes les interrogations et suscitent une réflexion à l'égard de plusieurs points (9).

1) Est-il vraiment réaliste d'attendre une correlation simple entre la toxicité "unicellulaire" *in vitro* et la toxicité systémique pour

laquelle l'influence de la pharmacocinétique et le profil métabolique des substances, difficiles à reproduire *in vitro*, sont souvent des aspects déterminants.

2) Lors des discussions sur les méthodes dites alternatives, la base de données *in vivo* servant aux fins de comparaison est-elle adéquate; la toxicité aiguë n'est-elle pas trop étroitement identifiée à la détermination du LD_{50}.

3) Dans le contexte de préoccupations écotoxicologiques, il est pertinent de se demander si les concentrations de contaminants dans l'environnement trouvent leur équivalence *in vitro*?

A ce stade d'avancement, il peut être jugé essentiel de poursuivre nos efforts de validation dans l'optique de mieux cerner les conditions pour atteindre une correspondance *in vivo - in vitro* satisfaisante, ceci dans le but d'acquérir des outils à valeur prédictive, utilisables sur une base quantitative plutôt que qualitative, comme cela semble être le cas à l'heure actuelle.

Une telle orientation pour le futur apparaît raisonnable. Cependant, il ne faut pas perdre de vue que les systèmes *in vitro*, au stade actuel de leur développement, fournissent déjà des moyens tout à fait uniques d'obtenir de l'information concernant les évènements qui définissent les mécanismes d'action à l'échelle cellulaire et subcellulaire, ce qui est difficilement accessible avec les animaux entiers. A travers le cheminement des dernières années, les systèmes *in vitro* ont acquis une "personnalité" qui leur est propre. Ainsi, pour que le plus juste profit en soit tiré, ils ne devraient plus être strictement considérés comme des outils de remplacement des modèles existants, mais aussi, comme des outils à part entière, fournissant une approche nouvelle d'une tout autre dimension. Appliqués judicieusement, les systèmes *in vitro* pourraient même servir de guides efficaces dans l'investigation des effets toxiques *in vivo*.

POTENTIEL CANCERIGENE

Dans la seconde partie de la présente discussion, il convient de porter une attention toute spéciale aux systèmes conçus pour la détection du potentiel cancérigène. En effet, c'est avec ces systèmes qu'a été constituée la banque de données la plus considérable, ce qui réflète l'intérêt soutenu dont ils furent l'objet. Les tests de cancérogénicité dits à court terme forment quatre classes principales qui se distinguent d'après la nature des paramètres mesurés. Ceux-ci suivent soit l'induction 1) de mutation, 2) de dommages primaires à l'ADN, 3) d'effets chromosomiques ou 4) de la transformation cellulaire (10). Ces tests sont réalisés tant sur des cellules eucaryotes que procaryotes. On en a dénombré plus d'une centaine en utilisation dans divers laboratoires à travers le monde. Cependant, une sélection s'est effectuée en faveur d'un minimum d'entre eux qui sont devenus d'application répandue. Parmi les plus importants, mentionnons le test d'Ames, qui détecte une réversion de mutation sur la bactérie *Salmonella thyphimurium*; les tests sur des cellules de mammifères, le plus souvent de hamster chinois (V79, CHO) ou de souris (L517Y8), qui suivent l'apparition de mutations à des locus spécifiques, les aberrations chromosomiques ou l'échange de chromatides soeurs; les tests qui mesurent les dommages et la réparation de l'ADN - dont le système hepatocyte/synthèse non programmée de l'ADN; les systèmes de transformation cellulaire *in vitro* sur des lignées de fibroblastes (C3H/10T1/2, BALB/c 3T3).

En rapport avec les essais en écotoxicologie, aucun système cellulaire spécifique de détection du potentiel cancérigène n'a encore été suffisamment développé pour que soit atteint le stade d'une utilisation courante. Toutefois, il semble que ce soit avec les cellules de poisson que le plus de travail ait été réalisé, incluant les hépatocytes de truite arc-en-ciel (22, 23). Dans ces systèmes, mise à part l'origine différente des cellules, les approches ont été les mêmes qu'avec les autres systèmes:

induction de mutation, échange de chromatides soeurs, synthèse de réparation de l'ADN ont servi d'indicateurs de génotoxicité (22, 23).

Comme nous l'avons précédemment évoqué, ces tests de potentiel cancérigène *in vitro* sont sortis gagnants à cause de plusieurs de leurs caractéristiques nettement avantageuses. Ils sont tout d'abord économiques, en comparaison avec les modèles animaux traditionnels. A l'heure actuelle, la réalisation des tests de mutagénicité ou de cancérogénicité chez des animaux intacts coûte entre 250,000 et 1, 000,000 $ par produit (11). De plus, les installations requises pour les protocoles impliqués sont sophistiquées et se retrouvent à peu d'exemplaires dans le monde. Il n'est donc pas réaliste d'envisager des études à grande échelle sur les animaux, tant pour les produits purs que pour les échantillons environnementaux complexes, même si cela était la meilleure approche pour déceler les sources potentielles de risque pour l'homme. Pour leur part, les tests à court terme présentent des coûts qui peuvent varier entre 1 000$ et 10,000$ par produit (11), ce qui est beaucoup moindre qu'avec les premiers. Reliée à la notion de coût est celle de la rapidité. Les tests à court terme fournissent des réponses sur une période de quelques heures, sauf pour les essais de transformation qui requièrent quelques semaines. L'évaluation sur les animaux nécessite des temps d'attente en général de l'ordre de plusieurs mois à plusieurs années.

Malgré les avantages que nous venons de décrire, il convient de nous intérroger sur la valeur ultime des essais de cancérogénicité *in vitro*, c'est-à-dire sur leur capacité réelle de prédire les risques avec justesse. La question fondamentale de la correspondance entre les résultats *in vitro* et les effets *in vivo* a suscité d'importants efforts de validation, et au cours de la dernière décennie, plusieurs études ont été menées dans cette perspective. Les études de validation ont porté sur des séries de composés, pour lesquels des données concernant leur cancérogénicité (ou non cancérogénicité) "in vivo" étaient disponibles;

elles ont comparé ces données à celles obtenues avec une sélection de tests *in vitro.* Deux indices utiles de performance ont été employés, soit *la sensibilité*, représentant la proportion de résultats positifs parmi les cancérigènes testés, et la *spécificité*, représentant la proportion de résultats négatifs parmi les non cancérigènes testés (12). Le Tableau (III), bien qu'il ne présente malheureusement pas une revue exhaustive, fait référence à une suite d'études d'envergure ayant eu trait à la validation (avec le test d'Ames) depuis 1975. Entre autres récemment, Shelby et Stasiewicz (12) ont retenu 70 composés n'ayant pas fourni d'évidence d'induction du cancer sur les rats et souris, mâles ou femelles. Le calcul de l'indice de spécificité réalisé à partir de leur compilation donne des valeurs qui sont assez éloignées de la valeur optimale de 100%. Il y aurait donc ici un nombre assez élevé de "faux positifs". Appliquant une approche similaire, Dunkel et coll. (13) ont sélectionné un total de 63 substances et évalué leur mutagénicité à l'aide de systèmes microbiens. Ici, 25 cancérigènes sur 33 ont donné une réponse positive, alors que 14 non cancérigènes sur 20 ont été négatifs.

Tout dernièrement, les résultats d'une évaluation approfondie des relations entre 4 mesures de génotoxicité *in vitro* (par des tests à court terme bien connus) et la capacité de substances chimiques d'induire la néoplasie chez les rongeurs, étaient publiés (Tennant et coll., 1987). Ces résultats montraient qu'une faible proportion (environ 50%) des cancérigènes furent détectés comme mutagènes *in vitro.*

Ce qui frappe, à l'examen des exemples précédents et également à la lumière de l'ensemble des valeurs des indices de performance, des tests de cancérogénicité, ce sont les écarts certains qui découlent des évaluations *in vitro* et *in vivo.* Une telle situation n'est pas sans soulever à nouveau d'importantes interrogations. Les spécialistes du domaine ont discuté de multiples difficultés qui pourraient empêcher une correspondance parfaite:

1) Le métabolisme des toxiques recréé *in vitro* (e.g. par des fractions microsomiques) est-il suffisamment près de celui qui se produit *in vivo*?

2) Les données *in vivo* servant à la comparaison sont-elles toujours elles-mêmes sûres? Les critères de cancérogénicité sont-ils constants? De plus, il semble plus facile de démontrer avec certitude la cancérogénicité que la non cancérogénicité. La question de la spécificité serait donc plus délicate à élaborer. Dans une perspective d'évaluation des essais de toxicologie génétique et ainsi en réponse à un urgent besoin, le groupe du "Gene-Tox Program" américain procédait récemment à l'établissement d'une vaste base de données "in vivo", à partir d'une revue et analyse en profondeur de la littérature pertinente (14).

3) Une sélection plus judicieuse des tests constituant la "batterie" amènerait-elle une meilleure prédictivité? Par exemple, les données rapportées par Shelby et Stasiewicz (11) ont été reprises par Ennever et Rosenkranz (15) et analysées avec une batterie de composition différente. Celle-ci a fourni une spécificité de 80% au lieu de 50%.

4) Quelle est la signification biologique des paramètres mesurés dans les tests? Pour ce qui est de la mutagénicité, une justification historique est fournie par la théorie de la mutation somatique du cancer. Cependant, cette théorie est-elle parfaitement cohérente à l'égard de tous les aspects de la cancérogénèse. Plusieurs agents pour lesquels aucune évidence d'interaction avec l'ADN n'existe, sont capables de produire des tumeurs.

5) Le point précédent conduit directement à la question cruciale des mécanismes de la cancérogénèse: notre compréhension de ces mécanismes est-elle assez détaillée. Nos connaissances fondamentales fournissent-elles une base assez solide, nécessaire au succès des efforts de recherche appliquée? De l'avis de plusieurs scientifiques, l'approfondissement des mécanismes est au coeur de la problématique.

C'est là que nos chances de progrès les plus significatifs seraient les meilleures. Récemment, Flamm et Lorentzen (16) étaient invités à commenter sur l'utilisation des méthodes *in vitro* dans l'évaluation des toxiques à des fins sécuritaires. Ces auteurs terminaient leur propos en indiquant que c'est par rapport aux mécanismes d'action que l'avenir de la toxicologie *in vitro* "brille avec le plus d'éclat" et ceci s'applique tout particulièrement au domaine de la cancérogénèse chimique. Il s'agit là de l'expression d'un consensus qu semble se dessiner à l'heure actuelle au sein de la communauté scientifique. Il apparait donc que, même si l'impact des systèmes *in vitro* n'avait été que de nous conduire à une vision plus juste des voies à poursuivre, les efforts pour les développer auraient été pleinement légitimés.

CONCLUSION

Dans le contexte de l'évaluation toxicologique, les modèles *in vitro* offrent une opportunité unique de consolider et d'accroître nos acquis. Un processus interactif *in vivo - in vitro* a été mis en branle; l'apport réciproque et dynamique d'une méthodologie au profit de l'autre constitue un potentiel de progrès des plus considérables pour l'avenir.

A propos de l'utilisation d'animaux de laboratoire, Goldberg (17), Balls (4) et d'autres scientifiques ont fait référence à ce qu'il pourrait être convenu d'appeler le "trio" des R pour exprimer la nécessité d'une rationalisation des ressources: Réduction, Raffinement et Remplacement. Dans ce contexte, les modèles *in vitro* prennent une signification particulière. Ils rendent sans contredit possible la réduction du nombre d'animaux, incitent au raffinement de nos protocoles expérimentaux et dans certains cas, pourront amener un remplacement peut-être complet. Néanmoins, l'activité en ce domaine ne pourra que puiser son inspiration

des efforts de recherche pour laquelle la méthodologie *in vitro* est parfaitement indiquée, comme nous l'avons déjà fait valoir en soulignant la question de l'élucidation des mécanismes d'action.

De plus, au cours des dernières années, le champ de la culture des cellules a connu un avancement marqué. Par exemple, des milieux exempts de serum, de composition définie ont pu être mis au point grâce à une meilleure connaissance, entre autres, des facteurs de croissance; la performance des substrats a pu être améliorée par l'emploi de composants de la matrice extra-cellulaire, qui ont pu être purifiés et mieux caractérisés. Il ne fait pas de doute que ces résultats de recherche auront un impact favorable sur le perfectionnement des outils de la toxicologie développée sur les modèles cellulaires *in vitro*.

Enfin, il est à souhaiter que la règlementation, dont l'objectif est d'assurer la meilleure protection de la santé de l'homme et de son environnement, s'appuie de plus en plus sur les progrès réalisés dans le domaine de l'évaluation toxicologie. Au Canada, les voies récemment proposées par les Ministères de l'Environnement et de la Santé et du Bien-Etre Social, qui retiennent l'utilisation des tests de mutagénicité pour l'évaluation toxicologique des produits chimiques (11), sont un encouragement en ce sens.

Au trio premier, pourraient donc légitimement s'ajouter deux nouveaux membres: Recherche et Réglementation.

TABLEAU I

TYPES DE CELLULES PERFORMANTS POUR LA CYTOTOXICITE

CULTURES PRIMAIRES	LIGNEES
MACROPHAGES PULMONAIRES	HeLa
EPITHELIALES DE TRACHEE	NCTC 2544
HEPATOCYTES	WI-38
	CHO
	V79
	RTG-2

TABLEAU II

CYTOTOXICITE: EXEMPLE D'APPROCHE DE CORRELATION *IN VIVO - IN VITRO*

SUBSTANCE	SI_{ld}
METHADONE	6
PHENOBARBITAL	3
QUINIDINE	0.2

Réf. Ekwall. B. Ann. N.Y. Acad. Sci. 407, 64-77 (1983) (8).

TABLEAU III

PERFORMANCE DES ESSAIS DE POTENTIEL CANCERIGENE

ANNEE	GROUPE	PRODUITS n	SENSIBILITE %	SPECIFICITE %
1975	McCann et coll. (*) (18)	30	90	87
1976	Sugimura et coll. (*) (19)	241	93	77
1981	Bridges et coll. (*) (20)	42	45-70	60-80
1982	EPA Gene Tox (*)	122	63	
1984	Shelby et Stasiewicz (12)	70		< 50
1985	Dunkel et coll. (13)	60	80	70
1987	Tennant et coll. (24)	73	45	86

* Données présentées par Brusick, D. Ann. N.Y. Acad. Sci. 407, 164-176 (1983) (21).

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FOURTH SESSION/QUATRIEME SEANCE

TOXIC EFFECTS IN AQUATIC ORGANISMS/
EFFETS TOXIQUES SUR LES ORGANISMES AQUATIQUES

CHAIRPERSON: Dr. C. Blaise, Environnement Canada, Longueuil, PQ
MODERATOR: Dr. J.S.S. Lakshminarayama, University of Moncton
Moncton, NB

L'ANTAGONISME SELENIUM MERCURE CHEZ LES INVERTEBRES MARINS: MYTHE OU REALITE?

Emilien Pelletier
Institut National de la Recherche Scientifique
INRS-Océanologie, 310 allée des Ursulines, Rimouski, Québec G5L 3A5

INTRODUCTION

Le Conseil canadien de la recherche sur l'évaluation environnementale, chargé de conseiller les gouvernements et l'industrie sur l'évaluation des incidences environnementales (EIE), a produit récemment un document synthèse sur les grandes orientations de recherche en matière d'évaluation des effets cumulatifs (CCREE, 1988). Ce document, bref et dense, a le mérite de tenter de définir et de classifier les effets cumulatifs environnementaux malgré des difficultés typologiques évidentes. Le phénomène des effets cumulatifs est connu depuis longtemps mais il a souvent été décrit en termes différents selon l'approche scientifique utilisée ou selon la nature même du phénomène étudié (effet de serre, pluies acides, toxicité d'effluents industriels, etc...). Parmi les effets cumulatifs les moins difficiles à cerner et à définir, on note les effets itératifs (incidences fréquentes et répétitives sur un même écosystème), les effets avec seuil de tolérance (incidences sur les processus écologiques) et les effets combinés (effets synergiques ou antagonistes). L'évaluation des effets cumulatifs présente un défi de taille aux chercheurs autant au point de vue méthodologique (définition des niveaux de base, choix des organismes contrôles,...) qu'opérationnel (détection des effets, longueur des expériences,...). Dans certains cas, il est cependant possible de définir une approche réductionniste viable qui permette d'étudier les effets combinés de plus d'un processus sur, par exemple, un seul organisme à la fois en tentant de garder les autres facteurs constants ou à incidence minimale. Le cas de l'antagonisme sélénium-mercure traité ci-après se prête bien à ce type de recherche fondamentale.

L'idée qu'il puisse exister dans les écosystèmes naturels des mécanismes de protection et de défense contre l'agression de certaines substances toxiques (anthropiques ou non) continue de fasciner les écotoxicologistes. L'antagonisme sélénium-mercure, découvert à la fin des années 60 (Parizek et Ostadalova, 1967), est un exemple typique de ce genre de mécanisme. Cet antagonisme a été reconnu chez les rats et lapins de laboratoire, quelques volailles, une algue phytoplanctonique et peut-être chez quelques mammifères marins (Pelletier, 1985). L'effet antagoniste du sélénium a été défini par Magos et Webb (1980) comme "une protection effective contre la toxicité aigue du mercure et de ses dérivés". Il importe ici de distinguer entre un antagonisme réel tel que défini ci-haut et une interaction chimique ou biochimique qui pourrait avoir des effets divers autres qu'antagonistes ou même synergiques.

Au cours des cinq dernières années, nous avons travaillé à identifier les effets cumulatifs du sélénium et du mercure, sous différentes formes chimiques, chez quelques invertébrés marins avec une attention toute particulière à l'existence d'un antagonisme sélénium-mercure similaire à celui observé chez plusieurs vertébrés.

BIOACCUMULATION DU SELENIUM ET DU MERCURE CHEZ Mytilus edulis

Le premier travail de ce programme de recherche a été une étude des effets cumulatifs liés au processus de la bioaccumulation simultanée du sélénium et du mercure chez un mollusque marin filtreur, Mytilus edulis, en utilisant des doses quasi-léthales de mercure (Pelletier, 1986). Nous avons mesuré les vitesses de bioaccumulation du sélénite de sodium dissous et d'un sélénium organique (le disélénium de bis(2-carboxybenzyl), et nous avons déterminé les effets de la présence de mercure inorganique dissous et de méthyl mercure dans le milieu. L'étude des effets individuels et combinés de ces quatre composés chimiques a nécessité l'utilisation de 9 bacs expérimentaux de 11 L chacun (incluant un contrôle) à circulation en continu d'eau de mer pendant 50 jours.

On observe d'abord que les moules accumulent lentement le sélénium inorganique (0,12 $ng.g^{-1}.d^{-1}$) administré seul à raison d'une forte dose de 50 $ug.L^{-1}$ tandis que le sélénium organique seul n'est pas accumulé. Quand du mercure inorganique (30 $ug.L^{-1}$) est ajouté à la circulation d'eau, l'accumulation du sélénium inorganique double sa valeur alors que cette vitesse est triplée (0,40 $ng.g^{-1}.d^{-1}$) si on ajoute du méthyl mercure à une concentration 3 $ug.g^{-1}$. Même le sélénium organique devient biodisponible en présence de méthyl mercure et son taux d'assimilation passe de zéro à 0,15 $ng.g^{-1}.d^{-1}$. Cependant, le phénomène n'est pas réciproque et la présence du sélénium n'a pas eu d'effet sur les vitesses d'accumulation du mercure quelles que soient la concentration et la nature chimique du sélénium ajouté. Aucun effet toxique aigu n'a été observé pour le sélénium seul mais la toxicité aigue du mercure a été évidente dans tous les cas. Le sélénium n'a montré aucun des effets antagonistes déjà observés chez d'autres organismes. Ces résultats plutôt encourageants, au moins au chapitre des interactions, nous ont poussé à poursuivre l'investigation au niveau des mécanismes de bioaccumulation du mercure et du sélénium.

ASSIMILATION ET TOXICITE AIGUE DES COMPLEXES DU METHYL MERCURE

La deuxième série d'expériences dans cette étude a porté sur la toxicité aigue à moyen terme (32 jours) de certains complexes du méthylmercure et la recherche d'une possible activité protectrice du sélénium dissous et organique (Pelletier, 1988). Les principaux résultats de cette expérience peuvent se résumer ainsi:

1) les différentes formes chimiques du méthyl mercure, possédant des solubilités différentes dans l'eau de mer, ont toutes été accumulées par la moules;
2) la charge corporelle totale en mercure n'est pas influencée par la présence du sélénium et le sélénium (faible dosage) ne fut pas bioaccumulé de façon significative quelle que soit sa forme chimique;
3) la forte toxicité de tous les complexes du méthyl mercure a été observée après 4 semaines d'exposition à une dose intermédiaire de 3 $ug.L^{-1}$;
4) comme dans la première série de bioessais, le sélénium n'a montré aucun effet antagoniste évident mais la proportion relative du mercure accumulé dans le manteau de la moule a été légèrement mais significativement accrue en présence de sélénium dissous dans l'eau de mer.

L'un des aspects le plus intéressant de l'interprétation de ces résultats a été la possibilité de discuter deux mécanismes d'assimilation des complexes du méthyl mercure selon que ceux-ci sont dissous ou particulaires dans l'eau filtrée par la moule. Le premier mécanisme proposé s'applique à un composé dissous et implique le déplacement direct d'un ligand (-SR) par un autre ligand libre (HS*R) via un intermédiaire à 3 coordinats:

$$CH_3Hg\text{-}SR + HS^*R \rightleftharpoons CH_3Hg\begin{matrix}S^*R\\ \cdot\\ SR\end{matrix} \rightleftharpoons CH_3Hg\text{-}S^*R + HSR \quad (\text{éq.1})$$

Le second mécanisme fait intervenir une dissociation en milieu acide suivie d'une réaction avec un ligand anionique ($^-S^*R$):

$$CH_3Hg\text{-}SR + H^+ \rightleftharpoons CH_3Hg^+ + HSR \quad (\text{éq.2})$$

$$CH_3Hg^+ + {}^-S^*R \rightleftharpoons CH_3Hg\text{-}S^*R \quad (\text{éq.3})$$

Les deux mécanismes conduisent au même résultat final, soit l'assimilation du méthyl mercure mais la prédominance de l'un ou l'autre des deux processus peut avoir des consequences importantes sur les vitesses de biotransfert du mercure complexé. Le premier mécanisme s'applique d'abord au cas où il y a contact entre le complexe dissous et un tissu biologique qui possède des sites d'échange HS*R. Comme ce type de sites est très fréquent et relativement accessible à une molécule dissoute, il y aura incorporation de la molécule étrangère par le mécanisme de l'équation 1. Il faut noter que le sélénium devrait interférer sur ce mécanisme puisse qu'il peut compétitionner pour les mêmes sites HS*R quand il se trouve sous forme réduite pour former un pont R-Se-S*R. Or, l'expérience avec les moules n'a pas permis de confirmer un tel phénomène.

Il devient nécessaire d'introduire le second mécanisme pour expliquer l'accumulation du méthyl mercure à partir d'un complexe insoluble dans l'eau. En effet, l'un des complexes utilisés dans cette étude était complètement insoluble dans l'eau; pourtant, sa vitesse de bioaccumulation a été à peine inférieure à celle du complexe soluble. Le premier mécanisme est peu efficace dans le cas d'un composé insoluble pour des raisons d'encombrement stérique autour du site de liaison; cependant, le deuxième mécanisme est beaucoup plus plausible parce qu'il fait appel à une dissociation en milieu acide. L'ion CH_3Hg^+ devient alors soluble et peut réagir avec un pont S-S ou un ligand anionique. Ce mécanisme ne peut se produire qu'en présence de protons qui initient la première réaction (éq.2) et de telles conditions acides se retrouvent dans le tractus digestif de l'animal. De nouveau, on devrait pouvoir observer une interférence du sélénium sur ce deuxième mécanisme pour les mêmes raisons que le premier. Il n'y a pas d'évidences absolues d'une interaction du sélénium; cependant, on remarque un accroissement significatif de l'entreposage du mercure dans le manteau de la moule par rapport au reste de l'organisme ce qui peut signifier une action quelconque du sélénium au niveau du mécanisme de transport et/ou d'entreposage du mercure.

En conclusion, les résultats de cette expérience ont montré que la moule,

sous des conditions de laboratoire, n'est pas en mesure d'utiliser le sélénium inorganique ou organique pour sa protection contre la toxicité du mercure. A défaut d'un véritable antagonisme chez la moule, nous avons cherché à précuser le type d'interactions observables en poussant notre étude du côté des indicateurs biochimiques du stress environnemental.

REPONSE ENZYMATIQUE AU STRESS TOXIQUE DU SELENIUM ET DU MERCURE

Cette troisième série de bioessais avec la moule a été entreprise avec l'objectif de tenter d'observer les effets individuels et cumulatifs du mercure et du sélénium sur l'activité de la malate déshydrogénase (MDH), une enzyme-clé du métabolisme (Pellerin-Massicotte et Pelletier, 1987; Pellerin-Massicotte, Pelletier, Rouleau et Paquet, 1988). L'utilisation de doses plus faibles de mercure a permis d'observer une nouvelle empreinte (pattern) de bioaccumulation du mercure. Dans le cas d'une dose de 0,3 $ug.g^{-1}$ (10 fois moins élevée que lors des travaux précédents), on observe un taux de bioaccumulation d'environ 0,1 $ug.g^{-1}.d^{-1}$ pour les trois premières semaines suivi d'une stabilisation de la concentration du mercure dans les tissus pour les trois semaines suivantes. Dans le cas d'une dose très faible (0,01 $ug.g^{-1}$), on n'observe aucune bioaccumulation supérieure à 0,5 $ug.g^{-1}$ pendant les 45 jours de l'expérience. Dans ces deux cas, la présence du sélénium inorganique à raison de 125 $ug.L^{-1}$ n'induit aucune difference significative dans les courbes de bioaccumulation.

Par contre, du côté de la MDH, l'activité de l'enzyme et son affinité pour le substrat (Km) sont modifiées de facon significative à la fois par les deux dosages de méthyl mercure (3,0 et 0,3 $ug.g^{-1}$) et aussi de façon encore plus marquée par le sélénium seul (figure 1). De nouveau, la présence du sélénium en combinaison avec le méthyl mercure ne modifie ni l'activité ni l'affinité de l'enzyme par rapport au mercure seul mais, en retour, la présence du mercure semble réduire fortement l'effet perturbateur du sélenium seul.

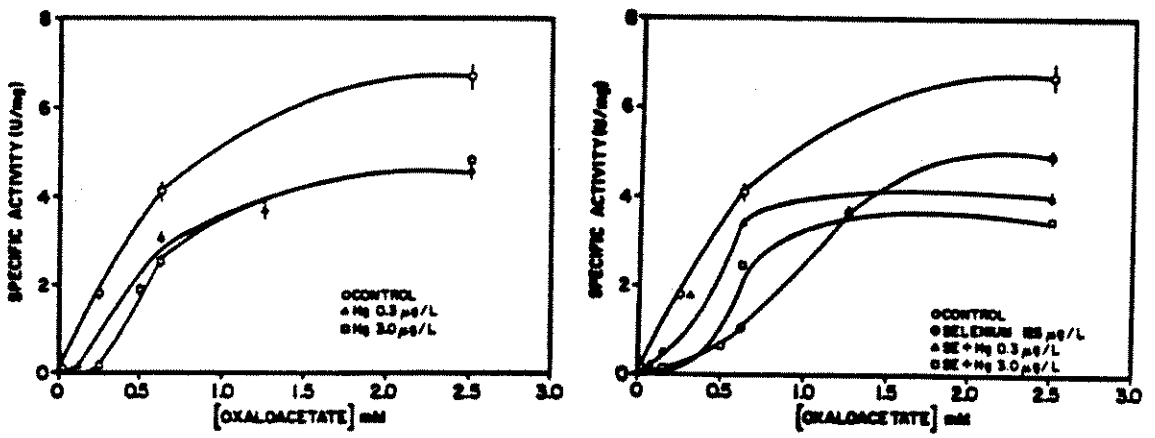

Figure 1: Effets cumulatifs du mercure et du sélénium sur l'activité spécifique de la malate déshydrogénase dans le manteau de la moule.

Ce résultat obtenu presque 4 ans après le début des travaux sur le sujet vient confirmer, d'une part, que c'est le mercure qui exerce une interaction

sur le sélénium et non l'inverse tel que supposé au départ et que, d'autre part, le manteau de la moule est certainement un organe cible au niveau du processus interactif entre le sélénium et le mercure.

BIOACCUMULATION DU SELENIUM ET DU MERCURE CHEZ Pandalus borealis.

Quoique les travaux mentionnés jusqu'ici aient tous portés sur la moule, nous avons aussi exploré le même problème avec la crevette Pandalus borealis que l'on retrouve en abondance dans le golfe Saint-Laurent ainsi que dans le fjord du Saguenay. Or, le Saguenay a été fortement contaminé par le mercure au cours des quarante dernières années et les crevettes qui s'y trouvent possèdent encore des taux de mercure de l'ordre de 0,5 $ug.g^{-1}$ dans leurs tissus (Cossa et Desjardins, 1984; Pelletier, Rouleau et Canuel, 1989). Des crevettes ont été capturées dans le fjord et gardées dans un vivier dans nos laboratoires pendant plusieurs semaines afin de procéder à des expériences de contamination par la nourriture à partir d'une diète contenant différentes doses de méthyl mercure (0,5 à 6 $ug.g^{-1}$) et de sélénium (jusqu'à 2,6 $ug.g^{-1}$) suivant un protocole expérimental similaire à celui utilisé pour les moules. Les empreintes de bioaccumulation du mercure, relativement semblables a celles observées pour les moules ne sont pas non plus modifiées par la présence du sélénium dans la diète. Le sélénium ne s'accumule pas chez la crevette dans les gammes de concentration utilisées. De plus, l'activité d'une enzyme, la glutamate déshydrogénase (GDH), a été suivie sur la durée de l'expérience pour détecter de possibles effets cumulatifs du sélénium et du mercure. Contrairement à la MDH chez la moule, on ne constate que peu d'effets sur l'activité de la GDH chez la crevette ni pour le mercure ni pour le sélénium.

SOMMAIRE ET CONCLUSION

L'antagonisme sélénium-mercure que certains ont pu imaginer comme un mécanisme d'auto-défense universel semble beaucoup plus un rève qu'une réalité chez les invertébrés marins étudiés. Cependant, il est certain qu'il existe une ou plusieurs "interactions passives" entre le sélénium et le mercure. Des travaux réalisés simultanément aux nôtres par une équipe anglaise (Micaleff et Tyler, 1987) ont montré que l'on peut induire une "protection apparente" à l'exposition au mercure inorganique chez Mytilus edulis en utilisant une quantité équimolaire de sélénium; cependant, l'effet demeura faiblement défini et aucune redistribution du mercure ne fut observée dans les tissus analysés.

Au point de vue fondamentale, nos travaux nous ont conduit à la formulation d'hypothèses nouvelles sur le mécanisme d'assimilation des complexes du mercure. Ces résultats ouvrent la voie à une étude mécanistique plus poussée sur l'assimilation du méthyl mercure et des autres organo-métalliques (organo-étains, organo-plombs,...) avec comme objectif principal de trouver le moyen chimique ou biochimique d'intervenir sur ce processus. Deux avenues semblent s'ouvrir actuellement à nous:

1) chercher un complexant qui réagisse directement avec le contaminant et le rende inoffensif en modifiant ses propriétés chimiques et biochimiques;
2) chercher une molécule capable de bloquer temporairement les sites cibles du contaminant sur les proteines et enzymes ou d'empêcher son transport à

travers les membranes cellulaires.

L'étude des effets cumulatifs de deux ou plusieurs contaminants sur un écosystème, aussi simple soit-il, présente un tâche pratiquement insurmontable aux chercheurs de la fin du 20ème siècle parce que notre connaissance des mécanismes fondamentaux d'interactions entre les contaminants anthropogéniques et les processus vitaux est insuffisante. Les progrès fulgurants de la biologie moléculaire et de la biotechnologie devraient nous être d'une très grande utilité dans les prochaines décennies.

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PHARMACOKINETICS OF ^{3}H-BENZO(A)PYRENE ADMINISTERED TO WHITE SUCKERS FROM POLLUTED AND REFERENCE SITES IN THE GREAT LAKES

G.M. Kirby, I.R. Smith, M.A. Hayes, Department of Pathology, University of Guelph, Guelph, Ontario N1G 2W1

The presence of hepatocellular carcinomas and biliary tract tumors in bottom-dwelling fish from highly industrialized areas of Lake Ontario suggests that tumors may be due to exposure to environmental chemical carcinogens. We examined the pharmacokinetics of a carcinogenic polyaromatic hydrocarbon (PAH), benzo(a)pyrene (BaP), which is found in high concentrations in contaminated lake sediment. The metabolism and excretion of ^{3}H-BaP, administered orally to White Suckers (*Catostomus commersoni*) obtained from a polluted site (Oakville, Ont.) and a reference site (Owen Sound, Ont.), was examined over a 24-hour period. The distribution of ^{3}H-BaP into aqueous and non-aqueous phases of various tissues indicated that BaP is excreted almost entirely in the bile as water-soluble metabolites. Suckers from polluted areas excreted ^{3}H-BaP at twice the rate of reference fish (1.14 vs 0.85 nmoles BaP/g liver in 24 hours). However, excretion rates of Oakville fish decreased to reference levels after 6 weeks in well water under laboratory conditions. These data suggest that BaP-metabolizing enzymes were induced in fish from polluted sites. Incubation of bile samples with ß-glucuronidase or aryl sulfatase gave an indication of the relative proportion of BaP metabolites. Liver cytosolic glutathione S-transferase (GST) activity was higher in ^{3}H-BaP treated fish from the reference site when compared with similarly treated fish from the polluted site (1.08 vs 0.59 IU/mg protein/min). In comparison with high levels in surrounding liver, hepatic carcinomas were consistently deficient in immunoreactive-GST. Few preneoplastic lesions were present indicating that normal liver in White Suckers is relatively resistant to the genotoxic effect of BaP. However, the lower overall levels of hepatic GST activity and proportionately lower concentration of glutathione conjugates in BaP-exposed fish suggests that GSTs may be important in the progression of preneoplastic cells.

INTRODUCTION

Tumour development in certain species of wildlife may act as a bioindicator of cancer risk in humans as well as providing a natural model system within which to study the environmental carcinogens. The Hamilton Harbour region of Lake Ontario has a wide range of organic chemical pollutants accumulated in the sediments (Harlow and Hodson, 1988). In recent years, increased prevalences of various skin and liver neoplasms have been demonstrated in bottom-dwelling fish from the Hamilton Harbour region, especially in White Suckers (*Catostomus commersoni*) (Sonstergard and Leatherland, 1984; Smith and Ferguson, 1986; Metcalfe *et al*, 1987; Hayes *et al.*, 1987; V. Cairns, personal communication). The geographic association of these tumours with increased industrial pollution suggests that the affected fish may be exposed to carcinogenic pollutants, but the implied cause-effect relationship has

not been clearly demonstrated. Polycyclic aromatic hydrocarbons (PAH), combustion byproducts of organic matter, are one of the many of carcinogenic agents present in the polluted environment (Harlow and Hodson, 1988), many of which are known skin and hepatic carcinogens for mammals and fish (Slaga *et al*, 1980; Hendricks *et al*, 1985). It is reasonable, therefore to suspect that PAHs may play a role in the development of the neoplasms observed in these fish. This suspicion is reinforced by evidence that similar neoplasms in other benthic fish in Puget Sound (Myers *et al.*, 1987; Varanasi *et al.*, 1987) Lake Erie (Baumann and Harshbarger, 1985) and Lake Ontario (Metcalfe *et al*, 1987; Dunn *et al.*, 1988) are associated with increased exposure to PAHs.

Our recent studies have addressed the question that PAHs might be responsible for the liver tumours we have observed in White Suckers from the Hamilton region. We have been comparing the kinetics and metabolism of benzo(a)pyrene (BaP) as a typical carcinogenic PAH in fish from the Hamilton region and from less polluted reference sites in Lake Huron. These studies suggest that fish from the polluted site have an induced ability to activate BaP in the liver and to excrete it in the bile as glutathione (GSH), sulfate, and glucuronide conjugates. Furthermore, preliminary evidence indicates that the liver in White Suckers may be naturally resistant to PAHs because of the normal hepatic glutathione S-transferases (GSTs) which are important detoxification enzymes for PAHs in these fish (Nimmo. However, malignant hepatocellular adenomas and carcinomas which are present in fish from polluted sites are consistently deficient in GST when stained immunohistochemically using antibodies to Sucker GST. It appears therefore that developing liver neoplasms lose their normal GST enzymes and thereby become more sensitive PAH-induced genetic perturbations. Collectively, our observations support a hypothetical explanation for carcinogen-induced genetic alterations responsible for malignant transformation and cancer progression in cells that may have been originally quite resistant to PAHs and other carcinogens. An important aspect of this emerging concept is that repeated exposures to high doses of genotoxic carcinogens may cause malignancies in constitutively resistant tissues, individuals or species.

METHODS

1. Distribution and Excretion of Benzo(a)pyrene (BaP)

Male White Suckers were captured from Sixteen Mile Creek near Oakville, Lake Ontario (polluted site) and from Keefer's Creek, Lake Huron (reference site) during their spring spawning migrations and were maintained in laboratory holding tanks in clean well water. Fish were selected and given BaP (2 mg/kg) by gavage in a vehicle composed of distilled deionized water, DMSO (10%) and sodium deoxycholate (1%). The BaP preparation contained 50 µCi/mg of ^{3}H-BaP (New England Nuclear, Boston MA) as a radioactive tracer. Treated fish were killed after 3, 6, 12 and 24 hours and subjected to postmortem examination, during which samples of bile (gall bladder), liver, muscle, blood, kidney, intestine, intestinal contents and gill were collected for liquid scintillation counting (LSC) of the ^{3}H-tracer.

In a second experiment, White Suckers from Oakville were held for 6 weeks under clean laboratory conditions before BaP administration to determine if the rates of BaP metabolism were altered when the fish were no longer exposed to their polluted natural environment.

Samples of blood and bile (40 µl) were solubilized in 1 ml of Protosol (NEN) and counted in 10 ml of Aquasol (NEN). Samples of tissue (100 mg) were digested and extracted overnight in 2 ml 0.5N NaOH and 3 ml n-Hexane on a motorized rotater (Varanasi *et al.*, l978). An aliquot (1.5 ml) of the n-Hexane fraction containing non polar BaP metabolities and the NaOH fraction (1 ml) containing polar metabolites were then counted in 10 ml of Aquasol. Concentrations of BaP and derivatives were calculated as nmol/g tissue from liquid scintillation counts of vials after overnight dark-acclimation and correction for counting efficiency.

2. Metabolism of BaP

Samples of bile obtained from fish given ^{3}H-BaP were subjected to enzyme hydrolysis and HPLC analysis to determine the proportions of B(a)P excreted as polar metabolites and conjugates. Bile in 1 ml of distilled water was extracted initially with ethyl acetate to remove non polar BaP (parent compound). The residual aqueous phase was divided into 3 equal parts brought to 0.9 mls using sodium acetate buffer, incubated with arylsulfatase (Sigma Chemical Col., St. Louis, MO, 35 units/ml incubate containing 20 mM D-saccharic acid 1-4 lactone to inhibit glucuronidase activity) or β-glucuronidase (Glucurase,Sigma, 500 Sigma units/ml incubate) or sodium acetate buffer (0.2 mM, pH 5.0, 37°C, for 24 hrs). Each sample was further extracted with ethyl acetate (2 x 2 ml) to remove less polar hydrolyzed BaP intermediates. All extracts and fractions were subjected to LSC to determine percentages of hydrolysible and nonhydrolysible polar metabolites. Samples of bile (50 µl) from fish given ^{3}H-BaP 24 hours previously were analyzed by reverse phase HPLC using a stepwise water/methanol gradient on a C18 column (Biorad Hipore RP-318; 250 x 4.6 mm) on a Biorad 402 HPLC system. The mobile phase conditions were 0-30% methanol in 2 minutes, 30% methanol for 15 minutes,30-70% in 3 min and 70% methanol for 10 minutes at a flow rate of 1.0 ml/minute. The eluant was monitored by absorbance (430 nm) and also by fluorescence (excitation 380 nm; emission 430 nm) by a Shimadzu RF 5000 spectrofluorometer and collected as 0.25 ml fractions in a Gilson Model 203 microfraction collector. The distribution of ^{3}H radioactivity tracer in fractions was determined by LSC.

3. Determination of GST Activity and Expression in Fish Tissues

Samples of liver, kidney, muscle, intestine and gill were also collected for histopathologic examination (fixed in 10% formalin). Samples of liver were also collected on ice, and homogenized in 3 volumes of 0.25 M sucrose buffer (containing 50 mM HEPES pH 7.5) for determination of hepatic glutathione S-transferase (GST) activity. Liver homogenates were centrifuged at 100,000 g for 1 hour to obtain cytosol (supernatant) from which GST activity was determined by CDNB conjugation rates (pH 7.0, 30°C) by the method of Habig *et al.* (1974). Protein concentrations in cytosol were determined by the Lowry method.

GST isoenzymes were purified from liver cytosol from normal White Suckers by affinity binding to S-hexylglutathione-agarose (Sigma) and stepwise elution with 50 mM and 200 mM NaCl (to remove non specifically bound proteins) and then 5 mM S-hexylglutatione (to elute glutathione-binding cytoslic proteins). The latter fraction, containing approximately 80% of hepatic GST, was analyzed by SDS-PAGE under reducing conditions and was found to consist of 4 major protein subunits in the 26 kD

molecular weight range corresponding to reference samples of pure GSTs from rat liver. The purified GSTs were used to immunize rabbits to produce polyclonal antiserum specific for all 4 GST subunits in hepatic cytosol (by western blot analysis). This specific antiserum was used in routine peroxidase-antiperoxidase (PAP) immunocytochemical staining for GST protein expression in formalin-fixed sections of livers from various Oakville and Hamilton Harbour fish with previously diagnosed hepatocellular or bile duct neoplasms.

RESULTS

1. Distribution and Excretion of BaP

Fish from Oakville excreted BaP twice as fast as reference fish when they were exposed to ^{3}H-BaP (2mg/kg) within 7 days of capture from the wild (Figure 1).

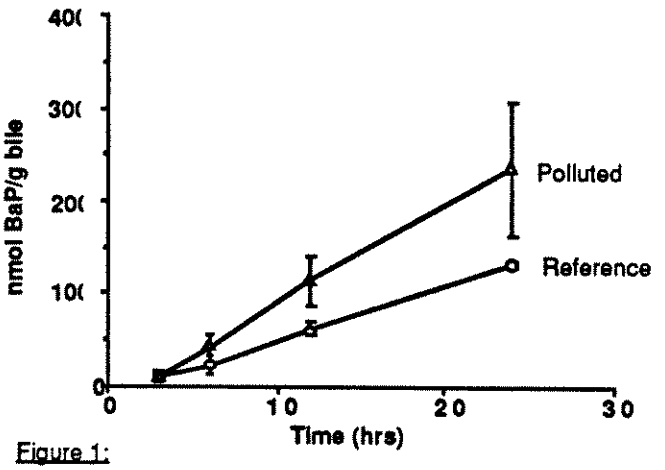

Figure 1:
Concentration of B(a)P in the bile of White suckers from polluted and reference areas at various times after oral dosing with B(a)P (2mg/kg). Values determined by total radioactivity in NaOH extracts of bile (100 µl) expressed as nmol/g of B(a)P equivalents per gram of bile (mean ± SE)

The vast majority of polar BaP metabolites were found in the bile. BaP-derived radioactivity in muscle, blood,gill, and kidney were negligible in both populations (less than 1.0 % of total) (Figures 2 & 3). Large amounts of radioactivity were present in liver and intestine. However, further analysis of these latter samples by NaOH/n-Hexane extraction (see below) revealed that the intestine contained mainly unabsorbed parent compound whereas the liver contained mainly polar metabolites of similar types to those found in the bile.

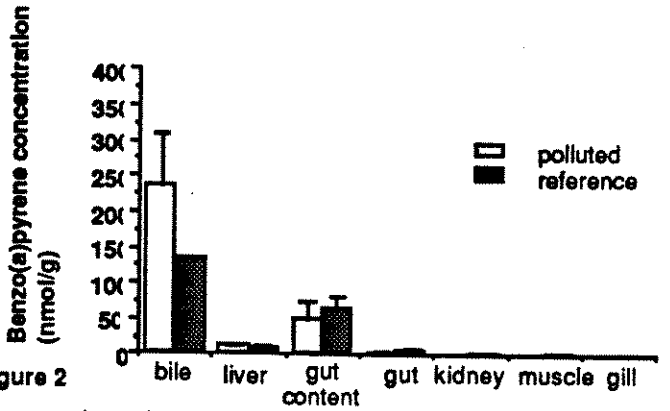

Figure 2

Concentrations of water-soluble BaP metabolites (NaOH extractable) in tissues and fluids of polluted and reference White Suckers 24 hours after oral exposure to tritiatedBaP

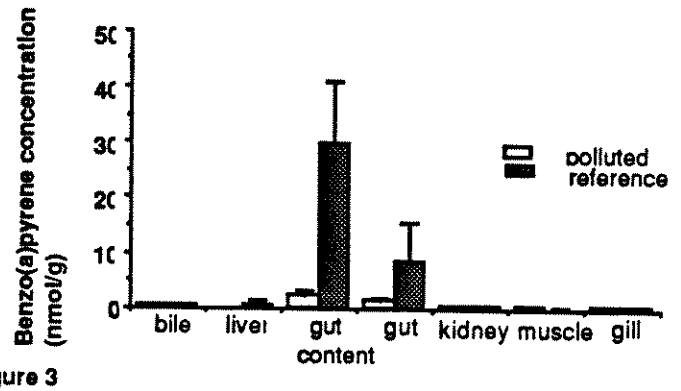

Figure 3

Concentrations of organic-soluble BaP metabolites and parent compound (n-Hexane extractable) in tissues and fluids of polluted and reference White Suckers 24 hours after oral exposure to tritiated BaP

The fish from Oakville exposed to BaP after 6 weeks in captivity excreted BaP at a lower rate similar to that observed in fish from the reference site at the time of capture (Table 1)

Table 1 : ^{3}H-BaP EXCRETION IN BILE AT 24 HOURS POST ADMINISTRATION

Reference	Polluted	Polluted (6 weeks)*
140.4 ± 3.2	250.8 ± 42.2	120.4 ± 37.0

Values are expressed as nmoles of BaP equivalents/g bile± SEM
*White suckers held in clean water tanks for 6 weeks after capture from polluted site.

2. Metabolism of B(a)P

The majority of radioactivity in bile occurred as more polar metabolites that could not be extracted from the aqueous phase by ethyl acetate. The aqueous phase of bile from Oakville and reference fish contained some metabolites that were hydrolysed by aryl sulfatase or β-glucuronidase (Table 2).

Table 2: CONCENTRATION OF BaP METABOLITES IN BILE OF WHITE SUCKERS AS DETERMINED BY ENZYME HYDROLYSIS

Metabolic Derivative	Reference sites	Polluted sites	Polluted/Reference
Ethyl acetate extractable metabolites	20.2 ± 0.1	20.7 ± 0.2	1.2
Sulfates	0.6 ± 0.0	9.7 ± 0.2	16.2
Glucuronides	14.7 ± 0.4	23.4 ± 0.4	1.6
Other	22.6 ±0.5	45.7 ± 0.5	2.0

Values expressed as nmoles of BaP/g bile ± SEM

These experiments indicated that Oakville fish had an increased proportion of sulfated conjugates (16.2 fold), but a similar proportion of glucuronides as reference fish (Table 2). Non hydrolysible polar metabolites, presumably containing glutathione and other conjugates, comprised a substantial proportion of the ^{3}H-BaP in bile from each group.

The distribution of fluorescent and ^{3}H-labelled metabolites of BaP in HPLC analyses of whole unextracted bile indicated that fish from polluted and reference sites had a similar complex profile of moderately polar and highly polar BaP metabolites . No parent (non polar) BaP was detectable in these bile samples. While there were minor, as yet uncharacterized, differences in some of the metabolites, the observations were consistent with excretion studies (Table 1) which indicated an overall increased rate of BaP metabolism and elimination of BaP in bile.

3. Determination of GST Activity and Expression in Fish Tissue

Activities of GST in hepatic cytosol from White Suckers exposed to BaP in these experiments were substantially lower than GST activities in White Suckers that had not been given BaP (Table 3).

Table 3: GLUTATHIONE S-TRANSFERASE ACTIVITY IN LIVER OF BaP-TREATED AND NON TREATED WHITE SUCKERS

^{3}H-BaP-treated		Untreated
Polluted site	Reference site	Polluted site
0.63 ± 0.14	1.12 ± 0.39	2.76 ± 0.14

Values expressed as IU/mg protein ± SEM

These observations are consistent with an interpretation that BaP metabolites compete with or inactivate GST in liver. Fish from Oakville had approximately one half the GST activity when compared with fish from the reference site.

Immunocytochemical stains of tissues from White Suckers revealed that GST proteins are present in substantial amounts in hepatocytes, bile duct epithelium, gill epithelium, intestinal epithelium, renal tubules and erythrocytes. All hepatocellular and bile duct adenomas or carcinomas examined in fish from Hamilton Harbour or Oakville were markedly deficient in GST proteins in comparison with surrounding non-neoplastic hepatocytes or major bile ducts. Preneoplastic hepatocellular lesions (foci of altered hepatocytes) which were rarely observed in livers of fish with neoplasms, on the whole had similar amounts of GST as did normal hepatocytes, but occasionally, GST deficient foci of hepatocytes were observed. No preneoplastic or neoplastic lesions with induced GST were observed.

DISCUSSION

These studies indicate that White Suckers from the industrially polluted western region of Lake Ontario have evidence of an induced ability to metabolize and rapidly eliminate BaP in the bile. This induction is transient and subsides when fish are held for some time in an uncontaminated environment. This evidence supports the view that these fish may be exposed to xenobiotics that induce various hepatic cytochromes P-450 and detoxification enzymes involved in elimination of xenobtiotics. A reasonable interpretation of these findings is that fish are influenced by the polluted environment in western Lake Ontario, and that this influence aids in the excretion of greater amounts of PAHs than can fish from less polluted regions.

The observations that White Suckers have numerous biliary metabolites of BaP are in accordance with observations that English sole from Puget Sound also excrete BaP by multiple pathways (Varanasi *et al.*, 1987). Our evidence suggests that conjugation with GSH by hepatic GST activity is a major detoxification mechanism for BaP in White Suckers. Because these fish are capable of rapidly excreting relatively large experimental dosages of BaP, especially when they have lived near or in the Hamilton Harbour that is contaminated with many PAHs (Harlow and Hodson, 1988), it is reasonable to consider that White Suckers are relatively resistant to BaP by virtue of their natural GST and other detoxification pathways. Such an interpretation is

consistent with our observations (Hayes *et al*, 1987) and those of others (V.Cairns, personal communication) that hepatic neoplasms are observed in fewer than 10% of White Suckers that have likely been exposed to the Hamilton environment for many years. Moreover, preneoplastic foci of altered hepatocytes, of the type considered to be initiated by PAHs and other genotoxic carcinogens in rodents (Farber and Sarma, 1986) and fish (Hendricks *et al.* 1985; Myers *et al.* 1987), were rarely observed in these fish inspite of their presumably chronic exposure to PAHs and other potentially genotoxic carcinogens. Accordingly, it is reasonable to conclude that White Suckers exposed to the Hamilton environment are rather resistant to any initiating effects of the PAHs to which they are exposed.

Because there is an apparently high rate of malignant progression (conversion) of preneoplastic lesions to hepatocellular adenomas and carcinomas, these fish must develop an increased susceptibility to tumorigenesis at some stage after the initiation step. In laboratory rodents, few of the numerous foci of altered hepatocytes initiated by brief exposures to genotoxic carcinogens actually progress to malignancy (Farber and Sarma, 1986). By comparison, preneoplastic epidermal papillomas initiated by PAHs in mice exhibit a high rate of malignant conversion when they are subsequently exposed repeatedly to genotoxic carcinogens, including PAHs (Hennings *et al*, 1983). One of several reasonable explanations for the consistent GST-deficient phenotype in neoplasms that have progressed in White Suckers is that multiple subsequent "hits" by PAHs or other carcinogens may have been involved in the later stages of carcinogenesis. Because all advanced hepatic neoplasms had markedly reduced GST resistance, it is plausible that loss of GST expression in rare initiated cells would render them more prone to further DNA damage by agents such as BaP for which normal hepatocyte GSTs are protective.

This hypothetical concept that a reduction in normal cellular resistance to carcinogens could favour tumour progression is important from an epidemiological viewpoint. Our findings suggest that environmental mutagenic chemicals such as PAHs, which can be normally detoxified by GSTs in these fish, are perhaps more likely to play a role in the later stages (malignant progression) of cancer development in cells that have lost GST-dependent resistance mechanisms. This hypothesis implies that long-term exposure to PAHs may be necessary to cause neoplasms in tissues that are initially resistant to them. This view also implies that some species or individuals with genetic deficiencies in specific protective mechanisms would be more susceptible to carcinogens that otherwise would be detoxified. Further investigation of this hypothesis and its implications for human suscepibility to the carcinogenic effects of environmental PAHs are currently underway. The availability of fish exposed continuously to PAHs under natural conditions provides a means of understanding the circumstances under which PAHs may be carcinogenic to humans. This knowledge is essential to a sound assessment of cancer risk in humans exposed to environmental PAHs in contaminated water, air or diet.

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PHARMACODYNAMIQUE DU ^{3}H-BENZO (A)PYRENE ADMINISTRE A DES MEUNIERS NOIRS PRELEVES SUR DES SITES POLLUES ET TEMOINS DANS LES GRANDS LACS.

G.M. Kirby, I.R. Smith, M.A. Hayes, Department of Pathology, University of Guelph, Guelph, Ontario N1G 2W1

RESUME

La présence de carcinomes hépatocellulaires et de tumeurs du canal biliaire chez le poisson de fond, dans la partie fortement industrialisée du Lac Ontario, laissent supposer que les tumeurs peuvent être dues à l'exposition à des produits chimiques cancérigènes présents dans l'environnement. Nous avons étudié la pharmacodynamique d'un hydrocarbure polyaromatique (HPA) cancérigène, le benzo(a)pyrène (BaP), qui est trouvé en concentrations élevées dans le sédiment contaminé du lac. Le métabolisme et l'excrétion du ^{3}H-BaP, administré par voie orale aux meuniers noirs (Catostomus commersoni), capturés dans un endroit pollué (Oakville, Ont.) et dans un site de référence, a été étudié sur une période de 24 heures. La répartition du ^{3}H-BaP dans les phases aqueuses et non aqueuses des divers tissus a démontré que le BaP est excrété presqu'entièrement dans la bile, comme métabolites solubles dans l'eau. Le groupe de meuniers provenant des régions polluées a excrété le ^{3}H-BaP à un taux deux fois plus élevé que le groupe de référence (1.14 vs 0.85 nmoles BaP/g de foie en 24 heures). Cependant, les taux d'excrétion du groupe de poissons d'Oakville ont diminué aux niveaux des taux du groupe témoin, après 6 semaines dans de l'eau pure et dans des conditions de laboratoire. Ces résultats semblent indiquer que les enzymes métabolisantes du BaP ont été induites chez les poissons des sites pollués. L'incubation des échantillons de bile contenant de la B-glucuronidase ou de l'aryl sulfatase a donné une indication sur la proportion relative des métabolites du BaP. L'activité hépatique cellulaire de la GST (Glutathione S-Transférase) était plus forte chez le poisson traité au ^{3}H-BaP provenant du site de référence, comparativement au poisson provenant d'un site pollué (1.08 vs 0.59 UI/mg de protéine/min) ayant reçu le même traitement. En comparant avec les fortes concentrations dans le foie avoisinant, les carcinomes hépatiques étaient tous déficients en GST immunoréactive. Quelques lésions pré-néoplasiques étaient présentes, indiquant que le foie normal chez les meuniers nois est relativement résistant à l'effet génotoxique du BaP. Cependant, les plus bas niveaux de l'activité de la GST hépatique et proportionnellement une plus faible concentration des congénères de glutathion dans le poisson exposé au BaP laissent supposer que les GST peuvent être importantes dans la progression des cellules pré-néoplasiques.

PERSISTENCE, FATE AND INVERTEBRATE TOXICITY OF TETRACHLOROPHENOL IN AQUATIC ENCLOSURES.

K. Liber, N.K. Kaushik, University of Guelph, Guelph, Ontario; K.R. Solomon, Canadian Centre for Toxicology, Guelph, Ontario; J.H. Carey, National Water Research Institute, Burlington, Ontario.

The environmental fate and non-target toxicity of technical 2,3,4,6-tetrachlorophenol (TTCP) was studied in a small mesotrophic lake using 125 m^3 aquatic enclosures (limnocorrals). Photolysis was found to be the main mode of degradation yielding an approximate environmental half-life of 90 hours during sunny days in mid-august. Laboratory experiments confirm the significance of photolysis, with a half-life of only 2 hours when irradiated at 3000 A with a light intensity of 4.66×10^{17} quanta/sec.

In field experiments Rotifera species were found to be more sensitive indicators of environmental stress than Cladocera and Copepoda species, exhibiting numerical declines to approximately 1 organism/l after treatment with 1 mg/l technical TTCP. This maximum impact was observed at 7 days post-treatment. Zooplankton populations recovered completely by 42 days post-treatment.

PERSISTANCE, DEVENIR ET TOXICITE SUR INVERTEBRES DU TETRACHLOROPHENOL, SUIVI DANS DES ENCLOS AQUATIQUES.

K. Liber, N.K. Kaushik, University of Guelph, Guelph, Ontario; K.R. Solomon, Canadian Centre for Toxicology, Guelph, Ontario; J.H. Carey National Water Research Institute, Burlington, Ontario.

RESUME

Le devenir environnemental, ainsi que l'évolution imprévue de la toxicité du 2,3,4,6-tétrachlorophénol (TTCP) de grade technique ont été étudiées dans un petit lac mésotrophe en utilisant des enclos aquatiques de 125 m^3 (limnocoraux). Il a été trouvé que la photolyse était le principal mode de dégradation, la demie-vie environnementale étant réduite à 90 jours au cours des journées ensoleillées de la mi-août. Les essais en laboratoire ont confirmé l'importance de la photolyse avec une demie-vie de seulement 2 heures lorsqu'irradié à 3000A avec une intensité lumineuse de 4.66×10^{17} quanta/sec.

Dans les essais de terrain, les espèces de rotifères se sont avérées être des indicateurs de stress environnemental plus sensibles que les cladocères et les copépodes, montrant une diminution numérique d'environ 1 organisme/L après traitement avec 1 mg/L de TTCP de grade technique. Cet impact majeur a été observé 7 jours après le traitement. Les polulations de zooplancton ont récupéré complètement 42 jours après le traitement.

THE CONCEPT OF AN INTERNAL CRITICAL BODY BURDEN IN AQUATIC TOXICOLOGY

S.M. McGeachy, B.E. Hickie and D.G. Dixon
Department of Biology, University of Waterloo
Waterloo, Ontario

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TOXICOKINETICS OF ORGANIC CHEMICALS IN AQUATIC ORGANISMS

Frank, A.P.C. Gobas
The Great Lakes Institute
University of Windsor
Windsor, Ontario, N9B 3P4

ABSTRACT

Recent developments in the field of toxicokinetics of organic chemicals in aquatic organisms are reviewed. Techniques for exposure assessment of organic chemicals are presented and illustrated with examples for trichlorobenzene and mirex.

INTRODUCTION

One of the most important issues presently addressed in aquatic toxicology is the interpretation of aqueous concentrations of toxic organic chemicals in terms of exposure and effects to aquatic life. This is also of particular interest to governments, who are confronted with questions regarding the exposure and effects of many chemicals in a large number of water bodies.

This study will explore exposure assessment methods for organic chemicals in aquatic organisms. It reviews recent studies of the chemical uptake and elimination kinetics of organic chemicals in fish and discusses methods by which chemical uptake in aquatic organisms can be determined and estimated. It further presents two examples, which illustrate exposure assessment techniques for organic chemicals in aquatic organisms.

UPTAKE, DEPURATION AND BIOACCUMULATION IN AQUATIC ORGANISMS

The expression describing the simultaneous uptake of chemical from food and water in aquatic organisms as well as the depuration of that chemical to the water (via the gills), into the faeces and by metabolic transformation can be expressed as

$$dC_F/dt = k_1.C_W - k_2.C_F + k_A.C_A - k_E.C_F - k_R.C_F \qquad (1)$$

where C is concentration (mol/m^3), t is time (h), and the subscripts W refer to water, A to food, E to faeces, and F to the whole organism (Gobas et al. 1988, 1989). The organism is defined as the whole organism excluding the gill compartment and the gastro-intestinal (GI) tract. k_1, k_2, k_A and k_E are respectively the rate constants (h^{-1}) of chemical uptake from the water, elimination to the water, uptake from food, and elimination by egestion in the faeces. k_R is the rate constant (h^{-1}) for metabolic transformation of the chemical in the organism.

Following the fugacity approach, discussed at great detail by Mackay and coworkers (Mackay and Paterson 1982), equation 1 can also be written as

$$V_F.Z_F.df_F/dt = D_F.(f_W - f_F) + D_A.f_A - D_E.f_F - D_R.f_F \qquad (2)$$

where V is volume (m^3), Z is the chemical's fugacity capacity ($mol/m^3.Pa$) in a phase, and f is the chemical's fugacity (Pa). D_F is the overall transport parameter (mol/Pa.h) for chemical transfer between water and fish across the respiratory surface (e.g. gills). D_A is the transport parameter for chemical uptake from food into the organism across the gastro-intestinal (GI)-tract. The transport parameter D_E (mol/Pa.h) describes chemical elimination in the faeces. D_R (mol/Pa.h) is the transformation parameter for metabolic transformation of chemical in the organism. The transport parameters D_F, D_A, and D_E include all transport processes involved in solute transfer between the water, food, and faeces, respectively, and the solute's final storage site in the fish.
Integration of equation 1 with a constant C_W and C_A, an initial C_F of zero and assuming a constant fish volume with time gives

$$C_F = \{C_W.[k_1/(k_2+k_E+k_R)] + C_A.[k_A/(k_2+k_E+k_R)]\}.\{1-\exp-(k_2+k_E+k_R).t\} \qquad (3)$$

This equation describes the chemical concentration in the organism with time when an uncontaminated organism, with no initial chemical concentration, is exposed to water with a constant concentration of the chemical for a certain length of time.

Since C_F is f_F/Z_F, C_W is f_W/Z_W and C_A is f_A/Z_A, it can be shown that equation 3 is equivalent to the integrated form of equation 2 with a constant f_A and f_W and an initial f_F of zero, i.e.,

$$f_F = \{f_W.[D_F/(D_F + D_E + D_R)] + f_A.[D_A/(D_F + D_E + D_R)]\}.\{1 -\exp[-(D_F + D_E + D_R).t/(V_F.Z_F)]\} \qquad (4)$$

The fugacity and the kinetic description of the bioconcentration process are similar in that they are based on the same assumptions, namely that (i) chemical transfer is passive and (ii) the organism is viewed as one, homogeneous compartment. The difference in the two approaches is that in the kinetic approach bioaccumulation is viewed as a balance between the rates of chemical uptake and elimination, whereas in the fugacity approach bioaccumulation is viewed as a process where the chemical is attempting (but not necessarily achieving) to reach a thermodynamic equilibrium. This thermodynamic equilibrium is characterized by equal fugacities of the chemical in the

organism, the water and the food consumed by the organism. The strength of the kinetic descriptions is that the rate constants can be measured directly from uptake and depuration experiments. The fugacity-equations, however, distinguish between thermodynamically controlled partitioning phenomena, characterized by the fugacity capacity values (i.e. Z) and pure transport phenomena, described by transport parameters (i.e. D). Fugacity expressions therefore often give an in-depth view of the actual mechanism of the bioaccumulation process. The two approaches complement each other, and are best combined. This can be easily achieved by comparing equations 3 and 4, from which it follows that

$$k_1 = D_F/V_F.Z_W \quad (5)$$

$$k_2 = D_F/V_F.Z_F \quad (6)$$

$$k_A = D_A/V_F.Z_A \quad (7)$$

$$k_E = D_E/V_F.Z_F \quad (8)$$

$$k_R = D_R/V_F.Z_F \quad (9)$$

Equations 3 and 4 show that at infinite exposure time an organism-water bioaccumulation factor, K_B can be defined for an organism simultaneously exposed to contaminated water and food as

$$K_B = C_F/C_W = (Z_F/Z_W).\{[D_F/(D_F+D_E+D_R)] + [(f_A/f_W).(D_A/(D_F+D_E+D_R)]\} \quad (10)$$

or

$$K_B = C_F/C_W = \{k_1/(k_2+k_E+k_R)\} + (C_A/C_W).\{k_A/(k_2+k_E+k_R)\} \quad (11)$$

It also follows from equations 3 and 4 that in food uptake experiments, when organisms are exposed to contaminated food but uncontaminated water, the ratio of organism to food concentrations, i.e., C_F/C_A or K_M, which is the biomagnification factor, can be expressed as

$$K_M = C_F/C_A = (Z_F/Z_A).\{D_A/(D_F+D_E+D_R)\} = k_A/(k_2+k_E+k_R) \quad (12)$$

The bioconcentration factor, K_C, which is defined as the ratio of fish and water concentrations at infinite exposure time for organisms exposed to contaminated water only (i.e., $C_A = f_A = 0$), is

$$K_C = C_F/C_W = (Z_F/Z_W).\{D_F/(D_F + D_E + D_R)\} = k_1/(k_2+k_E+k_R) \quad (13)$$

Equations 11 to 13 demonstrate that K_B, K_M, and K_C are not solely determined by the thermodynamic quantities, i.e., Z_F, Z_W

and Z_A, which reflect the affinities of the chemical for the organism, water, and food, but also by the relative rates of chemical uptake from water and food, release to the water, egestion with the faeces, and metabolic transformation. Equation 13 shows that even when no metabolic transformation occurs ($D_R = 0$), the bioconcentration factor only reflects organism-water partitioning when D_E is small compared to D_F. It thus follows that in order to make reliable predictions about the bioaccumulation potential of hydrophobic chemicals in aquatic organisms and the rate at which bioaccumulation is achieved in the organisms, knowledge is required about the processes controlling the exchange of solute between fish, water, food, and faeces.

Equation 3 illustrates that when a contaminated organism is introduced in clean, uncontaminated water (C_W is zero) and consumes uncontaminated food (C_A is zero), it will lose chemicals to the water resulting in a drop of C_F with time. The differential equation describing this process is again equation 1, but with a C_W and C_A of zero, i.e.

$$dC_F/dt = -(k_2 + k_E + k_R).C_F \qquad (14)$$

which after integration with an initial $C_{F,t=0}$ becomes

$$C_F = C_{F,t=0}.\{\exp(-(k_2 + k_E + k_R).t)\} \qquad (15)$$

or

$$\ln C_F = \ln C_{F,t=0} - (k_2 + k_E + k_R).t \qquad (16)$$

Equation 16 demonstrates that in a logarithmic plot $\ln C_F$ decreases linearly with time. The slope of this plot is the total depuration rate constant ($k_2 + k_E + k_R$) and has units of reciprocal time.

The rate at which chemicals are eliminated by organisms can also be expressed by the biological half-time in the fish. This is the time to reach half the initial concentration in the fish for an organism in uncontaminated water. It follows from equation 16 that the biological half-time, $t_{1/2}$ is

$$t_{1/2} = \ln 2/(k_2 + k_E + k_R) = 0.693/(k_2 + k_E + k_R) \qquad (17)$$

The time required to achieve steady state, i.e. a constant ratio between the chemical concentrations in the organism and the water or K_B, is also dependent on the rate constants for chemical depuration. It can be shown that the time required to reach 95% of the steady state t_{95} is

$$t_{95} = -\ln 0.05/(k_2 + k_E + k_R) = 3.0/(k_2 + k_E + k_R) \qquad (18)$$

This time t_{95} is of crucial importance since it determines how long the organism has to be exposed before the concentration in the organism reflects the concentration in the water through the

bioaccumulation factor. This time is different for each organism and for each chemical since the rate constants k_2, k_E and k_R are both chemical and organism specific. I will now discuss the kinetics of chemical uptake in aquatic organism and demonstrate the chemical dependence of the uptake and depuration rate constants.

CHEMICAL AND ORGANISM SPECIFIC RELATIONSHIPS FOR THE UPTAKE AND DEPURATION OF ORGANIC CHEMICALS IN AQUATIC ORGANISMS

Lipid-water mass transfer models were derived by Gobas and Mackay (1987), Gobas et al. (1986) and Mackay and Hughes (1984) to gain further insight into the processes controlling the exchange of chemical between aquatic organisms and water and to develop practical procedures to estimate the bioconcentration kinetics of chemicals in fish. The authors used the fugacity approach to derive the model equations but presented their final model in terms of rate constants.

The main feature of this model is that it views the exchange of solute chemical between the water and the organism to take place in a series of aqueous and lipid layers. All transport processes in water phases are therefore grouped together in one overall water phase transport parameter D_W. This overall water phase transport parameter contains all transport parameters $D_{W,i}$ in water phases. The transport parameters $D_{W,i}$ can refer to diffusion, in which case $D_{W,i}$ equals $k.A.Z_W$, where k is the mass transfer coefficient (m/s), A is area of diffusion and Z_W is the chemical's fugacity capacity in the water phase. It can also refer to non-diffusive transport, where the solute is conveyed by a fluid flow G (m^3/s) such that $D_{W,i}$ equals $G.Z_W$. The overall transport parameter D_W can therefore also be expressed as $Q_W.Z_W$, where the transport parameter Q_W (m^3/s) combines all k.A and flow rates G in water phases of the organism. The transport parameter Q_W is therefore sometimes viewed as an "hypothetical water flow rate" in the organism.

Similar to D_W all transport processes in lipid phases are combined in an overall lipid phase transport parameter D_L. This lipid phase parameter D_L can be designated $Q_L.Z_L$, where Q_L combines all k.A and flow rates G in lipid phases of the organism and can be considered the "hypothetical lipid flow rate" in the organism. The transport parameters D_W and D_L therefore refer to the net transport of chemical in respectively the water and the lipid phase of the organism.

Assuming that the water and lipid transport processes apply in series it follows that the transport parameter for chemical exchange between the water and the organism D_F can be expressed as

$$1/D_F = 1/D_W + 1/D_L = 1/Q_W.Z_W + 1/Q_L.Z_L \qquad (19)$$

Since D_F equals $k_1.V_F.Z_W$ or $k_2.V_F.Z_F$ (i.e. equations 5 and 6) and since bioaccumulation is predominantly in the lipids of the organisms Z_F can be expressed as a fraction L_F of the fugacity

capacity in the lipids i.e. as $L_F.Z_L$, where L_F is the lipid fraction of the organism (i.e. V_L/V_F), equation 19 can be rewritten as

$$1/k_2 = V_L.\{(Z_L/Q_W.Z_W) + 1/Q_L\} \quad (20)$$

$$1/k_1 = V_L.\{1/Q_W + (Z_W/Q_L.Z_L)\}/L_F \quad (21)$$

When the lipid-water partition coefficient Z_L/Z_W or K_L is replaced by the 1-octanol-water partition coefficient Z_O/Z_W or K_{OW} (thus assuming Z_L to be equal to Z_O) equations 20 and 21 become

$$1/k_2 = V_L.\{(K_{OW}/Q_W.Z_W) + 1/Q_L\} \quad (22)$$

$$1/k_1 = V_L.\{1/Q_W + (1/Q_L.K_{OW})\}/L_F \quad (23)$$

The ratios V_L/Q_L and V_L/Q_W can be viewed as the times of chemical transport in V_L m^3 of respectively lipids and water. However, if transport of a given amount of chemical requires a volume V of lipid, it will require a much larger volume i.e. $K_{OW}.V_L$ of water, since the chemical concentration in the water is a factor of K_{OW} lower than in the lipids. The time for the water phase in the organism to transport a certain amount of chemical is therefore K_{OW} times longer than that for the lipid phase. The transport time of the water phase is therefore multiplied with K_{OW} in equation 22 and alternatively the lipid transport time is divided by K_{OW} in equation 23. Since the lipid and water transport processes occur in series these times are additive and the longer time "controls" the bioconcentration kinetics.

The expressions 22 and 23 contain two types of variables namely, (i) biological parameters i.e. V_L, Q_W, Q_L, G_V and L_F, which are specific to a particular fish and its physiological condition and (ii) a chemical parameter K_{OW} expressing the chemical's tendency to partition between lipids and water.
When for a series of chemicals, varying in K_{OW}, rate constants or uptake efficiencies have been determined in a particular organism equations 22 and 23 can be fitted to the experimental data resulting in values for Q_W and Q_L. The data fitting involves a regression e.g. $1/E_O$ versus $1/K_{OW}$ or $1/k_2$ versus K_{OW}. Since K_{OW} usually varies over several orders of magnitude and would thus weigh heavily in favour of the data points with high K_{OW} simple linear regression (e.g. $1/E_O$ versus $1/K_{OW}$) leads to erroneous results. Instead regression should be performed on a semi-logarithmic basis e.g. $1/E_O$ versus log K_{OW} and $1/k_2$ versus log K_{OW} but using the linear equations 22 and 23. Fitting these equations to experimental data is therefore best performed by using non-linear regression techniques.
Gobas and Mackay (1987) and Gobas et al. (1989) fitted equations 22 and 23 successfully to kinetic data in various fish species i.e. the rainbow trout, goldfish and guppy resulting in values for Q_W and Q_L. It was also found that Q_W increases with increasing body weight of the organism as

$$Q_W = 1.4.M^{0.6} \quad (24)$$

Although conclusive data are presently lacking there seems to be a similar relationship for Q_L i.e.

$$Q_L = 0.014.M^{0.6} \quad (25)$$

Although equation 25 is less well established than equation 24 it is observed for higher K_{OW} chemicals (i.e. log K_{OW} > 3.5) the effect of Q_L on the uptake and elimination kinetics can usually be ignored with respect to Q_W.

Theoretical justification for the empirical relationship between Q_W and M can be obtained from studies on the influence of oxygen concentration on gill ventilation rates. Norstrom et al. (1976) showed that the gill ventilation volumetric rate G_V is a function of the oxygen concentration in the water C_{OX} (ml of O_2/ml of water), the oxygen transfer efficiency across the gills E_{OX}, the metabolic rate coefficient (ml of $O_2.h^{-1}.g^{-0.8}$) and the body weight of the organism to the power 0.8, i.e.

$$G_V = A.M^{0.8}/(E_{OX}.C_{OX}) \quad (26)$$

The similarity in body weight dependence of the gill ventilation volumetric rate and Q_W suggest a similarity in these two quantities. More importantly, the similarity in the actual values of Q_W and directly measured or estimated (i.e. equation 26) gill ventilation rates indicates that Q_W is dominated by the gill ventilation volumetric rate.

A similar lipid-water mass transfer model can also be developed for chemical uptake from consumed food and elimination to faecal matter in the GI-tract. The derivation of this model can be found in Gobas et al. 1988. It was shown that similar to equations 22 and 23 chemical specific relationships can be derived for the rate constants k_A and k_E and the chemical uptake efficiency from food E_{fO} i.e.

$$1/k_A = (V_F/G_I).\{(G_O.L_G/Q_{WF}).K_{OW} + (G_O.L_G/Q_{LF}) + 1\} \quad (27)$$

$$1/k_E = (V_F.L_F/G_O.L_G).\{(G_O.L_G/Q_{WF}).K_{OW}+(G_O.L_G/Q_{LF})+1\} \quad (28)$$

$$1/E_{fO} = (G_O.L_G/Q_{WF}).K_{OW} + G_O.L_G/Q_{LF} + 1 \quad (29)$$

where G_I is the volumetric feeding rate (in m^3 food per hour), G_O is the volumetric egestion rate (in m^3 faeces per hour), Q_{WF} is the water phase transport parameter for chemical exchange between the GI-tract and the final storage site in the organism (in m^3 per hour), Q_{LF} is the lipid phase transport parameter for chemical exchange between the GI-tract and the final storage site in the organism (in m^3 per hour) and L_G is the organic or "lipid" fraction of the gastro-intestinal contents (in grams of organic matter per gram of GI contents).

The reciprocal of k_A, i.e., $1/k_A$, can be viewed as the time needed to transport chemical from the food into the fish or as the total resistance for chemical transfer from the food into the fish. Likewise, $1/k_E$ is the time required to eliminate chemical from the fish into the faeces or the total resistance for chemical elimination to the faeces. The ratios $(V_F/G_I).(G_O.L_G/Q_W).K_{OW}$ and $(V_F/G_I).(G_O.L_G/Q_L)$ can be viewed as the solute's relative transport times in the water and lipid phases, respectively, of the fish or as the relative resistances that the solute encounters in the water and lipid phases of the fish on its route from the food phase in the GI-tract to the final storage site in the body lipid of the fish. When the solute's K_{OW} increases, and aqueous solubility thus decreases, the water phase of the fish can accommodate only a lower concentration of solute molecules. As a result, the time required to transport a certain amount of solute with this lower concentration increases. The resistance of the fish's water phase toward mass transfer thus increases, whereas it remains approximately constant in the lipid phase. For high K_{OW} chemicals, this implies that the uptake rate from food and elimination rate to the faeces and thus k_A, E_{fO} and k_E decrease with increasing K_{OW}. For low K_{OW} chemicals, uptake from food and elimination by excretion to the faeces is predominantly controlled by transport in lipid phases, and k_A, E_{fO}, and k_E are, therefore, expected to be approximately constant with respect to K_{OW}.

Equation 29 demonstrates that by experimental measurement of E_{fO} for a series of chemicals with varying K_{OW} under controlled conditions, i.e., a constant feeding rate and no uptake of chemical from the water, it is thus possible to determine the fundamental kinetic parameters Q_W and Q_L. Knowledge of these parameters is invaluable for reliable estimation of organic chemical bioaccumulation from contaminated food.
Gobas et al. (1988) showed that experimental data for dietary uptake of chemicals in fish fit this simple relationship with values for $(G_O.L_G/Q_W).K_{OW}$ of 5.3 (+/- 1.5).10^{-8} and for $(G_O.L_G/Q_L + 1)$ of 2.3 (+/- 0.3), thus resulting in the following relationship for E_{fO}, k_A and k_E

$$1/E_{fO} = 5.3.10^{-8} + 2.3 \qquad (30)$$

$$1/k_A = (V_F/G_I).(5.3.10^{-8} + 2.3) \qquad (31)$$

$$1/k_E = (V_F.L_F/G_O.L_G).(5.3.10^{-8} + 2.3) \qquad (32)$$

The relationships that have been presented above for the chemical uptake and elimination kinetics have been experimentally validated in several species of fish (Gobas and Mackay 1987, Gobas et al. 1988 & 1989). For other aquatic organisms relationships for the uptake and elimination rate constants similar to those discussed above have yet not been established because of a lack of experimental data. However, it is believed that when these experimental data become available similar

relationships can be derived for aquatic organisms other than fish.

THE USE OF KINETIC RATE CONSTANTS IN EXPOSURE ASSESSMENT

This section will demonstrate the role kinetic rate constants can play in the exposure assessment of organic chemicals for aquatic organisms. Before such an exposure assessment can be performed the chemical uptake and elimination kinetics must be established. Preferably, this is achieved by performing uptake and elimination experiments in the field, which is often very difficult, or in the laboratory. These experiments should involve the chemical(s) and organism(s) which are of interest. If it is impossible to perform these experimental measurements the rate constants can be derived with the expressions discussed earlier. This will now be shown with an illustrative example.

The example demonstrates the calculation of the chemical concentrations of trichlorobenzene (TCB) and mirex in a fish resulting from environmental exposure from the water and food. The fish is 5 gram (V_F is 0.005 L) in weight and has a lipid content of 6 %. To derive the rate constants for chemical uptake from and elimination to the water i.e. k_1 and k_2 equations 22 to 25 can be used. Equations 24 and 25 show that the Q_W and Q_L of the fish are respectively $1.4.5^{0.6}$ i.e. 3.7 L/d and $0.014.5^{0.6}$ i.e. 0.037 L/d. Substitution of Q_W and Q_L in equations 22 and 23 then results in

$$1/k_1 = 0.00136 + 0.136/K_{OW} \quad (33)$$

$$1/k_2 = 8.1.10^{-5}.K_{OW} + 0.0081 \quad (34)$$

Equations 33 and 34 demonstrate that for trichlorobenzene (TCB) with a log K_{OW} of 4.0, k_1 is 728 d^{-1} and k_2 is 1.2 d^{-1}. For mirex with a log K_{OW} of 7.5 these rate constants are respectively 735 d^{-1} and 0.0004 d^{-1}.

The rate constants for chemical uptake from food and elimination to the faeces i.e. k_A and k_E can be derived from equations 31 and 32. A typical feeding rate of a fish (G_I) is 1 % of the fish's own body weight per day. As a result it follows that k_A for TCB and mirex are respectively 0.0043 d^{-1} and 0.0025 d^{-1}. Assuming that the faecal egestion rate G_0 is one-third of the feeding G_I and L_F and L_G are approximately equal equation 32 shows that k_E can be calculated to give values of 0.0014 d^{-1} for TCB and 0.00083 d^{-1} for mirex.

When it is assumed that there is no metabolic transformation of TCB and mirex in the fish the total depuration rate constant k_T (i.e. the sum of k_2, k_E and k_R) can be determined as (1.2+0.0014) i.e. 1.2014 d^{-1} for TCB and (0.0004 + 0.00083) i.e. 0.00123 for mirex. These calculations demonstrate that TCB is predominantly eliminated to the water. Elimination of

TCB to the faeces is an unimportant route of depuration. For mirex the situation is quite different with 2/3 of the fish's body burden being eliminated in faecal matter and only 1/3 to the water.

Comparison of the uptake rate constants k_1 and k_A shows that for TCB chemical uptake from the food will become a significant route of exposure when the chemical concentration in the food C_A is approximately 150,000 (i.e. k_1/k_A) times higher than that in the water. In that case the chemical uptake rate from the water $k_1.C_W$ would equal the uptake rate from the food $k_A.C_A$ i.e. water and food are equally important exposure routes. For a chemical with a K_{OW} of only 10,000 it is extremely unlikely to have concentrations in food sources that exceed the chemical concentration in the water by that much. A reasonable estimate of the chemical concentration in the food can be derived by assuming a chemical equilibrium of the chemical in the food with that in the water e.g. C_A equals $0.05.K_{OW}.C_W$ (Mackay 1982). For TCB this means that C_A is approximately 500 times larger than C_W. It can thus be concluded that for TCB gill ventilation of water is the main exposure route. The TCB concentration in the fish thus reflects the concentration in the water. The relationship between the concentration in the fish and the water can now be established by substituting the calculated (or measured) values for k_1, k_2, k_E, k_A and k_R in equation 11 i.e.

$$K_B = C_F/C_W = \{728/(1.2+0.0014+0)\} + \{0.05.10^4.(0.0043/(1.2+0.0014+0))\} = 607 \quad (35)$$

From equation 35 it follows that a TCB concentration in the water of C_W will result in a concentration in the fish C_F that is 607 times larger than C_W i.e. $607*C_W$. It should be noted that this calculation is valid only when the organism and water are at steady-state. In practice, this steady-state will be reached after $3.0/(k_2 + k_E + k_R)$ i.e. 2.5 days assuming a chemical concentration which does not vary in time.

For mirex the situation is again quite different. With values for k_1 and k_A of respectively 735 d^{-1} and 0.0025 d^{-1} it follows that the chemical concentration in the food of the organism has to be approximately 300,000 (i.e. k_1/k_A) fold higher than the concentration in the water before food and water are equally important exposure routes for the fish. However, for a chemical with a K_{OW} of 32,000,000 simple partitioning of the chemical in the food source of the organism may cause such a large difference in chemical concentrations in the water and food. Assuming equilibrium partitioning of mirex between the food and the water it follows that C_A, i.e. $0.05.K_{OW}$, is 1,600,000 fold higher than C_W. In that case 84% of the total chemical exposure is chemical uptake from food and only 16% of the body burden of the organism is chemical taken up from the water. It thus follows that chemical uptake from food is the main exposure route for mirex in the fish.

The body burden of mirex in the fish therefore most closely reflects the concentration in the food source, not the water. In order to derive a reliable estimate of the mirex concentration in the fish it is necessary to know C_A or to make an estimate about the value for C_A. As discussed earlier, $0.05.K_{OW}.C_W$ is often a reasonable estimate for C_A. It then follows that K_B can be calculated as

$$K_B = C_F/C_W = \{735/(0.0004 + 0.00083 + 0)\} + \{0.05.10^{7.5}.(0.0025/(0.0004 + 0.00083 + 0))\} = 9.2*10^5 \quad (36)$$

Equation 36 thus shows that C_F is approximately $9.2.10^5$ times larger than C_W. The time required to reach this steady state is condition is $3.0/(k_2 + k_E + k_R)$ i.e. 2440 days. This shows that for mirex the fish has to be exposed for a very long time before equation 36 can be used to estimate C_F. It may even be possible that the fish will never reach this steady state condition within its life time. The body burden for mirex in the fish is thus a function of its exposure time t. This time function is given by equation 3, which after substitution of the values of the rate constants is

$$C_F = \{[735.C_W/(0.0004 + 0.00083 + 0)] + [0.05.10^{7.5}.C_W.(0.0025/(0.0004 + 0.00083 + 0))]\}* (1 - \exp-(0.0004 + 0.00083 + 0).t) \quad (37)$$

The example discussed above illustrates the role of toxicokinetics in exposure assessment. It is believed that with improving knowledge of the toxicokinetics of organic chemicals and especially its relationship with chemical properties and organism species reliable tools can be developed for chemical exposure assessment of organic chemicals in aquatic organisms.

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TOXICODYNAMIQUE DES PRODUITS CHIMIQUES ORGANIQUES DANS LES ORGANISMES AQUATIQUES.

Frank, A.P.C. Gobas
The Great Lakes Institute
Université de Windsor
Windsor, Ontario,
N9B 3P4

RESUME

De récents développements dans le domaine de la toxicodynamique des produits chimiques organiques chez les organismes aquatiques sont révisées. Les techniques pour la détermination de l'exposition de substances chimiques organiques sont présentées et illustrées avec des exemples pour le tricnloro-benzène et le mirex.

FIRST SPECIAL SESSION/PREMIERE SEANCE SPECIALE

UNCERTAINTY AND THE APPLICATION OF AQUATIC TOXICITY TEST DATA IN ENVIRONMENTAL PROTECTION

V.M. Brown
Alligator Rivers Research Institute
Darwin, Australia

PAPER UNAVAILABLE AT TIME OF PRINTING

SECOND SPECIAL SESSION/DEUXIEME SEANCE SPECIALE

CHAIRPERSON: N. Bermingham, Environnement Canada, Longueuil, PQ

METHODS FOR AQUATIC TOXICITY REDUCTION EVALUATIONS: PHASE 1 - TOXICITY CHARACTERIZATION PROCEDURES

L. Burkhard
Environmental Research Laboratory, EPA
Duluth, Minnesota

PAPER UNAVIALABLE AT TIME OF PRINTING

BIOLOGY IN THE NEW REGULATORY FRAMEWORK FOR AQUATIC PROTECTION: MAJOR MESSAGES AND RECOMMENDATIONS FROM THE ALLISTON WORKSHOP, APRIL 26-28, 1988*

K.E. Day[1], E.D. Ongley[1], R.P. Scroggins[2] and H. Eisenhauer[2]
[1]Environment Canada, Rivers Research Branch, National Water Research Institute, Burlington, Ontario
[2]Environment Canada, Place Vincent Massey, Hull, Quebec

INTRODUCTION

Recent legislative initiatives in Canada, such as the Canadian Environmental Protection Act (CEPA) and the Ontario MISA program, provide the policy framework for expanding the use of biological criteria for achieving aquatic environmental protection. This has significant implications for those government agencies which establish regulatory criteria, for those industries which will be subject to new forms of regulatory criteria, for private consultants and laboratory companies that will do much of the biological testing and evaluation, and for research organizations both in government and in the university sector.

The benefits of incorporating biological standards both into end-of-pipe criteria and for ambient effects assessment, are widely recognized. There are, however, differences of opinion about the nature of testing protocols, about the practicality of biological protocols, and of the level of scientific knowledge and practical expertise available to formulate and implement such regulations at this time. While government views biological testing of effluents as a cost-effective method of achieving environmental objectives, industry has reservations about such tests for process control of waste treatment plants.

The consulting and laboratory service industry has a significant role to play in implementing any biotesting or assessment program. Questions remain, however, over the precise nature of prescribed tests, the level of participation of the private sector and, indeed, whether or not government is truly committed to biological assessment and is prepared to invest in the R&D to achieve appropriate measures of biological integrity in receiving waters. Quality assurance and laboratory certification in the biotesting industry must be considered as part of any regulatory agenda; however, the role of government has not been defined. Expansion of biotesting requirements in Canada has the potential for creating additional expertise which can augment the competitive position of the Canadian environmental industries sector.

A significant research capacity exists within government to meet its own requirements. The relationship between government laboratories and the private sector must be defined so that activities are complementary and government research is supportive of the private sector and expands its competitive position. Because of increasing demand both for knowledge in this field and for trained expertise, the university sector has also an important role to play.

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*Full proceedings of the Alliston Workshop will be issued in a separate document by Environment Canada in 1989.

The Alliston Workshop (held at the Nottawasaga Inn, Alliston, Ontario, April 26-28, 1988) was created to provide a forum for discussion amongst regulators, regulated industrial groups, environmental consultants and the laboratory services sector, and government and university researchers. The intent of this Workshop was to discuss issues of broad policy significant so to ensure that governments have the benefit of advice during the development of regulatory criteria and to ensure that other government activities, such as research and development, is carried out in a manner which is consistent with and supportive of the broadest possible constituency. It was not the intention of this Workshop to discuss or debate the details of specific test procedures.

Participation in the Workshop was by invitation in order to limit the size to manageable proportions, and to ensure national representation by the various stakeholders. The format included formal presentations to provide necessary background information, and four concurrent working groups which addressed specific issues and formulated a concensus for presentation to the full Workshop.

OVERVIEW OF RECOMMENDATIONS FROM THE WORKING GROUPS

Each working group consisted of a mix of representatives from the federal and provincial governments (i.e., regulators, scientists and managers), industry, environmental consultants and academia. Thus a broad range of viewpoints were represented in each of the working groups. Working independently under the direction of a chairperson, the members of each working group were asked to address four questions as follows:

1. What are the benefits and limitations of biological testing/monitoring strategies as compared to other possible methods and strategies (e.g., the chemical-specific approach?)
2. What should be the role of government laboratories in biological testing and monitoring research and development?
3. Should government have a quality assurance/quality control (QA/QC) role in regulatory programs where biological testing and/or monitoring is a component?
4. What role should government play in ambient bio-effects assessment?

The summary notes of each rapporteur's report were reviewed by the workshop organizational committee. Major points of discussion and/or conclusions common to all four working groups are outlined below.

The responses to the four questions are as follows:

Question #1. What are the benefits and limitations of biological testing/monitoring strategies as compared to other possible methods and strategies (i.e., chemical-specific approach)?

The generic needs and recommendations identified by all four working groups are summarized as follows:

It was recommended that government develop a program framework and outline policy statements required to set biology-based regulatory standards and to expedite the development of standardized protocols for biological toxicity tests.

It is the responsibility of the government to set clear and precise requirements regarding the use of biological toxicity tests for regulations, guidelines, hazard assessment, etc. Standard protocols should be formalized by the federal governments for those tests that are already well-proven. These protocols should be incorporated into appropriate new and amended regulations or guidelines as soon as possible. Research and development should be concentrated on promising tests that are not yet proven and as newer, more sensitive, cost-effective and efficient tests mature, government should provide standard protocols for these tests.

Concern was expressed by industry that different jurisdictions may demand different biological testing methods or procedures for regulation within a region. Although it was recognized that some biological toxicity tests may be site-specific or industry-specific, it was recommended that protocols be standardized or harmonized as much as possible on a national basis and that they be related to other protocols required internationally.

Biological testing and monitoring must be integrated with chemistry in a multi-disciplinary manner when applied in hazard assessment and regulatory control.

A clear need was expressed for both laboratory and field biological toxicity tests to be used in conjunction with chemistry for regulatory purposes. The ideal protocol would consist of tiers of tests, with a "battery of tests" at each tier containing tests with quantified triggers leading to the next tier, if and only if, the trigger is tripped. Initial screening would be by simpler tests, i.e., short-term acute and chronic toxicity tests, followed by longer-term chronic and sublethal toxicity tests. More comprehensive ecotoxicological tests involving several trophic levels could be incorporated in the final tiers to assess ecosystem dysfunction. The tiered approach provides for the most effective use of funds and other resources but the selection of the tests at each tier requires a great deal of skill. At present, there are a limited number of toxicity tests available which provide sufficient sensitivity to detect sublethal ecosystem effects. In addition, field validation of such toxicity tests is rare but essential if these tests are to be considered to truly protect whole ecosystems.

Sublethal and chronic tests need to be developed to provide more sensitive monitoring tools for detecting toxic effects in ambient waters.

The detection of "far afield" effects of contaminants on individual species, populations and communities in ambient waters will, to a large extent, require different techniques than those used in effluent monitoring. There are some biological effluent monitoring tests currently available which are sensitive enough to detect "near-field effects" e.g., in the mixing zone of discharges in ambient waters. Generally speaking, however, high sensitivity sublethal and chronic monitoring techniques required for measuring far-afield effects are still in the early stages of development. For the most part, ambient testing involves separate philosophical implications because the cause/effect/solution relationships are seen to interact interactively through a number of linkages. For example, if biological toxicity tests are conducted on an ambient water sample and the results indicate toxicity, it may be difficult to identify a specific effluent discharge if there is more than one source of wastewater being

discharged in the vicinity. In theory, if all sources are regulated and if all are in compliance, there should be no problem with ambient water quality but this assumes "ideal" regulation.

Tests of ambient water quality should be tied to water quality objectives. At present there are some water quality objectives for ambient waters, e.g., in the Great Lakes, but these objectives are chemical-specific only. There is a need for biological criteria or objectives for ambient waters.

All four working groups strongly recommended that chronic and/or sublethal biological toxicity tests are essential when attempting to detect toxicity in ambient waters.

Several groups suggested that more flexible, site-specific biological testing and monitoring procedures may be required for ambient water impact assessment.

Mechanisms for improved communication, awareness and understanding of the applied uses of biological toxicity tests amongst the scientific community, regulatory agencies, industry and the public sector must be pursued.

Concern was expressed that neither government nor industry are trusted by the public in matters of environmental protection. Therefore, there should be an improved means of education and/or explanation of the significances and purposes of biological toxicity tests and their results. Such education would assist the public in their comprehension of regulations and to assure the public that reasonable attempts are being made to protect the environment and reduce risks.

There is a need to clarify nomenclature and/or definitions in order to have consistency amongst users and interpreters. This results from a confusion over the fact that some terms which are common in name have different meanings to human and environmental toxicologists, e.g., chronic, short-term, etc.

Other considerations and/or recommendations: In addition to the above points outlined as key issues of priority by all four working groups, several needs and/or recommendations were suggested by some of the working groups as follows:

- The need for the development of *in situ* toxicity tests.
- The need for objectivity, practicality and cost-efficiency.
- The need for the development of rapid in-plant monitoring systems.

Question #2. What should be the role of government laboratories in biological testing and monitoring research and development?

Government must maintain a strong research and development capability and continue to transfer technology developed in the field of biological testing and monitoring to the private sector.

The government is responsible for regulations regarding the use of biological toxicity tests and must undertake and maintain fundamental research programs in this area. This is necessary to ensure that government laboratories have expertise in biological toxicity tests and to maintain "state-of-the-art" research as well as viable in-house programs. In

addition, government must be qualified for the necessary role of auditing biotests conducted in the private sector.

Universities and other researchers within the private sector should be encouraged to collaborate with the government in the development of biological toxicity tests. Contracts should be assigned and funded to those with specific capabilities and expertise. The establishment of Centres of Excellence was discussed as one way to develop certain types of biological toxicity tests.

Governments should transfer the technology of biological toxicity tests to the private sector when such tests are fully developed. The private sector should have the opportunity to exploit such technology with the government remaining as an auditor for QA/QC.

Neither government nor university laboratories should enter into competition with the environmental service sector by providing biological monitoring or testing services to industry on a routine basis. Government's role in this area should be to provide QA/QC assistance and to supply its own needs.

The government should not necessarily bear the entire financial responsibility of payment for the development of biological toxicity tests. The polluter may be required to pay for R&D through revenues from fines imposed in violation of regulation.

Question #3. Should government have a QA/QC role in regulatory programs where biological testing and/or monitoring is a component?

The Federal Government should provide guidance and show leadership in the area of biological testing and monitoring QA/QC, e.g., policies, guidelines, laboratory accreditation, toxicologist certification, etc.

Strong QA/QC programs are essential to validate the results of laboratories in both the private and public sectors. Such programs would result in laboratory certification to identify those laboratories which have demonstrated reliability and reproducibility. The certification of toxicologists in Canada would be a spinoff from this program. This would increase the credibility of the results of testing and assure the public that there is no collusion between government and industry.

It was strongly recommended that the Federal government should provide a lead role in all QA/QC for biological toxicity tests. As well as providing very specific protocols for biotests, government should provide a manual of Good Laboratory Practices and enforce these practices. Self-assessment should be the rule within industry but the government should be responsible for periodic auditing.

Question #4. What role should government play in ambient bioeffects assessment?

Government is responsible for the maintenance of long-term ambient monitoring schemes while industry is responsible for the assessment of immediate areas of impact, e.g., end-of-pipe and mixing zones.

It is the government's role to assess or evaluate the effectiveness of existing regulations in the receiving environment. It is also the government's responsibility to identify emerging or unpredicted problems that need to be addressed by regulatory regimes. Therefore, the government must be

responsible for tests to assess the effects of contaminants in ambient waters.

The maintenance of long-term data sets for biomonitoring, state-of-the-environment reporting, and the monitoring of impacts on large areas must be conducted by the government. The only type of assessment that should be the primary responsibility of industry is the assessment of the immediate area of impact around its operation.

MAJOR MESSAGES

The purpose of this Workshop was to provide a forum for discussion of points of view by the various stakeholders. The following messages arose consistently from formal presentations and during informal discussions.

1. Industry views biological assessment as a useful supplementary activity but argue that chemical, rather than biological tools are more practical for process control of wastewater facilities.
2. Industry is concerned that standardized tests are inappropriate for compliance regulations but feel that they will be adopted by government for ease of implementation. Tests should be tailored to specific receiving waters.
3. Government views standardized acute toxicity tests as a reasonable regulatory compliance tool for generic first-level environmental protection, but feels that chronic toxicity tests should be required on a site-specific basis as warranted.
4. Industry considers that aquatic regulations should be factored into the total cost for protecting air, soil and water.
5. Historically, government regulations for the three environmental media have not been developed using an holistic approach.
6. Industry generally perceives that there are mixed and overlapping federal and provincial agendas.
7. Government extols the virtue of dialogue in the regulatory agenda.
8. Industry is sceptical that dialogue has much impact on regulators.
9. Consulting sector finds it easier to work in the United States than in Canada; they perceive that there are implicit interprovincial restrictions within Canada.
10. Consulting industry wants clear directions from government on testing protocols to be used.
11. Consultants are concerned that government will encourage the private sector to gear-up for biotesting for regulatory purposes but not follow through with specific requirements for biotesting in regulations. Should this situation develop, consultants will be forced to limit their development in this field or seek business opportunities in the United States where there is demand for biotesting.
12. Consulting industry is concerned that government will not honour its commitment to the concept of biological integrity as the measure of success of regulations; rather, government will revert to its traditional engineering approach using R&D funds for in-plant technology development without adequate R&D on biological assessment.
13. There is a concern on the part of the consulting industry that universities are training academic researchers rather than professionals useful to the consulting industry.

THIRD SPECIAL SESSION/TROISIEME SEANCE SPECIALE

ECOTOXICOLOGICAL TESTING IN AN INDUSTRIAL ABATEMENT STRATEGY (PERSPECTIVES FOR THE ST. LAWRENCE RIVER ACTION PLAN)

N. Bermingham[1] and M. Sinotte[2]
[1]Environnement Canada, Longueuil, PQ
[2]Environnement Quebéc, Ste-Foy, PQ

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FOURTH SPECIAL SESSION/QUATRIEME SEANCE SPECIALE

Joint meeting with the Society of Toxicology of Canada
Réunion conjointe avec la Société de Toxicologie du Canada

CHAIRPERSON: Dr. R. Van Coillie, Environnement Canada, Montréal, PQ

HISTORIQUE ET BUTS DE LA SOCIETE DE TOXICOLOGIE DU CANADA

J. Brodeur
Université de Montréal, Montréal, PQ

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THE CANADIAN ANNUAL AQUATIC TOXICITY WORKSHOP - GOALS AND HISTORY

Peter G. Wells
Conservation and Protection
Environment Canada
Dartmouth, Nova Scotia, B2Y 2N6

ABSTRACT

The Canadian Annual Aquatic Toxicity Workshop provides an annual focus in Canada on the principles, current problems, approaches, and issues in aquatic toxicology. Each workshop is an interdisciplinary forum that includes aquatic chemistry, toxicology, hazard assessment, and a consideration of applications in environmental monitoring, regulations and guidelines, water and sediment quality guidelines and objectives, and other approaches.

The paper describes the first workshop (Winnipeg 1974); the early years from the Toronto (1975) to Montreal workshops (1980); the establishment of the Corporation for the Canadian National Aquatic Toxicity Workshops; the growth in the field and the workshops to 1988, and events such as the multiple sponsorship, the Workshop Proceedings, the Continuity Chairman, and the continued Surveys of Aquatic Toxicologists that have led to the continued conduct and success of the Workshops. A synopsis of the topics covered during the 14 workshops with the active participation of scientific and managerial practitioners illustrates the contribution of the workshops to information exchange essential for the solution of pressing freshwater, estuarine and marine pollution problems.

1. WORKSHOP GOALS

The overall goal of the Workshop series is to meet annually to discuss new developments in aquatic and environmental toxicology, covering topics from:

- basic concepts and principles;
- critical reviews of the state of the art on specific topics;
- techniques of toxicity testing and biological assessment, in the laboratory and field;
- applications in environmental monitoring;
- applications in various strategies for environmental protection, including regulations and guidelines;
- applications in the development of water and sediment quality guidelines and objectives.

The Workshop provides an annual focus in Canada on the principles, current problems, approaches and issues in aquatic toxicology. The Workshop has broadened throughout the years to include aquatic chemistry, toxicology and hazard assessment, and the role of aquatic toxicology in environmental management.

The Workshop emphasizes an informal exchange of knowledge and ideas on the topic among interested persons from all sectors (government, industry, university, business). The Workshop offers opportunities to visit local institutions and relevant field sites, and it stimulates contacts between the diverse disciplines of environmental toxicology and its practitioners in the science, education and management.

The focus of the Workshop has expanded over the years from the important topic of toxicity testing methodologies, with which it is still concerned, to a comprehensive presentation of basic and applied studies on the fate and effects of chemicals entering aquatic ecosystems. This change reflects the significant developments of the field of environmental toxicology and chemistry, of which aquatic toxicology is a part, and the important role of aquatic science and aquatic toxicology in the hazard assessment, control and monitoring of chemicals, effluents and emissions.

2. HISTORY

The Workshops have been held at different locations, coast to coast, in centers where considerable work in aquatic toxicology is being conducted. To date, the workshops have taken place in seven provinces. This ensures that regional and national water pollution issues and research are discussed on a regular basis. Each meeting is organized and run by a different volunteer group, with funding coming from the participants (registration) and the agencies employing the volunteers. Hence, the specific objectives, presentations and character of each meeting vary with each local organizing group and location (Wong et al., 1979).

2.1 The First Workshop

The first meeting was held in Winnipeg in August 1974, organized by scientists at the Freshwater Institute, then part of the newly formed federal Department of the Environment, and at that time associated with the Fisheries Research Board of Canada. Its purpose was to bring together scientists, biologists and technicians who were practising aquatic toxicology, to discuss and compare toxicity testing methodologies for industrial wastes and microcontaminants. A new laboratory for aquatic toxicity research had been opened at the Freshwater Institute. Toxicity test development was part of the research activities of the Institute (Scherer 1979). The emphasis was on testing methods because a federal task force, chaired by Dr. R.D. Hamilton at the Institute, had concluded "that enforcement of new environmental laws would be limited due to the lack of sound testing procedures" (Scherer 1979). Approximately 45 persons attended in Winnipeg. A Compendium of Aquatic Toxicity Studies in Canada 1974 was produced, distributed to participants, but not formally published.

It is noteworthy that the first Canadian Annual Aquatic Toxicity Workshop occurred ten years after the Society of Toxicology of Canada was founded (1964); it was two years in advance of the first ASTM Annual Symposium on Aquatic Toxicology (October 1976); and it was six years before the formal establishment of SETAC (the Society of Environmental Toxicology and

Chemistry) with its first meeting in 1980. This is a reflection of the long history, many contributions and considerable foresight of Canadian scientists and environmental managers in water pollution research.

2.2 The Early Years

Between 1975 and 1980, the Workshops were held in Toronto, Halifax, Vancouver, Hamilton, Winnipeg and Montreal (Figure 1). With the exception of Montreal, they were mostly sponsored by single agencies, the Ontario Ministry of Environment (1975) and the (then) Department of Fisheries and Environment (1976-79). The Montreal Meeting (1980) marked the beginning of a broader sponsorship. Between 14 and 35 papers were given each year; attendance ranged from 100 to 149; and most of the meetings had three to six discussion workshops on key topics. Whereas the Toronto, Halifax and Vancouver meetings dealt primarily with methods (reference toxicants, rapid bioassays, field bioassays, experimental ecosystems and monitoring), the Hamilton, Winnipeg and Montreal meetings dealt with important regional problems (acid deposition, pesticides, industrial wastes, metals) and the applications of aquatic toxicology (environmental laws, environmental management). The Discussion Workshops debated techniques, bioaccumulation, applications of toxicology, fate pathways and the future of the science. A start was made to the survey of aquatic toxicity research and practice in Canada. Participants acknowledged that aquatic toxicology was changing and maturing, and that the annual meetings were essential for information transfer, discussion and direction.

2.3 Reaching Maturity - Establishing the Corporation: The Canadian National Aquatic Toxicity Workshop, 1980-1983

For several years, the Workshops had been organized and run, and kept running each year, with the guidance and interest of a small number of people. The participants of the Montreal Meeting, polled by Peter Hodson in 1980, after discussions at the Winnipeg Meeting the year before, voted for informality in the annual conduct of the Workshops, and did not support the formation of a new society of any affiliation with existing ones.

A key figure during this time was Sharon Leonhard of the Freshwater Institute, supported by others from across Canada, who acted as Secretariat for the Workshop series, keeping meticulous records, and organizing and chairing the first Steering Committee Meeting in 1980, in Montreal. This was to ensure that the next year's site was selected and an appropriate Chairperson and team were in place.

However, a structure was necessary to more formally guide the Workshops, to provide continuity from year to year, and to ensure that monies could be transferred from one Workshop Committee to the next correctly. Sharon Leonhard and Mr. William (Bill) Lake (Alberta Environment) produced terms of reference of the Corporation, drafted the by-laws, and made the formal application for incorporation. Hence, the wheels were put into motion in 1980 for a Steering Committee to lead the Workshop Series, behind the scenes.

The Corporation (The Canadian National Aquatic Toxicity Workshop) came into being in January 1984, after the Edmonton and Halifax Workshops, with the papers for incorporation being submitted in Alberta. The first Board of Directors had eight members: S. Leonhard, K. Solomon, W. Lake, P.G. Wells, M. Gilbertson, G. Geen, N. Bermingham, and P. Hodson).

The Board of Directors is composed now of six persons: the past two Chairpersons, the current Chairperson, the next two Chairpersons, and the Workshop Continuity Chairman from the Department of Fisheries and Oceans. The Board changes annually, reflecting ongoing workshops. The current Board (November 1988) is composed of Drs. J. Lakshiminarayana, P. Stokes, R. van Coillie, Sharon Leonhard, P. Chapman and A. Niimi.

There are at present nine Directors-At-Large, composed of past and future Chairpersons and continuity Chairpersons, and others appointed by the Board.

Hence, the full Board of the Workshop Steering Committee is currently composed of 15 persons, nine from government, five from university, and one from the private sector. Three more persons are being proposed to represent the private sector. We are honoured that one Director, Dr. Howard McCormick from the United States EPA, Duluth, MN, has served since the beginning, giving the Workshop a valuable United States' connection.

A Report from the Directors is given annually at each Workshop by the current Chairperson.

2.4 Growth of the Field of Aquatic Toxicology - Workshops 1981 to 1987

With the stimulation of the small and as yet unofficial Steering Committee, the workshops continued enthusiastically, locations often being chosen three years in advance.

Meetings were held in Guelph, Edmonton, Halifax, Vancouver, Thunder Bay, Moncton and Toronto between 1981 and 1987. Attendance was at an all-time high at the Guelph and Toronto meetings (around 180 persons), with attendance generally between 90 and 134 persons (Figure 2), and being influenced by location and meeting themes. Papers were supplemented with poster sessions; together they ranged between 35 and 80 contributions in the 2.5 day Workshop. Discussion workshops were very popular at Guelph and Edmonton, then declined, then picked up again for Thunder Bay and Moncton. The workshops continued to be sponsored by several agencies, up to 13 for Moncton's Meeting.

Themes became more specialized, and tended to reflect regional interests (energy concerns in Alberta, acid rain in Guelph, Arctic and offshore for the Halifax meeting, and mining and petroleum for Vancouver), as well as the latest developments and approaches. Sessions reflected the advancements in the field - toxicokinetics, biochemical toxicology, chemical contaminants and fish disease, sediment assays and assessment of particle-bound contaminants, and lab-to-field extrapolation. The meetings became ecotoxicology sessions by definition, with the thrust being towards understanding the most pressing water issues. Some attention was also being spent on where aquatic toxicology

vas headed as a discipline, an analysis also taking place at the US ASTM meetings.

The surveys of current aquatic research culminated in a Directory (Wells et al., 1985) published after the Halifax meeting.

Proceedings of all meetings to 1987 have been published. In addition, the Moncton workshop led to a special technical publication (Nriagu and Lakshiminarayana, 1988).

2.5 Significant Events - 1980 to Present

A number of events during this period are a reflection of hov ve are moving ahead as a successful but informal group:

2.5.1 Sponsors. Sponsorship of the Workshops expanded in this period, coming to represent two levels of government, universities, industry and the consulting sector. Environment Canada and the Department of Fisheries and Oceans co-sponsor the Workshops federally. The Workshop Committees during this period clearly shov the breadth of the sponsorship, from one to 13 sponsors, with a trend in recent years for multi-sectoral sponsorship.

2.5.2 Participation. Participants to the Workshops came from across Canada and notably from the United States and several other countries. The United States' participation has been very strong at several meetings (Vancouver, Halifax, Thunder Bay, Toronto). The Thunder Bay meeting vas co-sponsored by the USA EPA at Duluth, making it our first international gathering. Our guest speakers have been from as far avay as Australia.

2.5.3 The Continuity Chairman. The duties of the Continuity Chairman, supported by the Department of Fisheries and Oceans, are: (a) to provide a fixed address for inquiries to the Aquatic Toxicity Workshop; (b) to maintain the mailing list and provide it upon request to each year's local Workshop Steering Committee; (c) to publish and print the proceedings of the annual Workshop; and (d) to maintain a file of reprints of the workshop proceedings and distribute them upon request.

Three Continuity Chairman - Keith Marshall (1980-81), Michael Gilbertson (1981-86) and Dr. N.Y. Khan (1986-87) - filled the position until 1987. At that time, with the concurrence of the National Steering Committee, Fisheries and Oceans moved the position to the Canada Center for Inland Vaters, vhere Dr. Art Niimi currently continues this vital and essential role on behalf of the Vorkshops.

The preparation of each year's meeting has nov become streamlined, with clear sets of instructions, deadline dates, the mailing list and authors' instructions being passed from one Vorkshop to the next.

2.5.4 Mailing List. Between 1981 and 1984, the mailing list for the Vorkshop vas computerized, thanks to the efforts of Mike Gilbertson and DFO in Ottava, and it is nov available to all Vorkshop Steering Committees from the Continuity Chairman.

2.5.5 Surveys of Research in Aquatic Toxicology. Following from the efforts of several Workshops (starting in Hamilton in 1978) to present summaries of ongoing work in the field in Canada, a Canadian Directory of Aquatic Toxicologists and Related Specialists was organized and published by Environment Canada (Wells et al., 1985).

2.5.6 CAVPRC. The workshops gained another sponsor in 1986 - the Canadian Association for Water Pollution Research and Control (CAVPRC), the Canadian national body of the International Association for Water Pollution Research and Control (IAWPRC). This affiliation was considered important as it offers one possibility for a formal umbrella organization for the environmental/ aquatic toxicologists in Canada, and offers a journal (Water Pollution Research Journal of Canada) as one vehicle for the primary publication of papers presented at the Workshops.

2.5.7 SETAC. The Society of Environmental Toxicology and Chemistry was formed during the period 1978 to 1980, with its first meeting in November 1980. A number of Canadian toxicologists and environmental chemists were amongst its charter members, and several have been active in the organization and on the Editorial Board of its Journal. We are coordinating the times of our meetings with SETAC, and encouraging linkages between the two groups.

2.5.8 The Proceedings. Last, but probably most important, the Department of Fisheries and Oceans gave the National Steering Committee assurances for the continued use of their Technical Report Series, Canadian Technical Report of Fisheries and Aquatic Sciences, for the annual Workshop Proceedings. They are also financially supporting this publication, a major contribution to the Workshop's long-term financial and technical stability.

2.6 The Fifteenth Workshop, Montreal 1988

We are now in Montreal and we have come a long route over the past 14 years. Issues that we have to deal with as a group include:

- broadening of the disciplines represented at our annual meetings;
- affiliation with formal societies and other groups;
- selection of key and relevant themes, important to the problems we are facing with toxic chemicals;
- continuation of the Proceedings in its present or other format;
- ensuring that we keep moving the Workshop across Canada to represent regional as well as national research and regulatory priorities.

3. SUMMARY

The Annual Aquatic Toxicity Workshops have an important role to play in Canada. They are contributing to the maturing discipline of aquatic and environmental toxicology, and are providing crucial information for the resolution and control of major threats to water quality and aquatic ecosystems. There is still much to do, and the debate and discussion on future directions, especially with our colleagues from the Society of Toxicology of Canada, is most welcome. That we are meeting together is indeed

a sign that Aquatic Toxicology has come of age and has a bright and responsible future.

ACKNOWLEDGEMENTS

On behalf of the Board of Directors of the Canadian National Aquatic Toxicity Workshop, I would like to formally acknowledge efforts and dedication of the Workshop organizers, the Members of all local organizing committees since 1974, the two federal departments that sponsor the meeting and its publication (Environment Canada, Fisheries and Oceans), all other sponsors of the Workshops from all sectors, and the participants of all 15 Workshops for their technical contributions. I alone am responsible for any errors or oversights in the above historical overview.

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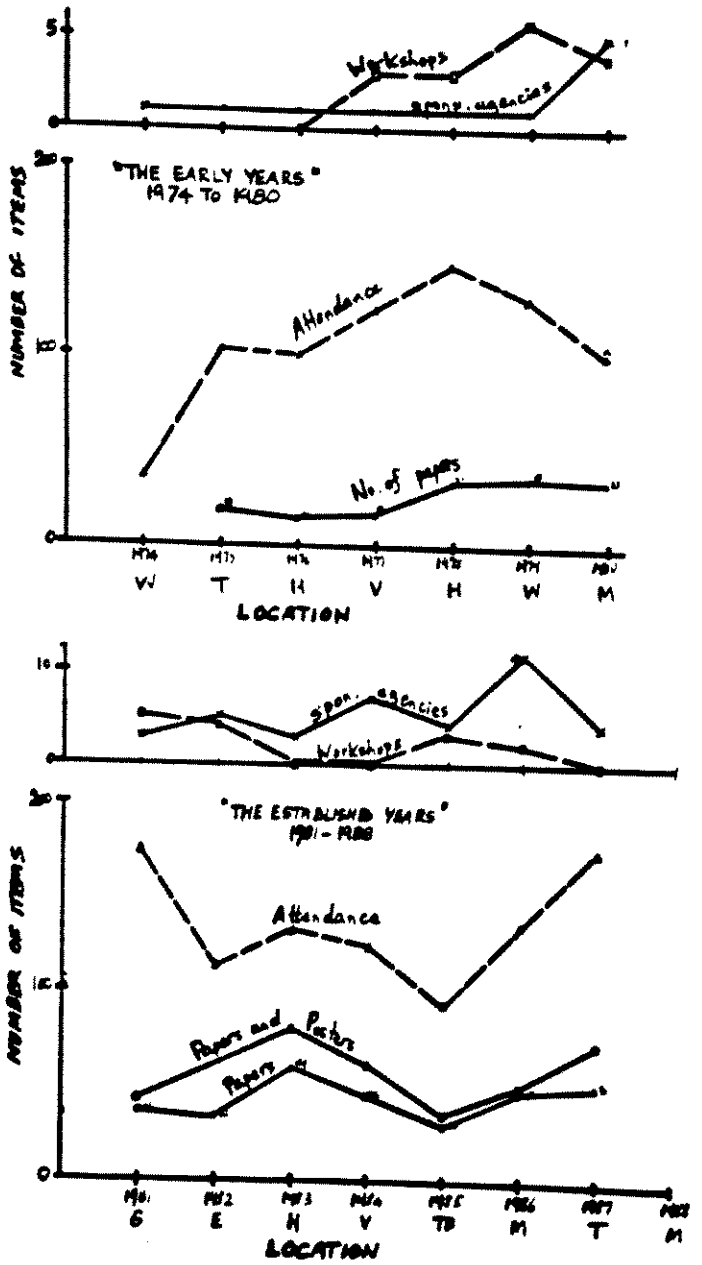


FIGURE 1. THE EARLY YEARS 1974-1980.

FIGURE 2. THE ESTABLISHED YEARS 1981-1988.

CURRENT STATE OF KNOWLEDGE IN HUMAN TOXICOLOGY

G. Plaa
Université de Montréal, Montréal, PQ

PAPER UNAVAILABLE AT TIME OF PRINTING

CURRENT STATE OF KNOWLEDGE IN ECO-TOXICOLOGY

P. Anderson
Concordia University, Montréal, PQ

PAPER UNAVAILABLE AT TIME OF PRINTING

USEFULNESS OF BIOTECHNOLOGY FOR TOXICOLOGY

C. Greer
Biotechnology Research Institute, NRC
Montréal, PQ

PAPER UNAVAIBLE AT TIME OF PRINTING

POSTER SESSION/SEANCES D'AFFICHAGE

Radiological, Chemical, and Biological Evaluations of Site Operations at Hanford, Washington, U.S.A.

Robert H. Gray
Office of Hanford Environment
Pacific Northwest Laboratory
Richland, WA 99352

SUMMARY

Environmental monitoring and surveillance are conducted on the U. S. Department of Energy's (DOE) Hanford Site to detect and assess potential impacts of facility operations on fish, wildlife, air, surface and ground water, foodstuffs, soils and natural vegetation. In addition to monitoring radioactivity in fish and wildlife, population numbers of key species are determined, usually during the breeding season. Data from monitoring efforts are used to estimate the overall radiological dose to humans onsite or residing in nearby communities. Results show that chinook salmon (Oncorhynchus tshawtscha) spawning in the Hanford reach of the Columbia River has increased dramatically in recent years with a concomitant increase in winter nesting activity of bald eagles (Haliaeetus leucocephalus). In addition, the Hanford area serves as a refuge for nesting Canada goose (Branta canadensis) and great blue heron (Ardea herodias), and various plants and other animals, e.g., elk (Cervus elaphus), mule deer (Odoeoileus hemionus), and coyote (Canis latrans).

In 1987, all measured Hanford Site perimeter concentrations of radionuclides were below applicable DOE or Environmental Protection Agency (EPA) guidelines. Tritium and nitrate continued to be the most widespread constituents in onsite ground water. Chromium, cyanide, fluoride and carbon tetrachloride were found in ground-water wells near operating areas. Concentrations of radionuclides identified at a municipal river water intake were well below concentration limits established for drinking water by EPA and the State of Washington. Nonradiological water quality parameters were in compliance with applicable standards. Foodstuffs irrigated with river water taken downstream of the site showed low levels of radionuclides that were similar to concentrations found in foodstuffs from control areas not irrigated with Columbia River water. Low levels of ^{90}Sr and ^{137}Cs found in some onsite wildlife samples were typical of those attributable to worldwide fallout. Although low concentrations of radionuclides were found in soils and vegetation from both onsite and offsite locations, there was no indication of radionuclide buildup attributable to Hanford.

Measured exposure to penetrating radiation and calculated radiation doses to the public from 1987 Hanford operations were well below applicable regulatory limits. The calculated effective dose potentially received by a maximally exposed individual (i.e., the individual receiving the maximum calculated radiation dose using worst-case assumptions for all routes of exposure) in 1987 was 0.05 mrem/yr, about half the dose calculated for 1985 and 1986. Hanford doses are well below applicable standards for radiation protection and are much less than those received from common sources such as natural background and consumer product radiation or radiation from medical diagnoses such as x-rays.

U.S. DOE Contract DE-AC06-76RLO-1830 with Battelle Memorial Institute.

EVALUATIONS RADIOLOGIQUES, CHIMIQUES ET BIOLOGIQUES DU SITE D'OPERATIONS A HANFORD, WASHINGTON, U.S.A.

Robert H. Gray
Bureau d'environnement d'Hanford
Laboratoire du Pacifique Nord-Ouest
Richland, Wa 99352

RESUME

Du monitorage et de la surveillance environnementale sont effectués sur le site Hanford du U.S. Department of Energy (DOE) afin de détecter et d'évaluer les impacts potentiels des installations opérationnelles sur le poisson, les organismes vivants dans le milieu, l'air, les eaux de surface et souterraines, les denrées alimentaires, les sols et la végétation naturelle. En plus de suivre la radioactivité dans le poisson et les organismes vivants dans le milieu, les dénombrements de populations des espèces clés sont déterminées normalement durant la saison de reproduction. Les données recueillies sont utilisées pour évaluer l'ensemble de la dose radiologique pour les humains qui vivent sur place ou résidant dans les communautés environnantes. Les résultats ont démontré que la fraie du saumon "Chinook" (Oncorhynchus tshawtscha) dans la rivière Columbia à proximité d'Hanford a augmenté de façon dramatique ces dernières années et ce, conjointement à un accroissement de l'activité de nidification hivernale des aigles à tête blanche (Haliaeetus leucocephalus). De plus, la région d'Hanford sert de refuge pour la nidification de l'oie du Canada (Branta canadensis), du grand héron bleu (Ardea herodias), et à de nombreuses plantes et animaux tels que l'élan (Cervus elaphus), le daim (Odoeoileus hemionus), et le coyote (Canis latrans).

En 1987, toutes les concentrations de radionucléïdes mesurées sur le périmètre d'Hanford étaient en dessous des normes établies par le DOE ou l'Agence de Protection de l'Environnement (EPA). Le tritium et les nitrates ont continué à être les constituants les plus répandus dans les eaux souterraines du site. Du chrome, du cyanure, du fluor et du tétrachlorure de carbone ont été retrouvés dans des puits souterrains près des lieux d'opération. Les concentrations de radionucléïdes identifiées à partir de la prise d'eau municipale située dans la rivière étaient très inférieures aux limites de concentration établies pour l'eau potable par l'EPA et l'Etat de Washington. Les paramètres non radiologiques de la qualité de l'eau étaient conformes aux normes en vigueur. Les aliments comestibles irrigués par l'eau de la rivière et prélevés en aval du site ont démontré de faibles niveaux de radionucléïdes, ce qui était comparable aux concentrations trouvées dans les aliments des zones témoins non irrigués

par l'eau de la rivière Columbia. De faibles concentrations de Sr^{90} et Cs^{137} trouvés dans quelques échantillons vivants prélevés sur le site étaient comparables aux moyennes des retombées mondiales. Bien que de faible concentrations de radionucléides aient été trouvées dans les sols et la végétation, à la fois sur le site et en dehors de celui-ci, il n'a pas été démontré d'accumulations de radionucléides attribuable à Hanford.

En ce qui concerne les opérations d'Hanford de 1987, l'exposition mesurée au niveau du public et relative à des doses de radiations pénétrantes et calculées étaient bien inférieures aux limites normatives applicables. La dose effective calculée, potentiellement reçue par un individu exposé au maximum (i.e. l'individu ayant reçu le maximum de radiations calculées à partir d'hypothèses les plus critiques pour toutes les voies d'exposition), était en 1987 de 0.05 mrem/an, soit la moitié de la dose calculée pour 1985 et 1986. Les doses de Hanford sont très inférieures aux normes applicables de protection pour les radiations et le sont beaucoup moins que celles reçues à partir de sources usuelles, telles que le bruit de fond naturel, les radiations de produits de consommation ou les radiations servant aux diagnostics médicaux, comme les rayons-X.

Phytoplankton and Periphyton Communities in a Shield Lake Receiving Acid Mine Drainage in NW Ontario

M.Kalin , M. Olaveson* and B. McIntyre
Boojum Research Ltd. Toronto, Ontario
*Algatax Toronto, Ontario

ABSTRACT

An account of the phytoplankton and periphyton communities in a shield lake, which has received acid drainage from a pyritic tailings deposit, for over a decade is presented. The algal populations in the lake exist at a pH of 4.3 and ranges of heavy metal concentrations of Cu <0.005 - 1.5 mg/l, Fe <0.01 - 62 mg/l, and Zn 3.7 - 19.3 mg/l. The phytoplankton diversity and biomass, show differences which can be related to acid and metal concentrations.

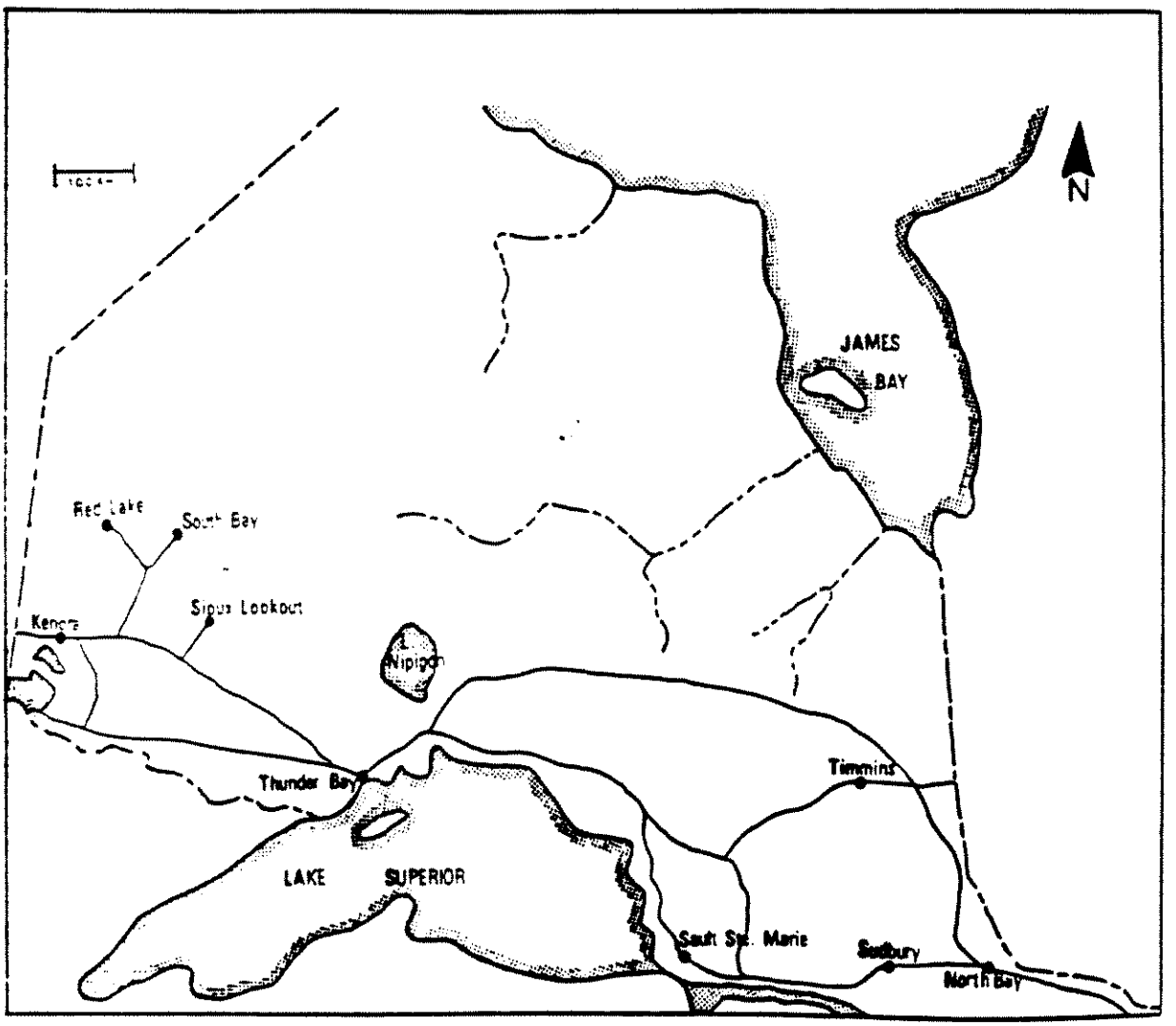
The presence/absence of desmids in the lake receiving discharge from our study lake, may be used as an indicator of water quality. The data suggest that acid tolerant algal communities exhibit tolerance to high levels of heavy metals. This is particularly apparent for the periphytic communities. Growth experiments have shown that the accumulation of periphyton on branches of dead fall can reach 20 g/100 g substrate (dry weights) in one summer (177 days), and up to 90 g in two seasons (496 days). The concentration of Zn in the algal complex varies from 328 to 7300 mg/l depending of length of exposure. Some tolerant species may even aid in monitoring the recovery of a stressed system.

INTRODUCTION

A shield lake (25 ha in surface area) located in northern western Ontario (Map 1), part of the English River drainage basin, receives acid mine drainage from a pyritic tailings deposit which resulted from the operation of a copper/zinc mine from 1971 to 1981.

The decommissioning activities after closure of the mine in 1981, included the evaluation of the phytoplankton and periphyton communities in the acidified lake. The purpose of the study was to compare the algal community of the acidified Boomerang Lake with

Map 1: Location of study site at South Bay in NW Ontario.



that of the receiving water body, Confederation Lake. The composition of the algal populations in each lake were evaluated with respect to the presence of pollution sensitive species to identify changes in water quality.

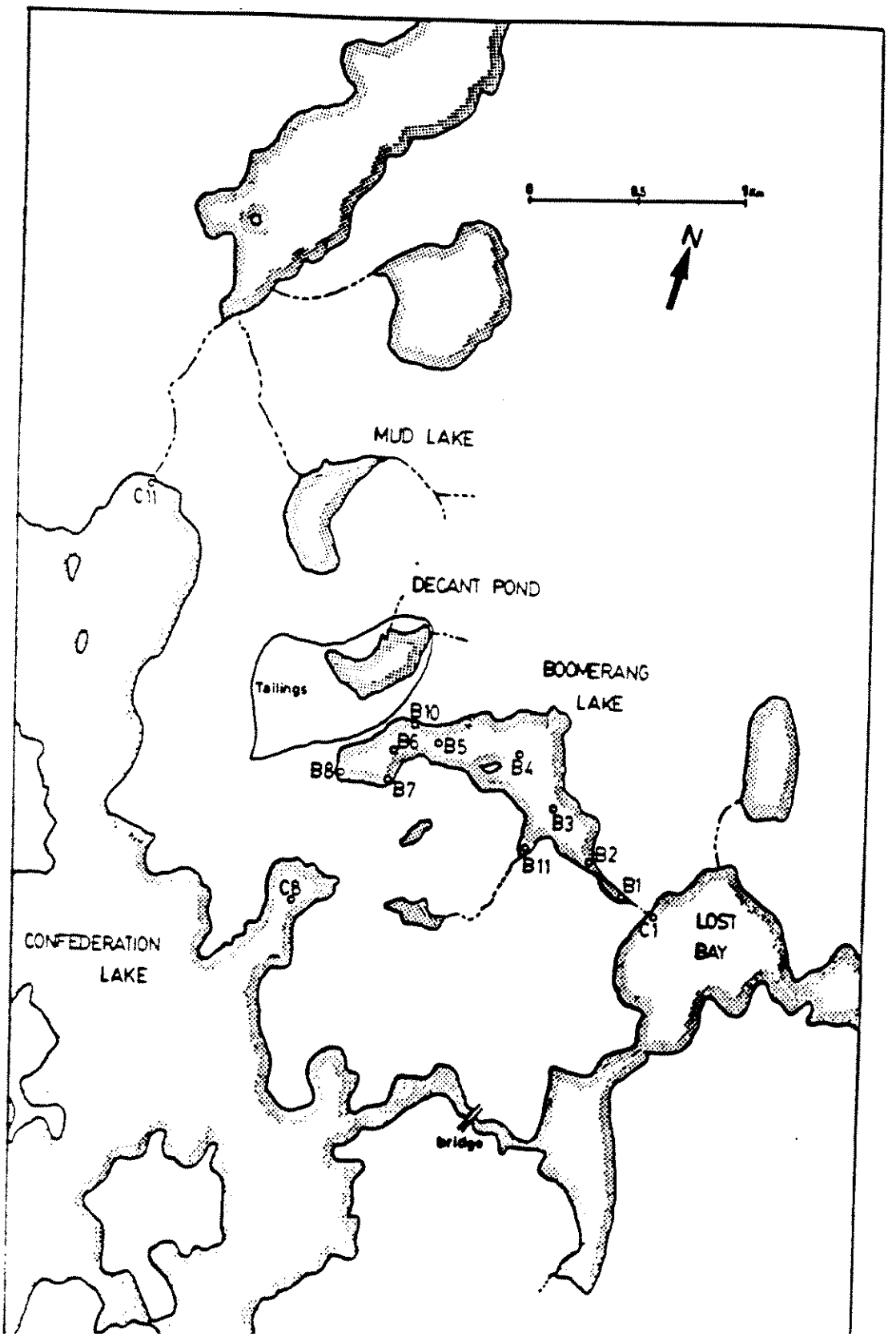
The composition of the phytoplankton community can be a useful indicator of the recovery of the lake ecosystem (Schindler, 1987). It is anticipated that favourable changes in water quality will occur as a direct result of Ecological Engineering and Biological Polishing measures implemented at the site (Kalin and Van Everdingen, 1988).

METHODS

The study was carried out of over a 3 year period (1986 - 1988). Sampling sites for this study were chosen at various locations in Boomerang Lake, B7, B8, B10 and B11 representing seepages or tailings spills and B6 to B1 stations in the open water of the Lake (Map 2). Station C1 represents the discharge from Boomerang into Confederation Lake.

At stations with water depth greater than 1 m (B6 to B1), an integrated phytoplankton sample was collected using a weighted tube sampler (3 cm diameter). The integrated sample represents the upper 2 to 3 m of the water column. At shallow locations (all other stations) surface grab samples were obtained. In all cases, a one litre sample was collected and fixed with Lugol's solution.

Map 2: Sampling locations in Boomerang and Confederation Lakes.



Each sample was allowed to settle for 5 days after which time an aliquot of 100 to 150 ml was collected and resettled for 2 days to arrive at a final concentrate volume of 20 mls. Phytoplankton identification was carried out to genus and where possible to species. For the data collected in 1986 determinations of cell concentrations and biomass were made using the standard Utermohl technique.

Periphytic algae samples were obtained from branches that were cut from dead fall that had been placed in Boomerang Lake. The algal complex or "jelly" was washed from the branches with a hand operated water spray gun. The jelly material and water mixture was settled for 24 hrs after which time, the water was decanted. All branches and subsamples of the needles were weighed and measured to determine surface area. All samples were dried at 60 °C. Biomass was recorded as either g/100g substrate or g/surface area.

Analysis of metal concentrations in water samples was carried out on filtered (0.45 um), acidified (conc. Nitric acid), water samples by ICP, at Assayers Ontario Limited. Periphytic algal collections were sorted to remove non-algal debris, and ground with a hand mortar. The pulverized material was wet oxidized for metal analysis by Assayers Ontario Limited.

RESULTS

Metal concentrations: Metal concentrations of Boomerang Lake water (pH and conductivity ranged from 3.54 to 5.4 and 240 to 600 umhos/cm^2 respectively) were consistent between the seasons and years. The lake is entirely mixed and does not display any stratification. Distinct differences in metal concentrations however, are evident between Boomerang Lake and Confederation Lake waters (pH and conductivity ranged from 5.85 to 7.46 and 38 to 215 umhos/cm^2 respectively), (Table 1 and 2). Of particular interest are the concentrations of Zn, with approximately 8 mg/l in Boomerang lake as compared to generally less then 1 mg/l in the effluent in Confederation lake. Since station C1 receives the effluent from Boomerang Lake, it could be expected that the phytoplankton community will be sensitive to these discharges, despite the significant dilution which appears to occur at the lake discharge.

Phytoplankton: The composition of the phytoplankton community collected in 1986 indicated that differences existed between areas of Boomerang lake and station C1 in Confederation Lake. The results suggested that Boomerang Lake could be divided into two regions, the area close to the tailings and the spills (locations B5, B6 , B7 , B8, B10 and B11) and the area close to TAble 1 and

Table 1: Means & Ranges of Cu, Fe & Zn in Boomerang Lake

Element	1986				1987				1988			
	n = 29				n = 19				n = 22			
(mg / l)	Mean	Std.	Min.	Max.	Mean	Std.	Min.	Max.	Mean	Std.	Min.	Max.
Cu	0.1636	0.047	<0.005	0.210	0.231	0.381	<0.005	1.5	0.0786	0.020	0.020	0.100
Fe	1.189	0.423	0.5	1.950	3.498	14.171	<0.01	62	0.7736	1.359	<0.01	6.600
Zn	8.3924	0.775	6.9	9.400	7.737	3.171	3.700	19.3	7.5045	0.832	5.800	10.000

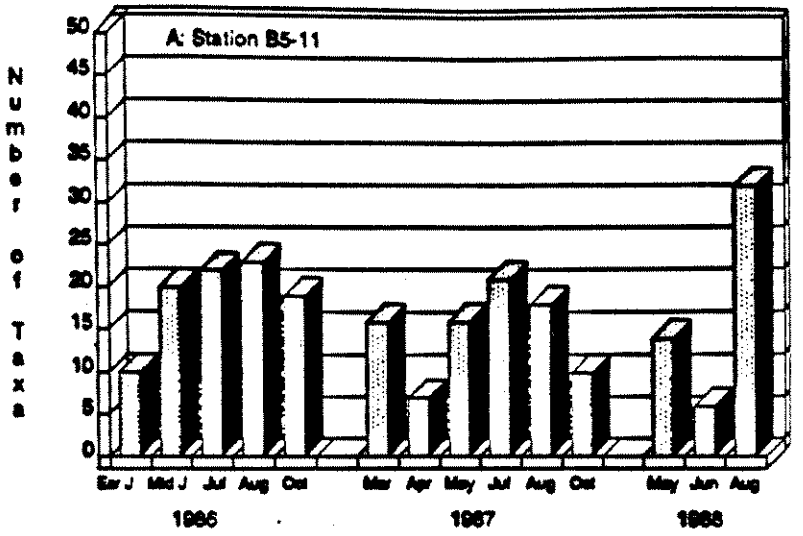
Table 2: Means & Ranges of Cu, Fe & Zn in Confederation Lake (Station C1)

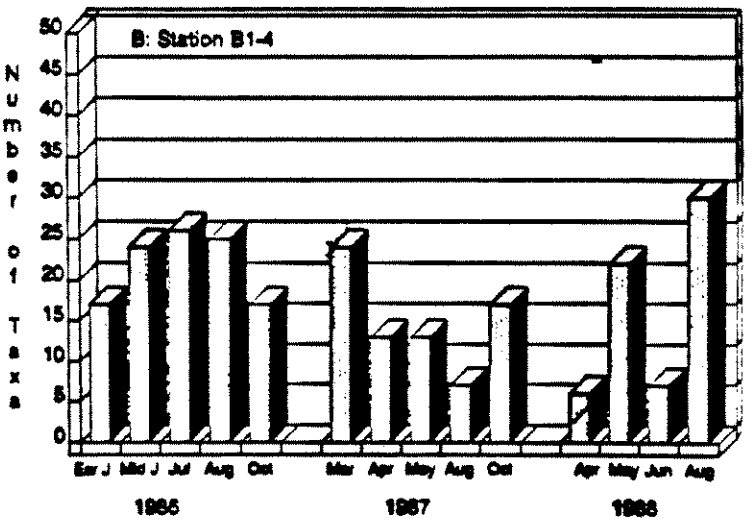
Element	1986				1987				1988			
	n = 4				n = 5				n = 4			
(mg / l)	Mean	Std.	Min.	Max.	Mean	Std.	Min.	Max.	Mean	Std.	Min.	Max.
Cu	0.056	0.103	<0.005	0.210	0.008	0.007	<0.005	0.020	0.010	0.000	<0.01	0.010
Fe	0.255	0.431	<0.01	0.900	2.410	5.362	<0.01	12.000	0.010	0.000	<0.01	0.010
Zn	0.640	0.779	0.030	1.700	2.090	2.783	<0.005	6.500	0.118	0.189	<0.01	0.400

the outflow (B1 to B4). Samples from stations in close proximity to the tailings or seepage had biomass estimates that ranged from 13.6 to 75.6 ug/l. This is low compared to a range of 83.5 to 165.1 ug/l near the discharge (B1 to B4) and distinctly lower than the range of 251.8 to 279.8 ug/l at station C1. Cell concentrations for late July displayed a similar trend with the lowest number of cell ranges in samples close to the tailings 14.0 to 79.3 $x10^4$ cells/l, somewhat higher ranges near the discharge with values of 27.7 to 104.4 $x10^4$ cells/l and the highest range at station C1 with 62.8 to 99.3 $x10^4$ cells/l.

It was based on these observations of phytoplankton communtiy composition that sampling areas were selected in which, for the years 1987 and 1988, presence/absence of taxa was determined. It appeared that this parameter might prove as a useful indicator of the state of the lake ecosystem, ie. ultimately indicating recovery of the lake due to the Ecological Engineering measures implemented.

The taxa diversity between the three monitoring locations increases as the distance from the tailings increases (Figure 1a to c). The increase in number of taxa is clearly expressed at station C1, the effluent in Confederation Lake. The lower metal concentrations at C1 are associated with an increased taxa diversity. The effects of the discharge of metals and acidity on the taxa diversity at station C1 appears to be minimal.





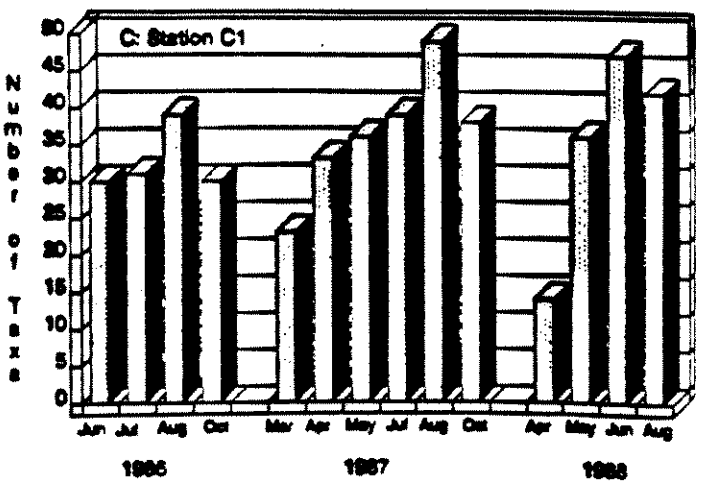


Figure 1: Seasonal changes in taxa diversity in Boomerang and Confederation Lakes

Slight changes between areas are noted for all three years within Boomerang Lake. These observations are of interest, as they suggest that changes in the biological system within Boomerang Lake appear to be occurring before any obvious changes in metal concentrations are detectable.

The seasonal trends indicate that the number of taxa present is highest in spring and fall. When comparing 1986 and 1988 the data suggest that, in general, diversity has increased. However, these differences in diversity are likely due to natural variation rather than improved conditions. Significant ecosystem recovery, expressed in phytoplankton diversity is not anticipated for several years. The changes in the presence of indicator genera within the areas in Boomerang Lake may be more sensitive as an indicator.

The presence of taxa identified at the presence in the respective sampling locations are summarized for all three years in Table 3. A distinct pattern in diversity is displayed within the taxa in terms of their absence from Boomerang Lake. Both the Cyanophyceae and desmids are poorly represented in Boomerang Lake.

A review of the information presented in Table 3 was carried out through comparison of similar phytoplankton diversity tables that focused on pollution tolerance and indicator species. The

TABLE 3: SUMMARY OF PRESENCE/ABSENCE OF ALGAL SPECIES BASED ON PHYTOPLANKTON SURVEY (May 1986 - August 1988)

	ALGAL TAXON	CONFEDERATION LAKE Open Water (C1,C11)			BOOMERANG LAKE Open Water (B1-B4)			BOOMERANG LAKE (B5-B11)		
		1986	1987	1988	1986	1987	1988	1986	1987	1988
	CYANOPHYCEAE									
	Anabaena spiroides	•	•							
	Anabaena spp.		•	•						
	Aphanizomenon sp.		•							
	Chroococcus minutus		•				•			
	Chroococcus spp.		•	•						
	Chroococcus turgidus		•							
	Coelosphaerium kuetzingianum	•	•	•						
	Gloeocapsa sp.		•	•					•	
	Gomphosphaeria sp.		•	•						
	Lyngbya sp.		•							
	Merismopedia spp.		•	•			•			
* a	Oscillatoria spp.		•	•					•	
	Rhabdoderma lineare		•	•						
	Tolypothrix sp.			•						
	Unidentified spp.	•	•	•	•					
	CHLOROPHYCEAE									
* a	Ankistrodesmus spp.		•	•			•		•	•
	Asterococcus sp.			•						
a	Binuclearia sp.		•							
a	Botryococcus braunii		•	•			•			
	Bulbochaete sp.			•						
* a	Chlamydomonas spp.	•	•	•	•	•	•	•	•	•
* a	Chlorella spp.		•	•		•	•		•	
	Chlorogonium sp.			•					•	
	Coelastrum spp.			•						
	Crucigenia sp.			•						
	Dictyosphaerium pulchellum			•						
	Elakatothrix sp.			•						
	Eremosphaera viridis			•						•
	Enastrum sp.			•						
	Gloeocystis spp.			•						•
	Gonium sp.			•						
	Kirchneriella sp.			•						
	Klebsormidium sp.		•							
a	Microspora sp.		•	•		•				
	Mougeotia spp.	•	•	•	•	•	•	•	•	•
	Oedogonium spp.		•	•		•				
a	Oocystis lacustris		•	•			•			•
a	Oocystis submarina	•	•	•	•			•	•	
	Pandorina sp.			•						
	Pediastrum duplex			•						
	Pediastrum spp.		•	•						
	Pediastrum tetras			•						
	Planktosphaeria sp.			•						
	Quadrigula sp.		•	•						
	Scenedesmus accuminatus		•	•						
	Scenedesmus acutus	•	•	•			•			
	Scenedesmus bijuga			•						
	Scenedesmus quadricauda		•	•		•	•			
* a	Scenedesmus spp.		•	•						
	Sphaerellopsis cylindrica		•	•	•	•	•	•	•	
a	Spirogyra spp.		•	•						
a	Stigeoclonium sp.					•				
	Tetraedris sp.			•						
a	Ulothrix spp.	•	•	•	•	•	•	•	•	•
	Unidentified spp.	•	•	•	•	•	•	•	•	•
	Zygnema spp.			•						

TABLE 3: SUMMARY OF PRESENCE/ABSENCE OF ALGAL SPECIES BASED ON PHYTOPLANKTON SURVEY (Continued)

	ALGAL TAXON	COMFEDERATION LAKE Open Water (C1,C11) 1986	1987	1988	BOONERANG LAKE Open Water (B1-B4) 1986	1987	1988	BOONERANG L (B5-B11) 1986	1987
	Desmids								
	Arthrodesmus incus	•	•	•					
	Arthrodesmus octocornis			•					
	Arthrodesmus spp.		•						
a	Closterium spp.		•	•					
a	Cosmarium spp.	•	•	•		•			
	Micrasterias sp.		•	•					
	Netrium sp.			•					
	Pleurotaenium sp.			•					
	Spondylosium sp.		•	•					
	Staurastrum sp. (10 arms)	•	•	•					
	Staurastrum sp. (6 arms)	•	•	•					
a	Staurastrum spp.			•			•		
	Xanthidium spp.		•	•					
	EUGLENOPHYCEAE								
a	Euglena mutabilis				•	•	•	•	•
• a	Euglena spp.	•	•	•	•	•	•	•	•
•	Phacus spp.		•	•					
a	Trachelomonas spp.		•	•	•			•	
	Trachelomonas volvocina					•	•		•
	CHRYSOPHYCEAE								
a	Chromulina spp.	•	•	•	•	•	•	•	•
	Chrysochromulina sp.			•					
	Chrysolkos spp.			•					
	Chrysosphaerella longispina			•					
	Dinobryon bavaricum		•	•				•	
	Dinobryon cylindricum		•	•					
	Dinobryon divergens		•	•					
a	Dinobryon sertularia		•	•			•	•	
	Kephyrion spp.			•					
	Mallomonas sp.		•	•				•	
a	Ochromonas spp.	•	•	•	•	•	•	•	•
	Pseudokephyrion sp.		•						
	Synura sp.		•	•		•	•	•	
	Unidentified spp.	•	•	•	•	•	•	•	•
	BACILLARIOPHYCEAE								
	Achnanthes linearis	•	•	•	•	•		•	•
a	Achnanthes minutissima	•		•	•	•	•	•	•
	Amphipleura sp.			•		•			
	Amphipora spp.		•	•					
	Asterionella formosa	•	•	•	•	•		•	
	Asterionella ralfsii	•	•	•					
a	Cocconeis sp.		•	•					
	Cyclotella bodanica		•	•					
	Cyclotella comta		•	•		•			
	Cymbella spp.		•	•			•		•
	Diploneis spp.		•	•					•
	Epithemia sp.		•	•					•
a	Eunotia fallax					•	•		•
a	Eunotia lunaris		•		•		•	•	•
a	Eunotia spp.		•	•					
	Fragilaria crotonensis	•	•	•	•		•		•
a	Fragilaria spp.	•	•	•		•		•	•
a	Frustulia rhomboides		•	•					
• a	Gomphonema spp.		•	•			•		
a	Melosira granulata		•	•					
	Melosira islandica	•	•	•	•		•	•	•
a	Melosira italica	•	•	•	•			•	•

TABLE 3: SUMMARY OF PRESENCE/ABSENCE OF ALGAL SPECIES BASED ON PHYTOPLANKTON SURVEY (Continued)

	ALGAL TAXON	CONFEDERATION LAKE Open Water (C1,C11) 1986	1987	1988	BOOMERANG LAKE Open Water (B1-B4) 1986	1987	1988	BOOMERANG LAKE (B5-B11) 1986	1987	1988
	BACILLARIOPHYCEAE (Continued)									
* a	Navicula spp.	◆	◆	◆	◆	◆	◆	◆	◆	◆
a	Neidium sp.			◆						
* a	Nitzschia spp.	◆	◆	◆	◆	◆	◆	◆	◆	◆
a	Pinnularia mesolepta	◆	◆	◆	◆	◆	◆	◆		◆
a	Pinnularia spp.	◆	◆	◆	◆	◆	◆	◆	◆	◆
	Rhizosolenia eriensis		◆	◆						
	Rhizosolenia longiseta	◆	◆	◆						
	Rhopalodia sp.	◆	◆	◆	◆		◆	◆	◆	
a	Stauroneis sp.	◆	◆	◆	◆	◆	◆	◆	◆	◆
a	Stephanodiscus sp.			◆			◆			
a	Surirella spp.		◆	◆			◆			
a	Synedra acus	◆	◆	◆	◆	◆	◆			
* a	Synedra spp.	◆	◆	◆	◆	◆	◆	◆	◆	◆
	Tabellaria fenestrata	◆	◆	◆	◆	◆	◆	◆	◆	
a	Tabellaria flocculosa	◆	◆	◆	◆	◆	◆	◆	◆	
	Tabellaria quadriseptata	◆	◆	◆						
	Unidentified spp.	◆	◆	◆	◆	◆	◆	◆	◆	◆
	CRYPTOPHYCEAE									
a	Chroomonas sp.		◆	◆						
a	Cryptomonas erosa	◆	◆	◆		◆	◆	◆	◆	◆
a	Cryptomonas ovata	◆	◆	◆	◆		◆			
	Cryptomonas rostriformis			◆						
	Rhodomonas lacustris	◆	◆	◆			◆			
	Rhodomonas minutus	◆	◆	◆					◆	
	DINOPHYCEAE									
	Ceratium hirundinella	◆	◆	◆						
	Glenodinium spp.		◆	◆		◆	◆			
a	Gymnodinium spp.		◆	◆		◆	◆			◆
a	Peridinium inconspicuum	◆	◆	◆		◆	◆		◆	◆
	Peridinium limbatum	◆	◆	◆			◆			
	Peridinium spp.	◆	◆	◆		◆				
	TOTAL TAXA REPORTED	46	102	125	22	60	69	34	60	25

* pollution tolerant genera (Palmer, 1968)

a species present in acid mine drainages (Parsons, 1968; Bennett, 1969; Warner, 1971; Hargreaves and Whitton, 1975)

letter (a) in Table 3 denotes that the genera was quoted as present in acid mine drainage water by several authors (Parsons, 1968, Bennett, 1969 , Warner 1971 , Hargreaves and Whitton 1975 in Olaveson, 1984). The star in table 3 denotes pollution tolerant genera after Palmer (1969).

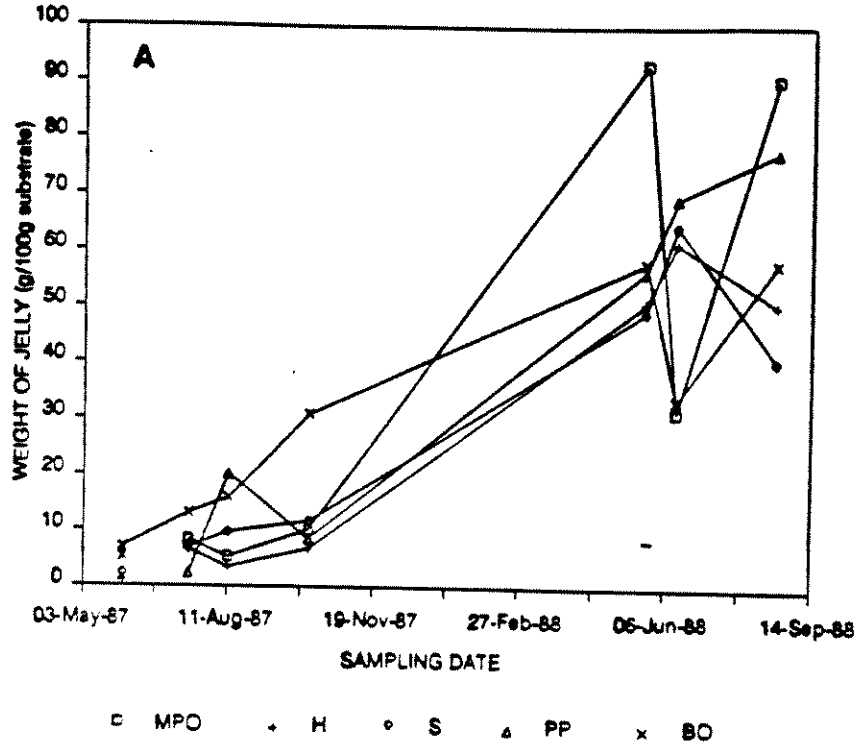
Two eugleniod organisms (Euglena mutabilis and Trachelomonas volvocina), indicators of acid mine drainage, occur in both areas of Boomerang Lake but not at station C1 and those species are classical indicators of acid mine drainage. As clean water indicators the data set suggest, several species of the Cyanophyceae and of the Chlorophyceae that occur at C1 but not in Boomerang Lake. This is especially the case with the desmids where only two genera out of 9 occurring at C1 appeared in Boomerang Lake (Table 3).

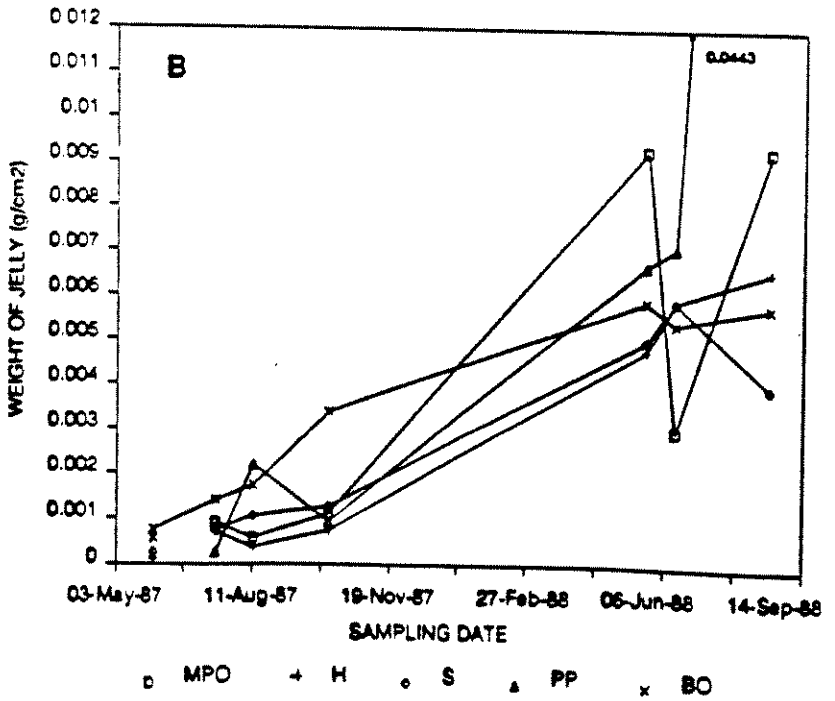
Periphyton: The dominant periphyton community in Boomerang Lake consisted of Mougeotia, Zygogonium, Achnanthes, and Eunotia. These attached algal mats, were suspended on branches of deadfall of Boomerang lake, and in some bays formed a mat over the sediments.

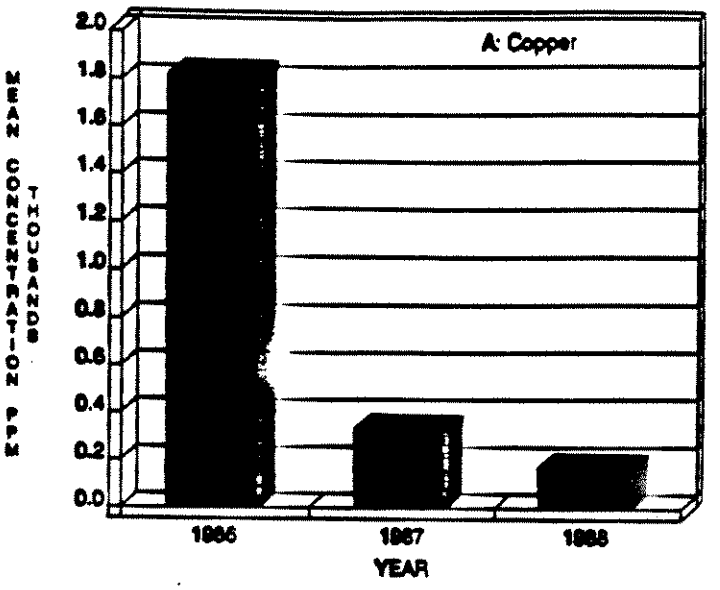
In 1987 growth experiments were initiated using deadfall and brush suspended behind log booms in Boomerang lake. The biomass of the periphytic complex was quantified for two years. Biomass was quantified as weight/ 100 gr branch and by surface area. A steady increase in biomass was evident using both means of quantification

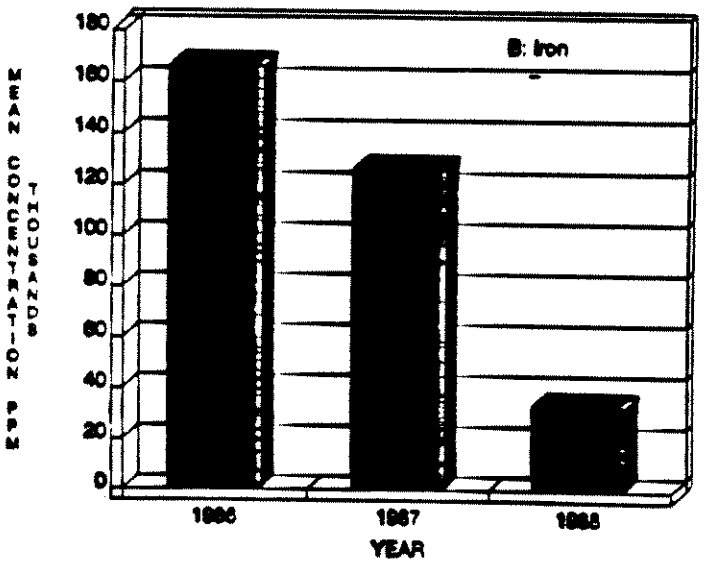
(Figure 2A and B). Metal analysis of these attached mats yielded significant concentrations of copper, iron and zinc (Figures 3A, B and C). The concentrations of these elements were as high as 1,825, 16,650 and 6,200 mg/kg (dry weight) respectively in the material collected in 1986 for which the time of accumulation and growth was unknown. The metal concentrations in the periphytic mats collected in 1987 and 1988 did not reach the same ranges for copper and Zinc as the original material collected in 1986. However, the concentrations of iron approach the range for the material collected in 1986 (12 % as compared to 16 %). The data indicate that over time both algal biomass and metal concentrations increase.

Figure 2: Growth of periphytic algae on submerged trees in Boomerang Lake , measured as g/100 g substrate (A) and g/cm^2 (B).









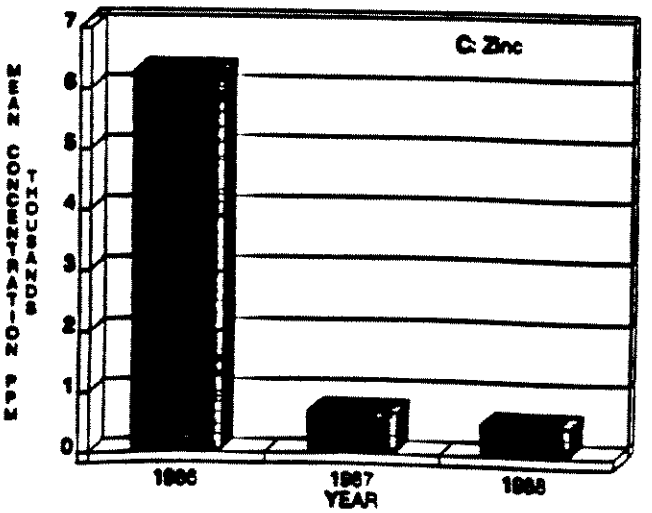


Figure 3: Concentrations of Copper, Iron and Zinc in algal material collected from Boomerang Lake

DISCUSSION

The composition of the phytoplankton community of Confederation lake is typical of other oligotrophic shield lakes. The presence of Oedogonium is indicative of reduced metal stress since this genus is sensitive to zinc (Whitton, 1970a). Crysophytes (eg. Dinobryon, Mallosmonas, Synura) were common in Confederation Lake, but absent from Boomerang Lake. Whitton (1970b) mentions, that many Chrysophytes are indeed sensitive to metal pollution, particularly Synura and Dinobryon. On the other hand desmids, mainly of the genera Arthrodesmus sp., Cosmarium sp., and Staurastrum sp., are indicators of high water quality (Liebmann, 1962 Palmer 1969) and are considered intolerant of high metal concentrations (Foster,1982).

The algal communities of Boomerang lake, which has received acid mine drainage, shows a shift to lower diversity and a change in dominance to new species which is characteristic of acidified waters (Whitton, 1970 a and 1970b). Similar trends are also associated with increases in the levels of heavy metals (Foster 1982). Euglena mutabilis an acidophilic Euglenoid that occurred frequently in Boomerang Lake is well documented as an indicator of acidic, high metal waters (Palmer 1959; Liebmann 1962, Bennett, 1969). Whitton (1970b) lists the following species as very tolerant to relatively high zinc concentrations: Lyngbya, Oocystis, Spirogyra, Chlamydomonas, Euglena, Trachelomonas, Cyribella,

Nitzschia and Synedra. These were consistently present in Boomerang Lake.

Most of the species considered tolerant to organic pollution appear, to display tolerance to metals and acidic conditions in Boomerang Lake as well as the conditions present at station C1 (Table 3). This is not the case for the desmids and as a result make this group ideal as an indicator of recovery in Boomerang Lake.

Oligotrophic lakes are generally inhabited by several or more species of desmids (Brooks 1982). Confederation Lake is classified as oligotrophic and contains more than ten species of desmids. Desmids as presence/absence indices, were originally described to identify organic pollution. In our situation desmids were consistently found in the waters at the discharge from Boomerang Lake and rarely in the lake itself. For these reasons the presence/absence of desmids may be a reliable indicator of changes in water quality in Confederation Lake.

The filamentous green algea Ulothirx, Microspora and Zygnematcean spp. were common in Boomerang lake. These species, which from the structural part of the periphytic complex growing on the deadfall, are known to be tolerant to acid conditions and heavy metals especially zinc (Whitton, 1970a and McLean and Jones, 1975). The diatoms Achnanthes and Eunotia, which dominate the "jelly complex"

have been reported has having the same characteristics especially in water with high zinc (Whitton 1970a). Whiton (1970a) quotes concentrations of > 10 mg/l which is close to the concentration of zinc in Boomerang lake at approximately 8 mg/l.

The periphytic mats on the deadfall in Boomerang lake were initially thought to be an accumulation of dead organic matter, as the metal concentrations in the material were very high. However, our growth experiments suggest, that indeed the algal complex is alive and grows at a substantial rate. Furthermore the metal concentrations accumulated in the periphytic mats, do not seem to be inhibiting continued growth. The mat is likely acting as an adsorptive surface for the metals, a situation that has been descrided for Cd and Cu (Xue, Stumm and Sigg 1988).

The periphytic algae growth in Boomerang lake, is anticipated to provide a continuous medium for the removal of metals. With the Ecological Engineering measures implemented at this site, the periphytic algae is acting as a Biological Polishing agent, to assist in the recovery of the lake.

ACKNOWLEDGEMENTS

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A COMPARISON OF LABORATORY AND IN SITU SEDIMENT BIOASSAYS

P.E. Ross[1] and M. Munawar[2]
[1]Illinois Natural History Survey, Champaign, IL
[2]Fisheries and Oceans, Burlington, ON

PAPER UNAVAILABLE AT TIME OF PRINTING

STUDY ON THE EFFECTS OF AN OIL SPILL AT COME BY CHANCE, NEWFOUNDLAND ON SEDIMENTS AND THE AMERICAN LOBSTER (Homarus americanus)

U.P. Williams[1], J.V. Kiceniuk[1], J.E. Ryder[1], and J.R. Botta[2]

1. Science Branch, 2. Inspection Services Branch
Department of Fisheries & Oceans
P.O. Box 5667
St. John's, Newfoundland

SUMMARY

In March 1988 an accidental spill of crude oil occured at the oil refinery at Come by Chance, Placentia Bay, Newfoundland. In this particular instance approximately 500 barrels of a light crude oil was spilled during offloading of a tanker. Concern was expressed by local fishermen as to the effect of this spill on the lobster fishery in the Bay. Studies of selected parameters were undertaken to determine the possible short and long term effects on the local lobster fishery. Sensory analyses were carried out to ascertain the effects of the oil on the taste and odor of the lobster. Lobster muscle and hepatopancreas were analyzed for PAH (polycyclic aromatic hydrocarbon) content and levels of gill browning were determined. Sediments were sampled at various location in the Bay and levels of PAHs were determined in an attempt to delineate the extent of contamination. Results from the sensory analyses indicate that there was no tainting of lobster samples obtained from the Come by Chance area. There was also no evidence for gill browning or for elevated levels of PAHs in either tail muscle or hepatopancreas. Elevated levels of PAHs were not detected in any of the sediments from the Bay. The results indicate that lobster were not contaminated as a result of the oil spill. Based on the results of analyses of sediments for PAHs, future contamination, as a result of the spill at the oil refinery wharf, is not anticipated.

ETUDE DES EFFETS D'UN DEVERSEMENT D'HUILE SUR LES SEDIMENTS ET LE HOMARD AMERICAIN (Homarus americanus) A COME BY CHANCE, TERRE-NEUVE.

U.P. Williams[1], J.W. Kiceniuk[1], J.E. Ryder[1], et J.R. Botta[2]

1. Division Science, 2. Division Services d'Inspection
Ministère des Pêches et Océans
P.O. Box 5667
St-Jean, Terre-Neuve

RESUME

En mars 1988, un déversement accidentel d'huile brute s'est produit à la raffinerie de " Come by Chance ", Placentia Bay, Terre-Neuve. Dans cet exemple particulier, environ 500 barils d'huile brute légère ont été répandus lors du déchargement d'un navire citerne. Les pêcheurs locaux ont manifesté une certaine inquiétude quant à l'effet de ce déversement sur la pêche au homard dans la Baie. Des études ont été entreprises sur des paramètres sélectionnés, afin de déterminer les effets possibles à court et à long terme sur la pêche locale du homard. Des analyses organoleptiques ont été effectuées pour vérifier les effets de l'huile sur le goût et l'odeur du homard. Le muscle et l'hépatopancréas du homard ont été analysés pour déterminer le contenu en HAP (hydrocarbures aromatiques polycycliques) et le degré de brunissement des branchies fut évalué. Des sédiments ont été prélevés à divers endroits dans la Baie et les taux de HAP ont été déterminés pour tenter de délimiter l'étendue de la contamination. Les résultats des analyses organoleptiques indiquent qu'il n'y avait pas de détérioration des échantillons de homard provenant de la région de " Come by Chance ". Il n'y avait aussi aucune évidence de brunissement des branchies ni de niveaux élevés de HAP dans le muscle de la queue ou l'hépato-pancréas. Aucune valeur élevé de HAP n'a été détectée dans aucun des sédiments provenant de la Baie. Les résultats indiquent que le homard ne fut pas contaminé suite à ce déversement. En se basant sur les résultats d'analyses de HAP dans les sédiments, il n'est pas prévu de contamination future au quai de la raffinerie comme conséquence de ce déversement.

ACIDIFICATION EFFECTS ON ZOOPLANKTON COMMUNITY STRUCTURE IN QUEBEC LAKES

Bernadette PINEL-ALLOUL

Département de Sciences biologiques

Université de Montréal

ABSTRACT

Abundances and biomass of 38 zooplankton species and accompanying morphometric, physical and chemical data from 54 Québec lakes were subjected to cluster and correlation analysis to determine 1) the characteristic zooplankton associations of co-occurring species, 2) the relative importance of abiotic variables in lake typology, and 3) the relationships between the integrated environmental factors derived from lake morphometry, water quality and acidification level, and the structure of zooplankton communities. The eight groups of species identified in the cluster analysis may be considered separate "species associations" characterized by distinct patterns of distribution in Québec lakes. Differences in mean pH and sulfate concentrations can be detected between groups of lakes representing the different species associations. Factor analysis produces seven factor scores which explain 28% of the total variance of the morphological, physical and chemical original variables. The distribution pattern of acidity or alkalinity represents the major feature in water chemistry variation but lake morphology and trophic status also account for the observed environmental variability. Each factor (hardness-alkalinity, lake depth, dystrophy, lake size, salinity-sulfate loading, lake volume development and nitrates loading) represents an integrated environmental property correlated to a set of colinear abiotic variables. These seven factors were correlated with zooplankton species abundances and biomass. The majority of species belonging to group 5A (*Diaphasonoma* sp., *Mesocyclops edax*, *Diacyclops bicuspidatus thomasi*, and *Daphnia galeata mendotae*) are linked to the hardness-alkalinity and salinity-sulfate factors. *Bosmina longirostris*, *Leptodiaptomus minutus*, *Keratella taurocephala*, *Polyarthra vulgaris*, and *Holopedium gibberum* represent the acid tolerant species group. The morphology and trophic features of lakes (lake depth, lake size, dystrophy), and the thermic preferences of species also influence the distribution of zooplankton species or groups. Furthermore, the community structure, whether expressed in size classes or in trophic groups tends to vary among set of lakes of different pH.

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Bernadette PINEL-ALLOUL Département de Sciences biologiques

Université de Montréal

RESUME

L'abondance et la biomasse du zooplancton dans 54 lacs du Québec ainsi que les caractéristiques morphométriques et physico-chimiques de ces lacs ont fait l'objet d'analyses factorielles et de corrélation visant à déterminer 1) les associations d'espèces représentatives de la variabilité du zooplancton, 2) l'importance relative des variables abiotiques pour la typologie des lacs et 3) les relations entre les facteurs environnementaux dérivés de la morphométrie des lacs, de la qualité physico-chimique des eaux et des niveaux d'acidification, et la structure des communautés zooplanctoniques. Les huit groupes d'espèces déterminés par groupement à liens complets peuvent être considérés comme des associations distinctes par leur patron de répartition dans les lacs du Québec. Des différences entre les valeurs moyennes de pH et de sulfates peuvent être détectées entre les groupes de lacs représentatifs des différentes associations d'espèces. L'analyse factorielle détermine sept facteurs environnementaux qui expliquent 28% de la variance totale de la morphométrie et de la physico-chimie des lacs. Le patron de distribution de l'acidification ou inversement de l'alcalinité, représente la majeure partie de la variabilité mais la morphométrie et la statut trophique des lacs influencent aussi la variabilité observée dans les conditions abiotiques. Chaque facteur (dureté-alcalinité, profondeur des lacs, dystrophie, taille des lacs, salinité-sulfates, développement du volume des lacs, apports en nitrates) représente une caractéristique environnementale composite corrélée à un ensemble de variables abiotiques colinéaires. Les sept facteurs ont été corrélés avec l'abondance et la biomasse des zooplanctontes. La majorité des espèces du groupe 5A (*Diaphanosoma* sp., *Mesocyclops edax*, *Diacyclops bicuspidatus thomasi* et *Daphnia galeata mendotae*) sont reliées aux facteurs dureté-alcalinité et salinité-sulfates. *Bosmina longirostris*, *Leptodiaptomus minutus*, *Keratella taurocephala*, *Polyarthra vulgaris* et *Holopedium gibberum* représentent le groupe d'espèces associées aux lacs acides. Toutefois, les caractéristiques morphométriques et trophiques des lacs (profondeur, taille, dystrophie) ainsi que les préférences thermiques des espèces influencent aussi la répartition des zooplanctontes. De plus, la structure de la communauté, exprimée soit en classes de taille ou en groupes trophiques est variable selon les groupes de lacs de pH différent.

Toxicity of Tributyltin: A Chesapeake Bay Perspective

Steven J. Bushong, Lenwood W. Hall, Jr., Michael C. Ziegenfuss, and Michael A. Unger

The Johns Hopkins University, Applied Physics Laboratory, Aquatic Ecology Section, Shady Side, MD 20764 USA

Summary:

Tributyltin (TBT) is primarily introduced into the aquatic environment as a biocide in antifouling paints. TBT is an effective biocide because it is extremely toxic at low concentrations. The increasing widespread use of TBT-based antifouling paints has raised concern over its potential toxic effect on nontarget organisms. Many countries have implemented or are considering restrictions on TBT usage.

A program was initiated at The Johns Hopkins University, Applied Physics Laboratory (JHU/APL) to evaluate the acute and chronic toxicity of TBT to selected Chesapeake Bay organisms. Tests were conducted using a Harvard syringe pump to introduce TBT stocks in a continuous-flow design. Polycarbonate and Teflon were used for contact surfaces to minimize adsorptive loss of TBT. TBT concentrations were measured in test aquaria during all experiments. Analyses were conducted by gas chromatography with flame photometric detection after sample extraction using either the method of Matthias et al. (1986) or Unger et al. (1986). Test concentrations as low as 0.006 (± 0.001) µg TBT/L have been maintained with this design.

A total of 14 acute toxicity experiments were conducted. The most sensitive fish species tested were larval inland silversides, Menidia beryllina (96-h LC50 = 3.0 µg TBT/L) and juvenile Atlantic menhaden, Brevoortia tyrannus (96-h LC50 = 4.5 µg TBT/L). The most sensitive invertebrates tested were the estuarine copepods, Eurytemora affinis (72-h LC50 = 0.6 µg TBT/L) and Acartia tonsa (48-h LC50 = 1.1 µg TBT/L). The most resistant species tested was the grass shrimp, Palaemonetes sp. (96-h LC50 > 31.0 µg TBT/L).

Chronic tests demonstrated effects of TBT at lower levels. In a 28 d test with larval M. beryllina, exposure to 0.093 µg TBT/L caused small but significant reductions in growth (22%). In chronic tests with B. tyrannus and the amphipod Gammarus sp., no significant sublethal effects were reported at the highest concentrations tested, 0.490 and 0.579 µg TBT/L, respectively. In a full life-cycle experiment with E. affinis, 0.088 µg TBT/L was found toxic to nauplii after chronic exposure. In repeated 6 day chronic experiments with A. tonsa nauplii (<48 h old), TBT concentrations of 0.023-0.024 µg TBT/L were the lowest observed effect concentration (LOEC). The no observed effect concentration (NOEC) in both A. tonsa experiments was 0.010-0.012 µg TBT/L.

Comparisons between laboratory toxicity data and environmental TBT concentrations reported in Chesapeake Bay can help assess the potential impact of TBT contamination. Monitoring studies conducted by JHU/APL in Maryland waters of Chesapeake Bay found peak TBT concentrations in marinas and associated waterways which exceeded the acute toxicity values for some sensitive species. TBT concentrations reported in non-marina areas are generally low or nondetectable; however, concentrations have been reported which approach or exceed the chronic effect levels for some sensitive organisms. The state of Maryland passed legislation restricting the use of TBT-based antifouling paints on recreational watercraft in 1988. The impact of this legislation on environmental TBT concentrations is currently being investigated by JHU/APL.

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TOXICITE DU TRIBUTYLTIN: UNE VUE DE LA BAIE DE CHESAPEAKE

Steven J. Bushong, Lenwood W. Hall, Jr., Michael C. Ziegenfuss, et Michael A. Unger

Université John Hopkins, Laboratoire de Physique Appliquée, Section Ecologie Aquatique, Shady Side, MD 20764 U.S.A.

RESUME

Le TBT (tributyltin) est principalement introduit dans l'environnement aquatique comme biocide dans les peintures antisalissures. Le TBT est un biocide efficace parce qu'il est extrêmement toxique à de faibles concentrations. L'usage de plus en plus répandu de peintures antisalissures à base de TBT a éveillé l'intérêt quant à son effet toxique potentiel sur des organismes non visés. Plusieurs pays ont réglementé ou envisagé des restrictions sur l'usage du TBT.

Un programme a été développé au laboratoire de physique appliquée de l'Université John Hopkins (JHU/APL) pour évaluer la toxicité aiguë et chronique du TBT sur des organismes cibles de la Baie de Chesapeake. Les tests ont été effectués en utilisant une pompe Harvard permettant d'introduire le TBT au moyen d'une seringue dans un montage à débit continu. Du polycarbonate et du teflon ont été utilisés pour les surfaces de contact afin de réduire la perte de TBT par adsorption. Les concentrations de TBT ont été mesurées dans les aquariums expérimentaux pendant toutes les expériences. Les analyses ont été faites par chromatographie en phase gazeuse avec détection photométrique à la flamme après extraction de l'échantillon, en utilisant soit la méthode de Matthias et al. (1986) ou Unger et al. (1986). Des concentrations expérimentales aussi faibles que 0.006 (±0.001) µg TBT/litre ont été maintenues au moyen de ce montage.

Un total de 14 expériences de toxicité aiguë ont été effectuées. Les espèces de poissons expérimentaux les plus sensibles ont été la larve amphibiotique de l'athérine cireuse (capucette cireuse), Menidia beryllina (LC50 96-h = 3.0 µg TBT/L) et le menhaden juvénile de l'Atlantique, Brevoortia tyrannus (LC50 96-h = 4.5 µg TBT/L). Les invertébrés expérimentaux les plus sensibles ont été le copépode estuarien Eurytemora affinis (LC50 72-h = 0.6 µg TBT/L) et Acartia tonsa (LC50 48-h = 1.1 µg TBT/L). L'espèce expérimentale la plus résistante fut la crevette herbière, Palaemonetes sp. (LC50 96-h > 31.0 µg TBT/L).

Des essais chroniques ont démontré les effets du TBT à des niveaux plus faibles. Dans un essai de 28 jours avec la larve M. beryllina, une exposition à 0.093 µg TBT/L a causé une diminution faible mais significative de la croissance (22%).

Dans des essais chroniques avec B. tyrannus et l'amphipode Gammarus sp., aucun effet sous-léthal significatif n'a été rapporté aux plus fortes concentrations utilisées, soit respectivement 0.490 et 0.579 µg TBT/L. Dans une expérience impliquant un cycle de vie complet avec E. Affinis, 0.088 µg TBT/L s'est avéré toxique aux nauplii après une exposition chronique. Dans des expériences chroniques répétées de 6 jours avec A. Tonsa au stade nauplii (Age < 48h), des concentrations de TBT de 0.023 - 0.024 µg/L ont été les plus faibles concentrations où un effet a été observé (LOEC). La concentration où aucun effet ne fut observé (NOEC) dans les deux expériences avec A. tonsa a été de 0.010 - 0.012 µg TBT/Litre.

Des comparaisons entre les données de toxicité en laboratoire et les concentrations de TBT rapportées dans la baie de Chesapeake peuvent aider à établir l'impact potentiel de la contamination au TBT. Des études de monitorage effectuées par le JHU/APL dans les eaux de la baie de Chesapeake du Maryland ont démontré des pics de concentration en TBT dans les marinas et les canaux de navigation communiquants qui exédaient les valeurs de toxicité aiguë pour quelques espèces sensibles. Les concentrations de TBT rapportées dans les zones extérieures aux marinas sont généralement faibles ou non détectables; cependant, des concentrations qui se rapprochent ou exèdent les seuils d'effets chroniques ont été rapportées pour certains organismes sensibles. L'Etat du Maryland a adopté, en 1988, une législation restreignant l'usage de peintures antisalissures à base de TBT sur les bateaux de plaisance. L'impact de cette législation sur les concentrations environnementales de TBT est actuellement étudiée par le JHU/APL.

COMPARING TOXICITY OF ST. LAVRENCE RIVER SEDIMENT ELUTRIATES AND INTERSTITIAL WATER USING A MICROBIOASSAY

M.A. Kiamos[1,2], J. Bureau[1], H. Sloterdijk[1], L. Veilleux[1] and P. Anderson[2]

1. Environnement Canada, Conservation and Protection, 1001 Pierre Dupuy, Longueuil, QC, J4K 1A1. 2. Concordia University, Ecotoxicology Program, 1455 de Maisonneuve West, Montreal, QC, H3G 1M8.

For several years now, Environment Canada has been studying the toxicity of sediments in the St. Lavrence River. These evaluations were made using chemical analyses of sediment samples, and bioassays, which were performed on sediment elutriates. However, recently more and more attention is drawn to the toxicity of the interstitial water within the sediments. There is some question as to whether the process of elutriation liberates more contaminants into the water than is normally contained in the interstitial water. The purpose of this study is to compare sediment elutriate toxicity to that of sediment interstitial water, and to determine experimentally which of the two is more toxic. The elutriate is prepared using a slurry of one part sediment and four parts surface water which is mixed vigourously and alloved to settle. The supernatant is then passed through a continuous flow centrifuge, from which the liquid effluent is filtered (0.2µm). The interstitial water is extracted from a wet sediment sample by centrifugation at 14000 RPM for 20 minutes. This supernatant is also passed through a 0.2µm filter. The toxicity of each of these preparations is determined by using the Microtox test system which measures the inhibition of bacterial bioluminescence (Photobacterium phosphoreum). The data are presented in terms of dose-effect curves which are subsequently used to compare the toxicities of sediment elutriate and interstitial water.

COMPARAISON DE LA TOXICITE D'ELUTRIATS SUR LE SEDIMENT ET D'ECHANTILLONS D'EAU INTERSTITIELLE DU FLEUVE ST-LAURENT AU MOYEN D'UN MICROBIOESSAI.

M.A. Kiamos[1,2], J. Bureau[1], H. Sloterdijk[1], L. Veilleux[1] et P. Anderson[2]

1. Environnement Canada, Conservation et Protection, 1001 Pierre Dupuy, Longueuil, Qc, J4K 1A1.
2. Université Concordia, Programme en Ecotoxicologie, 1455 De Maisonneuve Ouest, Montréal, Qc, H3G 1M8.

RESUME

Depuis plusieurs années déjà, Environnement Canada a étudié la toxicité de sédiments dans le fleuve St-Laurent. Ces évaluations ont été effectuées au moyen d'analyses chimiques sur des échantillons de sédiments et par des bioessais, qui ont été réalisés sur les élutriats de ces sédiments. Cependant, depuis quelque temps, de plus en plus d'attention est portée sur la toxicité de l'eau interstitielle à l'intérieur des sédiments. Il y a interrogation à savoir si le processus d'élutriation libère plus de contaminants dans l'eau que ce que contient normalement l'eau interstitielle. Le but de cette étude est de comparer la toxicité de l'élutriat du sédiment à celle de l'eau interstitielle du sédiment et de déterminer expérimentalement laquelle des deux est plus toxique. L'élutriat est préparé en utilisant un mélange d'une partie de sédiment et quatre parties d'eau de surface, lequel est mélangé vigoureusement puis laissé reposé. Le surnageant est ensuite passé dans une centrifuge à flot continu, de laquelle l'effluent liquide est filtré (0.2 μ). L'eau interstitielle est extraite d'un échantillon de sédiment humide par centrifugation à 14,000 RPM pendant 20 minutes. Ce surnageant est également filtré sur une membrane de 0.2 μM. La toxicité de chacune de ces préparations est déterminée en utilisant le test de Microtox, qui mesure l'inhibition de la bactérie bioluminescente (Photobacterium phosphoreum). Les données sont présentées sous forme de courbes dose-effet qui sont par la suite utilisées pour comparer les toxicités de l'élutriat du sédiment et de l'eau interstitielle.

TAUX DE RÉCUPÉRATION ET HYPERTROPHIE CELLULAIRE CHEZ *Selenastrum capricornutum* APRÈS UNE EXPOSITION AU MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) et au tébuthiuron

Donald St-Laurent[1], Christian Blaise[2] et Bertin Trottier[1]

1. Université du Québec à Montréal, Département des sciences biologiques, C.P. 8888, succ. A, Montréal (Québec), H3C 3P8.
2. Environnement Canada, Conservation et Protection, 1001 Pierre Dupuy, Longueuil (Québec), J4K 1A1.

RÉSUMÉ

Le déversement d'effluents industriels entraine, chez les communautés phytoplanctoniques, des réactions d'acclimatation et/ou de récupération dont la mesure a été suggérée pour améliorer la qualité de l'évaluation du risque environnemental. Dans cette perspective, les modes d'acclimatation et de récupération de l'algue *S. capricornutum*, suite à une exposition de 4, 24 et 96 heures au mutagène MNNG et à l'herbicide tébuthiuron, furent caractérisés par un suivi de l'évolution de variables structurelles (compte et volume cellulaires) et fonctionnelles (viabilité et teneur en ATP cellulaires).

Des résultats initiaux ont révélé que pour chacune des huit concentrations de MNNG étudiées (322 à 2575 ppb), lesquelles représentent seulement 2 à 20% de la CI50-96h, le taux de récupération diminue proportionnellement à l'augmentation de la concentration du toxique, et non en proportion de la durée de l'exposition. Dans le cas du tébuthiuron cependant, la population algale doit être traitée durant 96 heures à une concentration extrêmement élevée (i.e. 330 700 fois la CI50-96h) avant d'afficher un taux de récupération significativement inférieur à ceux des autres doses; ces derniers sont d'ailleurs tous regroupés autour de la même valeur. De plus, suivant une exposition de 96 heures à 644 ppb de MNNG, i.e. le minimum pour que le taux de récupération soit significativement inférieur au groupe témoin, la population algale se distingue par l'apparition d'une portion marquée de cellules hypertrophiées. Ainsi, avec une inhibition de croissance inférieure à 1,2% seulement, 14,8% des algues traitées possédaient un volume supérieur à 172 fL par rapport à 4,3% pour la population non-exposée.

Cette hypertrophie, de même que des taux de récupération nettement différents pour une inhibition de croissance comparable, seraient attribuables à des processus physiologiques, biochimiques et ultrastructuraux d'ajustement. L'ensemble des variables mesurant la réponse biologique post-traitement s'avèrent donc d'un apport informatif considérable à l'égard du mode d'acclimatation des algues. A ce titre, nous envisageons de poursuivre cette étude en intégrant la viabilité et la teneur en ATP celloulaires dans l'analyse des mécanismes adaptatifs de la phycopopulation agressée.

EFFECTS OF ALGAE ON THE AQUATIC PERSISTENCE OF FENITROTHION

T. D. Caunter and P. Weinberger. Dept. of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario. K1N 6N5

Most natural waters contain varying amounts of suspended particles of terrigenous and biogenous origins to which partitioning of various aquatic pollutants is likely to alter their photoreactivity and photoproducts (Zepp, 1980, in Dynamics Exposure and Hazard Assessment of Chemicals, Ed. Hague, Ann. Arbour Pub). Besides direct photolysis, involving light absorption by the chemical itself, there exists indirect or sensitized photolysis initiated through light absorption by other substances in the system, most likely phytoplankton (Zepp, 1980).

Fenitrothion (O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate), an organo-phosphorothioate pesticide representing 40% of all agricultural chemicals used in Canada (Symons, 1977, Pestic. Rev. 68: 1), can readily contaminate aquatic ecosystems. Rapid degradation in distilled and buffered solutions by hydrolysis and photolysis is well documented (Greenhalgh et al., 1980, J Agric. Food Chem. 28: 102; Maguire & Hales, 1980, J. Agric. Food Chem. 28: 372; Marshall et al., 1974, Pestic. Sci. 5: 781; Ohkawa et al., 1974, Agric.Biol. Chem. 38: 2247).

For the most part, the persistence and fate-transport studies have been carried out in laboratory microcosms devoid of phytoplankton. However, scattered data indicates that algae are capable of mediating photobiological transformations of chemicals (Zepp & Schlozhauer, 1983, Environ. Sci. Technol. 17: 462; Weinberger et al., 1982, Environ. Sci. Technol. 16: 470). Recently, our studies showed that algae act as 'active' sinks and modulate uptake, turnover, degradation and metabolism of fenitrothion in actively growing algal cultures (Weinberger et al., 1983, J. Environ. Sci. Health 18:269; 1981, In Stress Effects on Natural Ecosystems, Ed. barret & Rosenberg, J. Wiley & Sons).

Zepp and Schlotzhauer (1983) demonstrated that a variety of algae could accelerate the sunlight-induced transformations of some nonionic organic chemicals in a very selective fashion. Dixon and Wells (1987, Pestic. Sci. 21: 155) have shown that chlorophyll can act as a sensitiser in photodegradation of the pesticide pirimicarb. Choudry (1981, Toxicol. Environ. Chem. 4: 261) showed that humic acids could generate free electrons. From this, we hypothesized that light harvesting pigments of phytoplankton should also be capable of generating at least the same level of free electrons as the humic substances. If this is true, then the t½ of pesticides subject to photolysis should be substantially less in natural waters containing assemblages of phytobiota when compared with data obtained in phtobiotically cleared systems.

This present study was initiated to determine the effects of the algae Chlamydomonas segnis (Ettl) on the fate and persistence of fenitrothion.

Live algae in waters containing pesticide were maintained in natural sunlight (800 wm^{-2}) for 5 hours. Optimal uptake was obtained by 3.75 hours. At this time there was a 13.6 fold greater uptake of fenitrothion in the algae in the light versus the dark

treated sets held under comparable conditions. In sets exposed to Vita Lite® high intensity fluorescent lamps (Dura Test, 40 wm^{-2}, 290-700 nm), optimal uptake of fenitrothion was observed within 25 hours of exposure. This represented a significant 20 fold increase (at the 0.05 level) in pesticide uptake in light versus dark treated cells (Figure 1). In freeze-killed (dead) cells, uptake in natural sunlight and Vita Lite® lamps was significantly enhanced 10 and 12.6 fold, respectively, as compared with cells maintained in the dark.

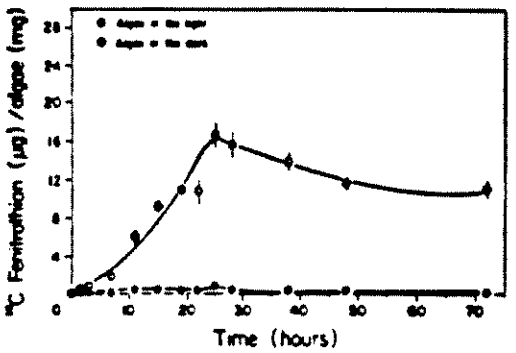

Fig 1 - Uptake of ^{14}C labelled Fenitrothion in live algae exposed to lamps.

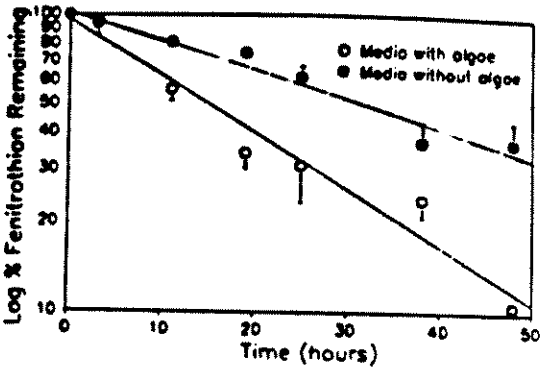

Fig 2 - Persistence of Fenitrothion in media with algae vs without algae in light.

Under Vita Lite® fluorescent lamps, the t½ of fenitrothion in the media was significantly lower in media with algae compared to media without algae. In media containing live algae, fenitrothion decreased with a rate constant of 0.44E-1 resulting in a t½ of 15.97 hours as compared to a decrease in rate of 0.22E-1 and t½ of 31.0 hours in media only (Figure 2). Under dark conditions, fenitrothion decreased from the media slowly without any significant differences between media with and without live algae (rate constants of 0.79E-2 and 0.61E-2, and t½'s of 88.7 and 113.7 hours, respectively). When freeze-killed algae were combined with fenitrothion in the media, the same phenomena was observed, although at a reduced level. Under Vita lights, fenitrothion decreased at a rate constant of 0.28E-1 resulting in a t½ of 24.39 hours. Under dark conditions, fenitrothion decreased with a rate constant below 0.6E-2 and had a t½ over 115 hours.

Sorption of the pesticide fenitrothion was augmented in algae exposed to light, thus affecting the apparent $t_{1/2}$ of the fenitrothion in the medium.

It was further observed that in natural sunlight the onset of chlorosis in the living and dead cells was obtained following 3.75 and 1.5 hours, respectively. Dead cells under Vita Lite®'s were obviously chlorotic following 15 hours of exposure, while live cells held under comparable conditions showed no visible chlorosis. The evidence to date indicates a possible photosensitization of fenitrothion mediated by the accessory pigments as well as chlorophyll and/or membrane lipid peroxidation, as there was continuing uptake of fenitrothion occurring even after chlorosis of the green pigments.

USE OF PHYSIOLOGICAL PARAMETERS RELATED TO GROWTH-RATE RECOVERY IN PHYTOPLANKTON: AN ECOTOXICOLOGICAL APPLICATION TO RIVER DISCHARGES FROM A CHLOR-ALKALAI PLANT

P.Couture[1], C. Thellen[2] and P.A. Thompson[1]
[1]Institut national de la recherche scientifique,
Université du Québec, 2700 rue Einstein, Ste-Foy, PQ
[2]Environnement Québec, Directions des laboratoires,
2700 rue Enstein, Ste-Foy, PQ

PAPER UNAVAILABLE AT TIME OF PRINTING

VERIFICATION OF A TOXICOKINETIC BASED MODEL FOR PLUSE AND FLUCTUATING EXPOSURE TOXICITY

B.E. Hickie, S.M. McGeachy and D.G. Dixon
Department of Biology, University of Waterloo
Waterloo, ON

PAPER UNAVAILABLE AT TIME OF PRINTING

ACCELERATING AN IN VIVO TROUT CARCINOGENESIS ASSAY WITH CARBON TETRACHLORIDE AND PARTIAL HEPATECTOMY.

N.Kotsanis and C.D.Metcalfe
Trent University, Peterborough, Ontario.

ABSTRACT-The rainbow trout microinjection assay has been shown to be a useful model for experimental carcinogenesis in fish because it offers an accurate dose administration, lower cost, safety, and the use of nanogram quantities of carcinogens. In this study we were able to further develop this model by reducing the test period to between 3 and 6 months with the hepatotoxic agent carbon tetrachloride (CCl_4) and partial hepatectomy (PH). A single microinjected dose of the hepatocarcinogen aflatoxin B1 (AFB1) at a concentration of 20ng/fry was given to rainbow trout sac-fry. Subsequent repeated intraperitoneal (I.P) injections with CCl_4 at a concentration of approximately 1ml/kg body weight were given to the fish every 21 days for a total of 7 I.P injections over six months. Partial hepatectomy (PH) was also used on 25 fish from all groups at 4 months post-injection with AFB1. Approximately 10% and 25% of the liver was removed during PH. Liver tumors were induced in trout necropsied at 3 months and 6 months in the groups treated with AFB_1 + CCl_4 and AFB_1+PH, at a higher incidence of neoplasms as compared to the AFB_1 group. Also we observed a greater number of neoplasms per liver in these groups as compared to the AFB1 group. In Table 1 and Table 2, the incidence of grossly visible tumors, and the incidence of histologically observed preneoplasms and neoplasms are tabulated for the 3 and 6 months necropsies, respectively. It is indicated that the promotors carbon tetrachloride, and partial hepatectomy led to a significant enhancement of liver tumor response. The AFB_1+CCl_4 and AFB_1+PH treatments had a higher incidence of hepatocarcinomas compared to the AFB_1 treament. Chi square analyses indicated that the incidence of hepatocarcinomas observed in these treatments were statistically different at 3 and 6 months. Table 3 and Table 4 list the frequency with which various numbers of carcinomas per liver were observed histologically in treatments where there was a tumor response. Although the PH treatment did not appear to increase the numbers of livers with multiple tumors, CCl_4 did produce a definite increase in the numbers of livers with multiple tumors.

Promotion is a new approach to aquatic carcinogenicity bioassays.It will be a useful assay technique to accelerate carcinogenicity assays and to identify environmental contaminants that act as promoters. In addition, these data show that the liver carcinogenesis model in fish is similar to the liver carcinogenesis model in rodent species.

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Table 1.- Incidence of basophilic hepatic lesions at the 3 month necropsy for rainbow trout treated as controls, or with DMSO, or Aflatoxin B_1 in DMSO (20ng/sac-fry), and repeated I.P. injections of CCl_4 (1ml/Kg body weight).

TREATMENT		VISUAL SURVEY N# of Fish with Gross Neoplasms	HISTOLOGICAL SURVEY PRENEOPLASMS N# of Fish with Basophilic Lesions	HISTOLOGICAL SURVEY NEOPLASMS N# of Fish with Basophilic Carcinomas	TOTAL* Visual Carcinomas + Histological Carcinomas
CONTROL	(n=50)	0	0	0	0
DMSO	(n=100)	0	0	0	0
DMSO+CCl_4	(n=100)	0	1	0	0
AFB_1	(n=150)	4	21	19	23 (15.3%)[a]
AFB_1+CCl_4	(n=165)	7	12	40	47 (28.5%)[a]

a) Frequencies significantly different in X^2 analysis (a=0.1).

* The total incidence of hepatocarcinomas observed visually and histologically.

Table 2.- Incidence of basophilic hepatic lesions at the 6 month necropsy for rainbow trout treated as controls , or DMSO, or Aflatoxin B_1 in DMSO (20ng/sac-fry), and repeated I.P.injections of CCl_4 (1.ml/Kg body weight), or submitted to partial hepatectomy.

TREATMENT	VISUAL SURVEY	HISTOLOGICAL SURVEY		TOTAL*
		PRENEOPLASMS	NEOPLASMS	
	N# of Fish with Gross Neoplasms	N# of Fish with Basophilic Lesions	N# of Fish with Basophilic Carcinomas	Visual Carcinomas + Histological Carcinomas
CONTROL (n=50)	0	0	0	0
CONTROL+PH (n=24)	0	0	0	0
DMSO (n=103)	0	0	0	0
DMSO+CCl_4 (n=82)	0	0	0	0
DMSO+PH (n=25)	0	0	0	0
DMSO+CCl_4+PH (n=25)	0	0	1	1
AFB_1 (n=158)	16	20	39	55 (34.17%)[a]
AFB_1+CCl_4 (n=131)	32	14	35	67 (51.14%)[b]
AFB_1+PH (n=39)	8	2	12	20 (51.28%)[b]
AFB_1+CCl_4+PH (n=38)	13	2	7	20 (52.63%)[b]

a) Frequency significantly different from AFB_1+CCl_4, AFB_1+PH, and AFB_1+CCl_4+PH X^2 (a=0.1).
b) Frequencies were not significantly different X^2 (a=0.10).
* The total incidence of hepatocarcinomas observed visually and histologically

Table 3.- The frequency with which various numbers of hepatocarcinomas were observed per liver in the AFB_1 and AFB_1+CCl_4 groups at the 3 months necropsy.

	Number of Tumors / Liver		
GROUPS	1	2	3
AFB_1 (n=150)	22	1	0[a]
AFB_1+CCl_4 (n=165)	34	10	3[a]

a Frequencies are statistically different in X^2 tests (α=0.05)

Table 4.- The frequency with which various hepatocarcinomas were observed per liver in the AFB_1, AFB_1+CCl_4 AFB_1+PH, and AFB_1+CCl_4+PH groups at the 6 months necropsy.

	Number of Tumors / Liver						
GROUPS	1	2	3	4	5	6	7
DMSO+CCl_4+PH (n=25)	0	1	0	0	0	0	0
AFB_1 (n=158)	30	16	6	3	0	0	0
AFB_1+CCl_4 (n=131)	30	15	12	5	1	2	2[a]
AFB_1+PH (n=39)	16	4	0	0	0	0	0[a]
AFB_1+CCl_4+PH (n=38)	10	6	1	3	0	0	0[a]

a) Chi square analyses showed no statistical difference between these groups with the AFB_1 treatment ($X^2_{0.05}$).

ESSAI IN VIVO DE CARCINOGENESE ACCELEREE CHEZ LA TRUITE AU MOYEN DU TETRACHLORURE DE CARBONE ET D'UNE HEPATECTOMIE PARTIELLE.

N. Kotsanis et C.D. Metcalfe
Université Trent, Peterborough, Ontario.

RESUME

L'essai de microinjection chez la truite arc-en-ciel s'est avéré un modèle utile pour la carcinogénèse expérimentale chez le poisson à cause de la précision de la dose injectée, de son coût moins élevé, de la sécurité de la méthode et de l'utilisation de quantités de l'ordre du nanogramme de produits carcinogènes. Dans cette étude, il a été possible de développer par la suite ce modèle en réduisant la durée du test à une période entre 3 et 6 mois avec l'agent hépatotoxique tétrachlorure de carbone (CCl_4) et une hépatectomie partielle (H.P.). Une seule dose microinjectée de l'hépatocarcinogène aflatoxine B1 (AFB_1) à une concentration de 20 ng/alevin a été administrée aux alevins vésiculés de truite arc-en-ciel. Subséquemment, des injections intrapéritonéales (I.P.) répétées de CCl_4 à une concentration d'environ 1 ml/Kg de poids ont été administrées aux poissons à tous les 21 jours pour un nombre total de 7 injections I.P. sur une période de 6 mois. Une hépatectomie partielle (H.P.) a aussi été effectuée sur 25 poissons de tous les groupes, 4 mois après l'injection de AFB_1. Environ 10% et 25% du foie a été enlevé pendant l'H.P. Des tumeurs au foie étaient induites chez la truite autopsiée à 3 mois et 6 mois chez les groupes traités avec le AFB_1 + CCl_4 et AFB_1 + H.P. avec une plus grande incidence de néoplasmes comparé au groupe AFB_1. Nous avons aussi observé un plus grand nombre de néoplasmes par foie dans ces groupes en comparaison au groupe AFB_1. Dans le tableau 1 et le tableau 2, l'incidence des tumeurs extrêmement visibles et l'incidence des prénéoplasmes et des néoplasmes observés en histologie sont compilées pour les autopsies de 3 et 6 mois respectivement. On peut remarquer que les inducteurs tétrachlorure de carbone et hépatectomie partielle ont condui à une augmentation significative de la réponse de tumeur au foie. Les traitements AFB_1 + CCl_4 et AFB_1 + H.P. ont eu une plus grande incidence d'hépatocarcinomes comparé au traitement AFB_1. Les analyses de Chi-carré ont indiqué que l'incidence des hépatocarcinomes observés dans ces traitements étaient statistiquement différents à 3 et 6 mois. Le tableau 3 et le tableau 4 énumèrent la fréquence avec laquelle un grand nombre de carcinomes par foie ont été observés en histologie dans les traitements où il y avait une réponse de tumeur. Bien que le traitement d'H.P. n'a pas semblé accroître le nombre de foies avec des tumeurs multiples, le CCl_4 a produit un accroissement marqué du nombre de foies avec tumeurs multiples.

Cette méthode est une nouvelle approche sur la carcinogénicité aquatique par les bioessais. Elle sera une technique utile d'essai pour accélérer la durée des essais de carcinogénicité et pour identifier les contaminants environnementaux qui agissent comme inducteurs. De plus, ces données démontrent que le modèle de carcinogénèse du foie chez le poisson est semblable au modèle de carcinogénèse chez des espèces de rongeurs.

LIMITATIONS ON THE APPLICATION OF BCF IN CHEMICAL HAZARD ASSESSMENT

A.J. Niimi. Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington, Ontario, L7R 4A6.

Abstract

The relationship between the bioconcentration factor (BCF) of a chemical in fish and its octanol-water partition coefficient (Kow) has been used to assess the hazard potential of organic chemicals. A reexamination of this concept does indicate the BCF of some chemicals are well below the general trend of increasing BCF with increasing Kow. Based on the limited results of an earlier study, the relationship between BCF and Kow was examined for 14 monochloro- to pentachloronitrobenzenes in rainbow trout through waterborne and dietary exposure studies. The results indicated BCF was not significantly correlated with Kow for this chemical group. Limited evidence also indicate other chemical groups like the PAHs and chloroguaiacols may also show a nonlinear response with Kow.

Comparaison entre les activités de la malate déshydrogénase provenant des fractions cytosoliques et mitochondriales du manteau de Mytilus edulis L. pour le choix d'un indicateur biochimique évaluant les effets sous-léthaux du méthylmercure

PELLERIN-MASSICOTTE, Jocelyne* [1]
et PELLETIER, Emilien [2].

[1] Univ. du Québec à Rimouski, 300 des Ursulines, Rimouski, Qué, G5L 3A1
[2] INRS-Océanologie, 310 des Ursulines, Rimouski, G5L 3A1.

INTRODUCTION

Notre recherche porte sur l'élaboration d'indicateurs biochimiques et cellulaires pouvant refléter les variations environnementales subies par les organismes présents dans un écosystème. Ces dernières années nous avons développé un indice biochimique capable de renseigner sur la performance biologique d'un organisme en présence de méthylmercure et de sélénium (Pellerin-Massicotte, 1988). La mesure de l'activité maximale de la malate déshydrogénase et de sa constante d'affinité (Km) dans la fraction cytosolique du manteau de la moule bleue, s'est avérée très sensible aux niveaux de pollution sous-léthale par le méthylmercure. L'objectif spécifique de ce travail est de comparer les niveaux d'activité et d'affinité de la malate déshydrogénase présente dans les fractions mitochondriale et cytosolique du manteau de la moule bleue Mytilus edulis L., en réponse à deux concentrations sous-léthales de méthylmercure (0.01 ug L^{-1} et 0.3 ug L^{-1}) dans le but de faire le choix du meilleur indicateur biochimique de pollution sous-léthale.

MATERIEL ET METHODES

Le protocole expérimental a déjà été décrit par Pelletier (1986) et Pellerin-Massicotte (1988). Il consiste à contaminer des moules bleues (Mytilus edulis L.) grâce au pompage en continu d'un mélange d'eau de mer, de phytoplancton, de méthylmercure et de sélénium. Des moules bleues (Mytilus edulis L.; 3.5+ .3 cm),ont subi une période de contamination de 45 jours suivie d'une décontamination de 14 jours. Les fractions mitochondriales et cytosoliques des homogénats tissulaires ont été obtenues par centrifugation différentielle et gardées ultérieurement à -70°C jusqu'à la réalisation des analyses enzymatiques. L'activité de la malate déshydrogénase a été évaluée en suivant l'oxydation de la NADH à 340 nm avec un spectrophotomètre Perkin-Elmer Coleman 575 (Bergmeyer, 1983). L'oxaloacétate a été utilisé comme substrat avec 5 ug de protéines par essai enzymatique, pour un volume total de 3.1 ml.

RESULTATS ET DISCUSSION

Au début de la période de contamination (Jour 1), l'enzyme provenant des deux fractions voit son Km se déplacer significativement vers la gauche, démontrant ainsi une activation du métabolisme en

réponse aux polluants. La mise en présence des moules avec les polluants modifie donc les paramètres cinétiques de la malate déshydrogénase, par rapport aux moules contrôles. Celles-ci, subissent un stress important causé par la mise en bassin, l'affinité de l'enzyme pour son substrat diminuant significativement. La variation du Km observée en présence des polluants est proportionnelle à la concentration des contaminants, la plus faible concentration ne déplaçant la courbe que légèrement vers la gauche, tandis que la concentration la plus forte, déplace de 5 fois le Km de la MDH dans les deux fractions.

Les processus de détoxification sont enclenchés dès le premier jour du processus de contamination et engendrent une activation du métabolisme, ce qui maintient chez les moules contaminées, une valeur de Km similaire à celle observée avant la mise en bassins. Par contre, les moules contrôles ne subissent pour leur part, que le stress de la mise en bassins et cet effet s'observe par la perte d'affinité de l'enzyme pour son substrat. Aux jours 22 et 29 après le début de la contamination, le Km de l'enzyme présente dans la fraction cytosolique est augmenté de 5 fois mais sans modification du Vmax par rapport aux moules contrôles. Celles-ci retrouvent une activité métabolique normale, comparable aux activités retrouvées chez des moules échantillonnées à Pointe-Mitis. La MDH mitochondriale, pour sa part, a un Vmax diminue et une légère augmentation de son Km au jour 22. Par contre, 29 jours après le début de la contamination, les deux concentrations de méthylmercure entraînent une forte perte de l'affinité de l'enzyme mitochondriale pour son substrat. Toutefois, les variations individuelles sont plus importantes dans la fraction mitochondriale. L'activité enzymatique dans les deux fractions montre une nette récupération après que les polluants aient été enlevés. L'évolution du Km dans le manteau des moules contrôles illustre bien la capacité de récupération et d'adaptation des moules à leur nouvel environnement (Bayne, 1985). En effet, le Km de la MDH revient aux valeurs normales 22 jours après le début de la période de contamination. Par contre, la présence de méthylmercure affecte le métabolisme énergétique. Le polluant, dans la fraction cytosolique, en n'affectant que l'affinité de l'enzyme pour son substrat et non la capacité de transformation du substrat illustrée par le Vmax, démontre qu'il y a une inhibition compétitive exercée par le mercure, probablement par la liaison du polluant au niveau du site actif de l'enzyme sur les ponts disulfure. Ces résultats démontrent que le choix de la fraction cytosolique s'avère judicieux comme indicateur biochimique de pollution sous-léthale par sa réponse plus accentuée au jour 22 et pour sa fiabilité.

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AVIAN VITAMIN A DYNAMICS IN A DIOXIN-CONTAMINATED ENVIRONMENT

P. A. SPEAR and T. W. MOON

Department of Biology, University of Ottawa

Introduction:

Polyhalogenated aromatic hydrocarbons having similar molecular structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin elicit symptoms that are comparable to those typically associated with nutritional vitamin A imbalances. We have previously reported that liver vitamin A stores in rats and doves are severely depleted and that the degradative metabolism of vitamin A is induced by these contaminants (Spear *et al.* 1986; Spear *et al.* 1988). In the present poster, we demonstrate the first results indicating that this effect may be occurring in the environment. Laboratory experiments have revealed that embryo mortality in doves coincided with changes in egg yolk vitamin A (Spear *et al.* in press). Effects upon vitamin A concentrations in bird eggs in the field are presented.

Liver vitamin A stores:

Vitamin A stores were measured in nesting adult herring gulls, *Larus argentatus*, collected from 3 colonies on the Great Lakes and a marine colony located in New Brunswick. Significant differences in the concentrations of both retinol and retinyl palmitate were found between the sampling sites. Significant differences also occurred between the 3 Great Lakes sites, when the marine site was omitted. The liver vitamin A stores appeared to be inversely related to the concentration of 2,3,7,8-TCDD in the gull eggs. In several birds from a colony located on Lake Ontario, the liver vitamin A concentrations were critically low, i.e. below 10 ug retinol/g liver. Liver retinol concentration was significantly correlated with liver aryl hydrocarbon hydroxylase activity in both the gulls and in a controlled experiment with the ring dove, *Streptopelia risoria*, injected with 3,3',4,4'-tetrachlorobiphenyl. Vitamin A depletion is therefore attributed, in part, to enzyme induction and the catabolism of vitamin A.

The Vitamin A Ratio in Eggs:

A method was developed to analyse 5 different retinoids (forms of vitamin A) in egg yolk. In eggs of the herring gull collected from colonies on the Great Lakes, the retinyl palmitate concentration was significantly lower in contaminated sites compared with a relatively clean site. The molar ratio of retinol:retinyl palmitate was positively correlated with the 2,3,7,8-TCDD concentration in gull eggs. In contrast, the retinol:retinyl palmitate ratio was not related to the total concentration of vitamin A indicating that the correlation was not

a consequence of different vitamin A levels in food. Controlled experiments also demonstrated high ratios of retinol:retinyl palmitate in eggs laid by exposed ring doves. These studies indicate complex effects upon vitamin A dynamics during oogenesis, and possibly during the early stages of embryonic development. The "vitamin A ratio" may be useful as a sensitive indicator of the combined effects of the polyhalogenated aromatic hydrocarbons in the environment.

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CONCENTRATIONS AND EFFECT OF Unat, 210 Pb, 226 Ra, and 228 Th ON FISH EXPOSED TO URANIUM MINE TAILINGS

D.T. Waite 1, G. Wobesser 2, S.R. Joshi 3 and H. Sommerstad 1
1 Environment Canada, Regina, Saskatchewan
2 University of Saskatchewan
3 Environment Canada, Burlington, Ontario

From 1955 to 1964, the Gunnar Uranium Mine produced approximately $5x10^6$ tonnes of waste rock tailings. Large quantities of fine tailings material washed into Langley Bay, a shallow bay opening into Lake Athabasca, Saskatchewan, Canada. High concentrations of 210 Pb, 226 Ra, 228 Th and U were measured in samples of bone and gut contents from the resident whitefish population (Coregonus clupeaformis). The concentrations were approximately 100 x greater than those found in whitefish from a non-contaminated, control area. Blood hematocrit values for two populations were not statistically different.

An histopthological comparison of bone, gut and kidney tissues of the two fish populations will be presented and compared with individual radionuclide analyses of some of these tissues.

CONCENTRATIONS ET EFFET DE L'$U_{nat.}$, DU Pb^{210}, DU Ra^{226} ET DU Th^{228} SUR LE POISSON EXPOSE AUX REJETS D'UNE MINE D'URANIUM.

D. T. Waite[1], G. Wobesser[2], S. R. Joshi[3], et H. Sommerstad[1]
1. Environnement Canada, Régina, Saskatchewan
2. Université de Saskatchewan
3. Environnement Canada, Burlington, Ontario

RESUME

De 1955 à 1964, la mine d'uranium Gunnar a produit approximativement 5 X 10^6 tonnes de résidus rocheux. De grandes quantités de résidus fins ont été rejetés dans la baie de Langley, une baie peu profonde s'ouvrant sur le lac Athabaska, Saskatchewan, Canada. De fortes concentrations de Pb^{210}, de Ra^{226}, de Th^{228} et d'U ont été mesurées dans des échantillons d'os et les contenus intestinaux de la population locale de poisson blanc (Coregonus clupeaformis). Les concentrations étaient environ 100 fois plus élevées que celles trouvées chez le poisson blanc d'une région contrôle non contaminée. Les valeurs de l'hématocrite sanguine pour les deux populations ne démontraient aucune différence statistique.

Une comparaison histopathologique des tissus osseux, intestinaux et rénaux des deux populations de poissons sera présentée et comparée avec les analyses individuelles de radionucléides de quelques-uns de ces tissus.

Striped Bass Contaminant and Water Quality Studies in the Potomac River and Upper Chesapeake Bay

Michael C. Ziegenfuss, Lenwood W. Hall, Jr., Steven J. Bushong and Michael A. Unger

The Johns Hopkins University, Applied Physics Laboratory, Aquatic Ecology Section, Shady Side, MD 20764 USA

Striped bass, Morone saxatilis, have been declining along the Atlantic coast in recent years (Boreman and Austin, 1985). Adverse water quality and contaminants have been identified as possible factors influencing the decline in stocks. The major spawning habitat for Atlantic coast striped bass is located in Chesapeake Bay (Berggren and Lieberman, 1978). Since 1983 The Johns Hopkins University Applied Physics Laboratory has been conducting field studies to evaluate the survival of young striped bass in spawning habitats as related to water quality and presence of contaminants during the spawning season.

Three simultaneous 96 h on-site and in-situ striped bass prolarval survival studies and one simultaneous 28 d in-situ and on-site yearling survival study was conducted in the Potomac River during a major portion of the 1988 spawning season. The in-situ and on-site testing technique has previously been used successfully (Hall et al., 1985; Hall et al., 1987). Additionally, 28d in-situ yearling studies were conducted in the Susquehanna, Elk and Sassafras Rivers of upper Chesapeake Bay. During the Potomac River and Upper Bay studies, 11 water quality parameters, 11 inorganic contaminants, and 14 organic contaminants were monitored. At the end of 28d, surviving yearlings from all study sites were posted for hematological and histological evaluation.

Survival of striped bass prolarvae ranged from 1-20% in Potomac river water during the three concurrent in-situ and on-site toxicity tests. Control survival was 82% or greater in all experiments. Results from the in-situ and on-site tests were similar. Yearling survival was 15% or less during 28d in-situ tests at two of the Potomac river stations. Survival of yearlings in the controls was 100%. Significant mortality of prolarvae was likely caused by a combination of the following factors: cadmium (5 ug/L), lead (12 ug/L), chlordane (0.152 ug/L) and sudden drops in temperature (4-5°C in 48h). Mortality of yearlings was probably caused by a combination of cadmium (14 ug/L), lead (15 ug/L), zinc (310 ug/L), chlordane (0.152 ug/L), and ammonia (0.39-0.48 mg/L Total) likely associated with a point source discharge.

Survival of striped bass yearlings tested 28d in-situ in the Susquehanna, Elk and Sassafras Rivers was 57, 100, and 100%, respectively. Control survival was 100%. The low yearling

survival in the Susquehanna river may have been partly attributed to the presence of copper (13 ug/L) and lead (4 ug/L). However, it is doubtful that these two contaminants alone at the detected concentrations would have caused significant mortality. Adverse contaminant and water quality problems were not reported in the Elk or Sassafras Rivers.

Histological and hematological evaluation of yearlings from the Potomac River (in-situ and on-site tests) and Upper Chesapeake Bay (in-situ tests) studies suggested that fish exposed to habitat water were stressed when compared to control fish. Branchial lamellar telangiectasis and edema, hepatic fatty metamorphosis, and neutrophilia were observed in fish from habitat waters. Due to the non-specific stress related nature of the lesions, a specific cause and effect could not determined.

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ETUDE DE LA CONTAMINATION DE LA PERCHE RAYEE ET DE LA QUALITE DE L'EAU DANS LA RIVIERE POTOMAC ET LA PARTIE SUPERIEURE DE LA BAIE DE CHESAPEAKE.

Michael C. Ziegenfuss, Lenwood W. Hall, Jr.,
Steven J. Bushong et Michael A. Unger

Université John Hopkins, Laboratoire de Physique Appliquée,
Section Ecologie Aquatique, Shady Side, MD 20764 U.S.A.

RESUME

Au cours des dernières années, la population de la perche rayée Morone saxatilis, a décliné le long de la côte Atlantique (Boreman et Austin, 1985). Une mauvaise qualité de l'eau et la présence de contaminants ont été identifiés comme les facteurs possibles influençant ce déclin de la population. Le principal endroit de reproduction de la perche rayée pour la côte Atlantique se situe dans la baie de Chesapeake (Berggren & Lieberman, 1978). Depuis 1983, le laboratoire de physique appliquée de l'université John Hopkins a entrepris des études sur le terrain pour évaluer la survie de la jeune perche rayée dans les lieux de reproduction en relation avec la qualité de l'eau et la présence de contaminants durant la saison de reproduction.

Trois études de survie de 96 heures réalisées simultanément sur le terrain et in-situ avec la perche rayée au stade prolarvaire et une étude de survie de 28 jours réalisée simultanément in-situ et sur le terrain avec des jeunes d'un an ont été effectuées dans la rivière Potomac durant la majeure partie de la période de reproduction de 1988. La technique in-situ et sur le terrain avait déjà été utilisée précédemment avec succès (Hall & al, 1985; Hall & al. 1987). De plus, des études in-situ de 28 jours avec des jeunes d'un an ont été réalisées dans les rivières Susquehanna, Elk et Sassafras, dans la partie supérieure de la baie de Chesapeake. Pendant les études de la rivière Potomac et la partie supérieure de la baie, 11 paramètres de la qualité de l'eau, 11 contaminants inorganiques et 14 contaminants organiques ont été suivis. A la fin des 28 jours, les jeunes d'un an qui ont survécu, en provenance de tous les sites étudiés, ont été soumis à une évaluation hématologique et histologique.

Le taux de survie de la perche rayée au stade prolarvaire a varié de 1 à 20% dans l'eau de la rivière Potomac durant les trois essais simultanés de toxicité in-situ et sur le terrain. Le taux de survie du contrôle était de 82% ou plus dans toutes les expériences. Les résultats des essais in-situ et sur le terrain ont été similaires. La survie des jeunes d'un an était de 15% ou moins au cours des essais in-situ de 28 jours à deux stations de la rivière Potomac. La survie des jeunes d'un an

dans les contrôles était de 100%. Une mortalité significative au stade prolarvaire était possiblement dûe à une combinaison des facteurs suivants: cadmium (5 µg/L), plomb (12 µg/L), chlordane (0.152 µg/L) et des chutes soudaines de température (4-5°C en 48 heures). La mortalité des jeunes d'un an était probablement causée par une association de cadmium (14 µg/L), plomb (15 µg/L), zinc (310 µg/L), chlordane (0.152 µg/L) et ammoniaque (0.39 - 0.48 µg/L) possiblement reliée à un rejet localisé.

La survie des jeunes d'un an de la perche rayée expérimentée pendant le test in-situ de 28 jours dans les rivières Susquehanna, Elk et Sassafras a été respectivement de 57, 100 et 100%. La survie du contrôle était de 100%. Le faible taux de survie des jeunes d'un an dans la rivière Susquehanna peut avoir été en partie attribué à la présence de cuivre (13 µg/L) et de plomb (4 µg/L). Cependant, il est incertain que seuls ces deux contaminants aux concentrations décelées puissent avoir causé une mortalité significative. Des problèmes de contaminant nuisible et de qualité de l'eau n'étaient pas signalés dans les rivières Elk et Sassafras.

Les études d'évaluation histologique et hématologique des jeunes d'un an de la rivière Potomac (tests in-situ & sur le terrain) et de la partie supérieure de la baie de Chesapeake (tests in-situ) ont laissé supposer que le poisson en contact avec l'eau du milieu était stressé lorsque comparé au poisson témoin. Une télangiectase branchiale lamellaire et de l'oedème, une métamorphose de la graisse hépatique et de la neutrophilie ont été observées chez les poissons des eaux du milieu. Puisque les lésions ne présentent pas de tensions spécifiques qui se rapportent à l'expérience, on ne peut déterminer de relation spécifique de cause à effet.

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LEACHING OF CHROMIUM, COPPER AND ARSENIC FROM CCA-TREATED WOOD

J. Warner[1] and K.R. Solomon[2]
[1]Department of Environmental Biology
University of Guelph, Guelph, ON
[2]Canadian Centre for Toxicology, Guelph, ON

PAPER UNAVAILABLE AT TIME OF PRINTING

Effects of copper, aluminum and nickel,individually and in combination, on phytoplankton from Niagara-on-the Lake, Hamilton Harbour and Cataraqui River.

Wong, P.T.S., Y.K. Chau* and S. Rhamey. Department of Fisheries and Oceans, Bayfield Institute, Great Lakes Laboratory for Fisheries and Aquatic Sciences and *Department of the Environment, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario, L7R 4A6.

Relatively little information is available on the interactive effects of metal mixtures to phytoplankton. The response of phytoplankton to one metal may be significantly different than that to the metal mixtures. The objective of this study was on single and multiple metal (Cu, Al and Ni) toxicity to natural phytoplankton from areas with different water chemistry.

Metal toxicity experiments were carried out in Niagara-on-the-Lake, Hamilton Harbour and Cataraqui River near Kingston from May to August, 1985. Cu, Al and Ni in their respective sulfate forms were spiked at the Great Lakes Water Quality Objectives levels of 5, 25 and 100 ug/L respectively to the water containing phytoplankton. After exposure to the metals and metal mixture for 20 hr, the phytoplankton activities were measured with standard ^{14}-C technique. Cu, Al and Ni individually had no significant inhibitory effects on the productivity of phytoplankton from Hamilton Harbour and Cataraqui River. Ni also had no effect on phytoplankton from Niagara-on-the-Lake. In contrast, individual Cu and Al reduced the productivity by 47 and 29% respectivity in one experimental station while by 57 and 54% respectivity in another station as compared with the phytoplankton without exposure to the metal. In general, addition of the mixture of 3 metals caused synergistic toxicity effects. The complexation, phytoplankton biomass and water chemistry affect the metal toxicity.

COMPARISON OF THE TOXIC RESPONSES OF THREE BIOINDICATORS (BACTERIA, ALGAE AND FISH) TO PULP AND PAPER INDUSTRY EFFLUENTS

Christian Blaise[1], Raymond Van Coillie[2], Normand Bermingham[1] and Guy Coulombe[2].

[1]Environmental Conservation and Protection Service, Environment Canada, 1001 Pierre Dupuy, Longueuil, Quebec J4K 1A1
[2]Environmental Conservation and Protection Service, Environment Canada, 1179 Bleury, Montreal, Quebec H3B 3H9

ABSTRACT

Regulations have been instituted by the Canadian Government in order to control final effluent quality for several industrial sectors. Those specific to the pulp and paper industry - a vital economic activity for Canada - have been in place for over a decade.

In this context, the regulatory bioassay ensuring toxics control of liquid wastes for such mill process operations is the 96-hour LC50 fish bioassay conducted with rainbow trout (Salmo Gairdneri Richardson). Bioanalytical compliance and surveillance work in this industrial sector has allowed us to accumulate acute toxicity data based on the fish test for several years(1976-1984). Because sublethal effects have been frequently reported for the effluents, we felt it important to bolster our data bank by undertaking two additional complementary biotests.

In this way, we were able to study and compare the ecotoxicity of some fifty effluents from pulp and paper plants by estimating their toxicity at three trophic levels. Rainbow trout (Salmo Gairdneri), the unicellular alga Selenastrum capricornutum and the Photobacterium phosphoreum bacterium of the Microtox system were thus chosen as the biological indicators for this purpose. The addition of the latter two tests allowed us to compare their overall toxicity sensitivity responses to those of the fish test and to see whether such microbial tests could act as potentially useful screening tools for future investigations of similar wastes.

A first analysis of the data revealed the high hazard of these effluents as indicated by the EC50 (algal and Microtox biotests) and LC50 (trout biotest) results obtained. There was, as well, an excellent general agreement among the three biotests as shown by 1) a comparison of the number of toxic and non-toxic responses; 2) a percentage rank comparison of toxicity; and 3) a log rank comparison of toxicity.

A second analysis of the data showed that the algal biotest was most sensitive to the effluents analysed, followed by the Microtox biotest and then by the trout biotest. It is probable the greater sensitivity of the algal and Microtox biotests can be explained by the fact that their toxicity is expressed at the sublethal level, while the trout test measures mortality. In addition, the agreement in sensitivity proved better among algae and bacteria: this could be due to a closer phylogenetic connection between these two indicators. Nevertheless, the differences in sensitivity observed emphasizes the need to use more than one indicator to correctly determine the ecotoxicity of an effluent.

When the toxicity of the effluents studied was compared with regards to the type of 1) industrial process and 2) waste treatment, each biotest was able to differentiate noxiousness on the basis of these two variables. In short, the biotests and bioindicators used proved to be sensitive and reliable tools for determining the (sub)-lethal toxicity of these industrial liquid wastes. They can thus be recommended for integrated ecotoxicological evaluations of similar wastes in future studies.

MATERIAL AND METHODS

Samples

To ensure that samples of final effluent or process water were representative, each was composited over a period ranging from 8 to 24 hours (depending on mill operating methods) in accordance with a sampling protocol approved by Environment Canada (Vezeau, 1982). Four hundred or so litres were collected of each sample and transported to the laboratory in 60 L Rubbermaid barrels lined with polyethylene. Each sample was recomposited in the laboratory and then divided up for analysis as follows:

Bioassay with:	fish :	360 L
	algae :	2 L
	bacteria:	1 L
Supporting chemical analysis:		15 L

Bioanalysis

Static tests were performed with rainbow trout (Salmo Gairdneri Richardson) following the government procedure in effect (Environment Canada, 1980a).

The algal strain that was used, Selenastrum capricornutum (ATCC no 22662), is a single cell chlorophyte. The algal assay procedures were those proposed by Greene et al (1975), Miller et al (1978) and Joubert (1980) for determining algal growth inhibition in ecotoxic conditions. Allowance was made for the level of nutrients (N or P) that regulates algal growth (Shiroyama et al, 1975), the level being derived from the tested sample and the nutrient input; the latter ensured the presence of sufficient macro and micro elements for organism growth. Observed biomass measurements were compared with expected biomass for each concentration, and inhibition percentages were then calculated to determine EC50.

The subsamples were not pre-treated for the Microtox bacterial test except that, if the pH was not within the 6 to 9 range, it was adjusted to 7. Microtox Model 2055 (Toxicity Analyzer System, Beckman Instruments Inc, USA) was used, bioanalysis being done in accordance with the protocol described by Beckman (1980).

Data Reduction

Comparison of EC50 and LC50: after transferring all EC50 and LC50 values for each indicator to graph paper, it was possible to make a preliminary assessment of results by plotting the X and Y axes (curve = 1) and examining the distribution of data.

Comparison of toxic vs non-toxic: by attributing a positive sign to EC50 and LC50 values <100%, and a negative one to indicate that no toxicity had been found, it was possible to estimate the general correlation between the toxic responses of the three groups.

Percent rank comparison: this method (Bulich, 1982) gives a level of toxicity for each bioassay result on the following scale:

EC 50 or LC50	Category	Rank
<25%	toxic	1
25-100%	slightly toxic	2
>100%	non-toxic	3

Values can then be consolidated as in the following four examples:

EC50 Microtox	LC50 fish	Microtox rank	Fish rank	Rank difference
2.5	26	1	2	1
>100	80	3	2	1
15	>100	1	3	2
>100	>100	3	3	0

Log rank comparisons: this type of analysis was recommended by Kenaga (1978) and modified by Bulich (1982) to provide a system, based on half-log intervals, for distributing results among six classes, as shown in the following table:

Class	EC50 or LC50 within an interval (v/v%) of	Corresponding log interval
1	< 1.0%	< 0.0
2	>1.0 - 3.2%	>0.0 - 0.5
3	>3.2 - 10%	>0.5 - 1.0
4	>10 - 32%	>1.0 - 1.5
5	>32 - 100%	>1.5 - 2.0
6	>100%	>2.0

An example of the classification system follows:

EC50 Microtox	Class	LC50 Fish	Class	Difference between Classes
>100%	6	>100%	6	0
30%	4	23%	4	0
62%	5	>100%	6	1
75%	5	3%	2	3

Relative sensitivity of bioindicator groups: relative sensitivity to pulp and paper effluents was measured by determining:

1. a sensitivity rating (expressed in percentage terms) for each, based on total data;
2. the range of toxic responses in each group;
3. the overall toxic response of each group.

Evaluation of the toxicity of pulp and paper mill effluents, by type of process (regardless of effluent treatment method): mills were classified under six headings according to the industrial process that each used:

Group	Type of process
1	Fine paper making (non-integrated mills, with no pulp making)
2	Semi-chemical and/or chemi-thermomechanical pulping
3	Kraft pulping (integrated mills)
4	Fibre recycling and de-inking
5	Groundwood pulping and/or thermomechanical pulping and/or refiner mechanical pulping
6	Low yield sulphite pulping with or without mechanical pulping

Evaluation of toxicity of pulp and paper effluents vs effluent treatment method (regardless of industrial process): mills were classified by treatment type as follows:

Code	Treatment
0	None
1	primary
2	primary and secondary (lagooning)

RESULTS AND DISCUSSION

Comparison of the toxic response of three indicator groups to pulp and paper effluents

30 and LC50 values were compared for two groups at a time: trout vs algae; trout vs Microtox; and algae vs Microtox (figure 1). The following conclusions may be drawn from the distribution of points on figures 1A, B and C:

- a strong toxic response generally occurs in bioassays with pulp and paper effluent samples, a clear indication of their harmfulness. That harmfulness would seem to be multi-trophic since bacteria, algae and fish are affected in fairly similar proportions (algae: 54 positive toxic responses out of 55, or 98%; Microtox, 43 out of 51, or 84%; and trout 46 out of 55, or 84%;

- the three groups differ in their sensitivity to pulp and paper effluents: algae and bacteria are both more sensitive than trout , and algae are more sensitive than bacteria.

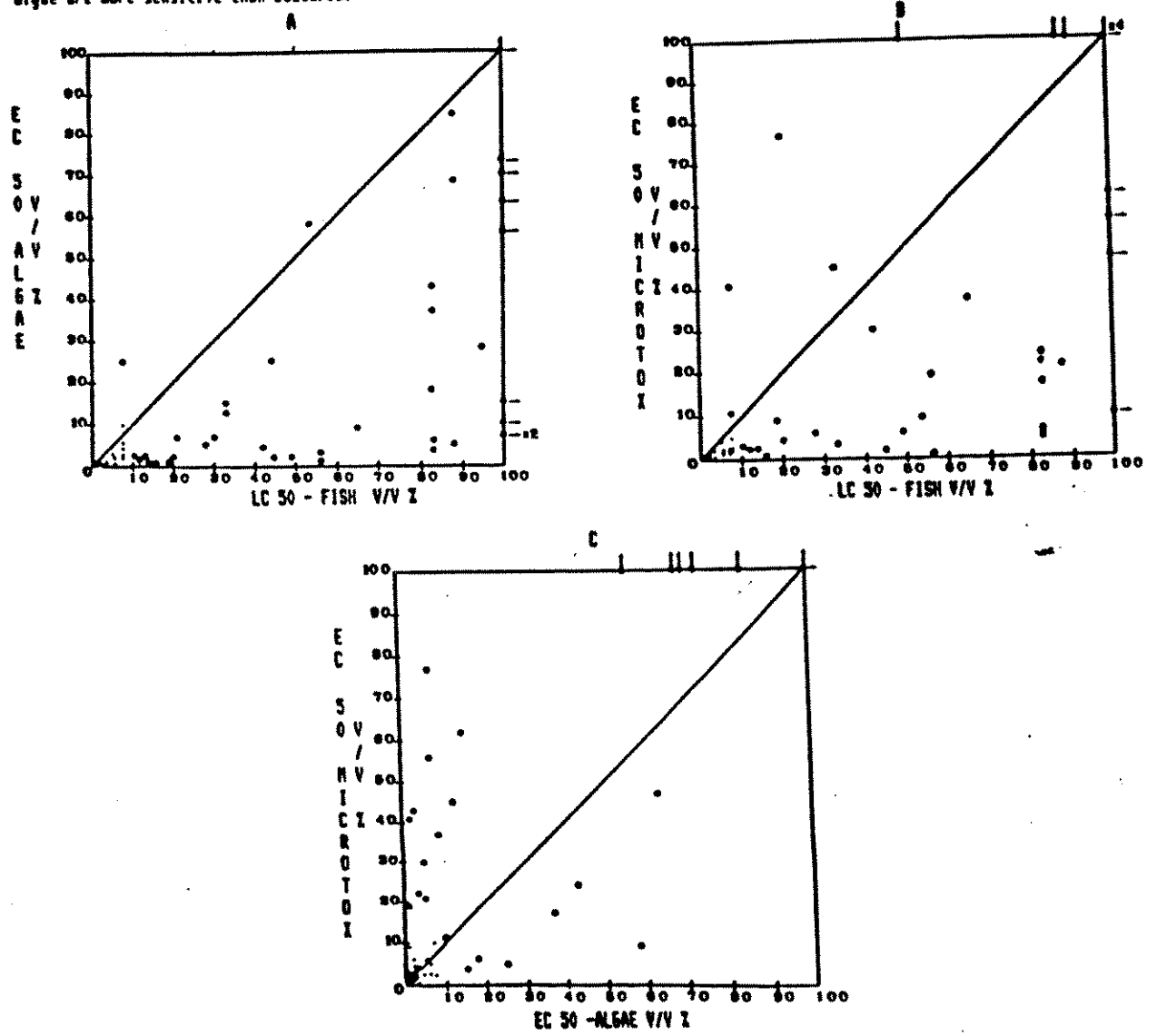


Figure 1. Comparative EC50 and LC50 data for each group of two indicators following exposure to pulp and paper effluents.

Quantitative comparison of toxic and non-toxic responses

Table 1, which compares the toxic and non-toxic responses of each two-indicator set, shows a fairly good correlation between them since 85%, 84% and 90% of trout/algae, trout/Microtox and algae/Microtox respectively responded positively or negatively to the effluents. Also evident is a high percentage of comparative data indicating a toxic response, providing confirmation of effluent toxicity. Results also tend to show that the algal or Microtox test can be relied upon to detect relative toxicity in samples from the pulp and paper industry. While it is impossible to predict the exact degree of toxicity to rainbow trout, the species used in biotests in Canada, algal and Microtox assays would appear capable of detecting the presence or absence of potential toxicity. Using either of those to the exclusion of all other assays could, however, result in the toxicity to trout being over - or underestimated. Thus, in 15% of cases, the algal test indicated a toxicity that could not be found in trout while, in 8% of cases, the Microtox test either over or underestimated toxicity to that species (table 1A and B). Moreover, when the Microtox was used to measure the phytoplanktonic toxicity of pulp and paper effluents, such toxicity was underestimated in 10% of cases (table 1C). Nevertheless, it will be noted that non-correlation, if it occurs, is most likely to do so for effluents of relatively low toxicity (EC or LC50 100v/v%; see figure 1). Consequently, underestimating toxicity to one indicator group is unlikely to have serious environmental consequences for either of the other two groups. Nevertheless, such findings highlight the inability of a single indicator group to accurately predict the toxicity that is likely to appear in another. That is further argument for using more than one bioindicator, where possible, to estimate toxic stress.

Table 1. Comparative toxic/non- toxic responses between indicator groups for pulp and paper effluents.

A. Toxic vs non-toxic response (trout vs algae)

Type of Response	Sample Number	Percentage	
T(-) A(-)	1	2%	) 85%
T(+) A(+)	44	83%	
T(+) A(-)	0	0%	
T(-) A(-)	8	15%	
Total	53	100%	

B. Toxic vs non-toxic response (trout vs Microtox)

Type of Response	Sample Number	Percentage	
T(-) M(-)	4	8%	) 84%
T(+) M(+)	37	76%	
T(+) M(-)	4	8%	
T(-) M(+)	4	8%	
Total	49	100%	

C. Toxic vs non-toxic response (algae vs Microtox)

Type of Response	Sample Number	Percentage	
A(-) M(-)	1	2%	) 90%
A(+) M(+)	43	88%	
A(+) M(-)	5	10%	
A(-) M(+)	0	0%	
Total	49	100%	

A=Algae; M=Microtox; T=trout. (+)=toxic sample; (-)=non-toxic sample.

Percent rank comparison

This method of data processing was used in order to obtain additional information on the degree of correlation among indicators on reporting effluent toxicity, as shown in table 2 below.

Table 2. Percent rank toxicity data classification for pulp and paper effluents.

Rank difference*	Correlation or Non-correlation (Δ) T-A	T-M	A-M
0	57%	53%	65%
	)93%	)98%	)100%
1	36%	45%	35%
2	7%	2%	0%

-T=Trout; A=Algae; M=Microtox;
-53, 49, 49 pairs of comparative data for T-A, T-M, and A-M respectively.
*Rank 1; EC50 or LC50 <25% (toxic).
Rank 2; EC50 or LC 50 between 25% an 100% (slightly toxic).
Rank 3; EC50 or LC50 >100% (non-toxic).

These results indicate the percentage of correlation (Δ = 0) and non-correlation (Δ) 1) for intergroup data. The degree of correlation for T-A, T-M and A-M is 57%, 53% and 65% respectively. Correlation and slight non-correlation (Δ = 1), together account for the vast majority of data for groups T-A (93%) and T-M (98%) and for all data for A-M (100%). Note that Bulich (1982) used this system and similar toxicity scale to compare the results of fish and Microtox assays of 235 samples of assorted effluents; he found that 86% of the data had a toxic rank difference of 1. We observed this difference in 98% of the cases for the T-M group during our experiments with pulp and paper effluent. It is also interesting that the correlation of toxicity for the A-M series (65%) is slightly more marked than for the T-M series (53%). The greater correlation in the A-M group may be attributable to the phylogenetic connection between the two indicators. A link between toxic response and the phylogenetic relationship between species has in fact been reported by Kenaga (1978), Suter et al (1983), and Leblanc (1984), who compared interspecies toxic responses after acute exposure to various chemical products (pesticides and metals).

Log rank comparison

The results of the comparison are given in table 3, examination of which shows that more than 50% of inter-indicator data fall within a log range of 0.5. That percentage is highest in the A-M group (82%) which lends support for the theory, referred to above, of a link between toxic response and phylogenetic closeness.

Table 3. Log rank comparison of data for pulp and paper effluents.

Log rank difference	Cumulative percentages of frequency T-A	T-M	A-M
0.5 LOG	60%	70%	82%
1.0 LOG	83%	92%	94%
1.5 LOG	100%	98%	98%
2.0 LOG	100%	100%	100%
Average log difference	0.67	0.51	0.48

-T=trout; A=algae; M=Microtox.
-53, 49 and 49 comparative data pairs for T-A, T-M and A-M respectively.

The average log difference for the T-M group (0.51) is virtually identical to that (0.50) reported by Bulich (1982), who calculated it from 235 comparative fish-Microtox data for assorted industrial effluents. Note too that, in his study as in ours, 98% of the fish-Microtox data fell within a log range of 1.5. Moreover, taking as a basis the hypothesis put forward by Kenaga (1978) that only a log range of 1 translates into significant variations in the toxic response of a single species to different substances, it appears that 83%, 92% and 94% of the data for the T--A, T-M and A-M groups reseeble each other. That is reason to believe that, for screening purposes, Microtox and Algal assays would definitely be helpful when assessing the toxicity of pulp and paper effluents, since they often produce a toxic response similar in magnitude to that of the trout bioassay.

Relative sensitivity of bioindicators

The sensitivity of trout, algal and Microtox bioassays was determined by combining the toxicity data of table 4 in three different ways. As the table shows, algae have the highest sensitivity to pulp and paper effluents and toxic response increases as follows: trout, Microtox and algae. Generally speaking, the difference in sensitivity is least marked between algae and Microtox bacteria which, as we said earlier, may be attributable to their phylogenetic closeness. They also represent the same ecotoxic level (sublethality), which gives them even greater affinity; the fish assay, by contrast, measures lethality. The overall toxic response of each of the three indicators confirms this clear difference between the lethality stage (trout: a total of 400 units) and the sublethality stage (Microtox and algae: totals of 1,600 and 2,900 toxic units).

Table 4. Comparative sensitivities of the three indicators in relation to pulp and paper effluents.

A. Comparative sensitivities of the three indicators

Order of sensitivity	Number of effluents (percentages) Trout(T) assay	Algae(A) assay	Microtox(M) assay
Greatest	1(2%)	27(66%)	13(32%)
Average	7(17%)	12(29%)	22(54%)
Least	33(81%)	2(5%)	6(14%)
Total	41(100%)	41(100%)	41(100%)

B. Range of toxicity for each indicator

Indicator	No. of effluents	EC50 or LC50 range (toxic units)[a]	Factor[b]
Trout	55	1.7 to >100 ((1 to 59)	-
Microtox	51	0.4 to >100 ((1 to 250)	4.25 x
Algae	55	0.1 to >100 ((1 to 1000)	4.0x(17x)[c]

a. Toxic units *= 100% divided by EC or LC50 v/v%
b. Difference factors in sensitivity between two adjacent indicators on the basis of the maximum responses.
c. Cumulative difference in sensitivity between algal and trout assays on the basis of the maximum toxic responses.

C. Overall toxic response by each indicator group to exposure to 47 effluents

Indicator	Toxic units	Factor*
Trout	400	-
Microtox	1,600	4.0x
Algae	2,900	1.8 (7.3x)**

* Multiple of increase in overall toxicity between adjacent indicators.
** Cumulative increase in overall toxicity between algal and trout bioassays.

Evaluation of pulp and paper effluent toxicity as a function of industrial process

On the basis of our findings for inter-indicator toxicity, we proceeded to examine the harmfulness of effluents in terms of the industrial process used in pulp and paper making. The results appear in ascending order of toxicity in table 5 and, to make them easier to visualize, they have also been transposed to figure 2. The reader will note that the toxic profile of each process is relative, since a number of factors (differences in the number of data compared, toxic variability of effluent, degree of effluent treatment, dilution caused by the addition of non-toxic water) can influence the final toxic response in bioassays. The point at issue here, therefore, was not to determine the extent of toxic stress associated with each particular process, but rather to demonstrate the ability of bioassays to show clear differences in effect. Process 1 would appear to be the least toxic to all three indicators, and process 6 most toxic. Between these two extremes, algae and Microtox vary in their toxic responses. Of the three indicators, algae appeared most sensitive, except in the case of process 3, where the Microtox reaction produced the strongest toxic response. In short, although the difference in effluent toxicity as a function of industrial process is still not properly understood, the bioassays that were used have shown themselves to be sensitive instruments for highlighting that difference.

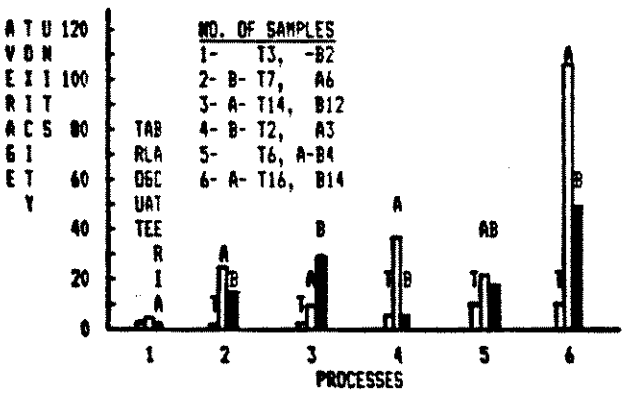
Table 5. Pulp and paper effluent toxicity in relation to mill process.

Processes*	No. of effluents	Toxic units/effluent			Total of toxic units / effluent
		T**	A**	M**	
1	3	1.3			9
	2		5.7	2.0	
2	7	1.7		13	36.7
	6		22		
3	14	2.1	9.3		40.4
	12			29	
4	3		35		43.3
	2	5.4		2.9	
5	6	9.7			53.7
	4		22	22	
6	16	11	110		169
	14			48	
	Total	31.2	204	116.9	

* Processes: 1. Fine paper (non-integrated, no pulp making).
2. Semi-chemical, and/or chemi-thermomechanical pulping.
3. Kraft pulping (integrated mills).
4. Fibre recycling and de-inking.
5. Groundwood pulping and/or thermomechanical pulping and/or refiner mechanical pulping.
6. Low yield sulphite pulping with or without mechanical pulping.

** T=trout; A=Algae; M=Microtox.

Figure 2.Pulp and paper effluent toxicity in relation to industrial mill process without considering the degree of effluent treatment.



Evaluation of the toxicity of pulp and paper effluents in terms of treatment method.

We also attempted to evaluate effluent toxicity according to its method of treatment. Table 6, shows the outcome of that exercise. The main conclusion to be drawn is clear: the more extensive the treatment, the greater the reduction in toxicity for the three indicators. That conclusion also supports the opinion that biological treatment is an effective way of decreasing the toxic load of pulp and paper effluent (Rainville et al, 1975; Beak Consultants, 1978; Hutchins, 1979; Besner and Van Coillie, 1981).

Table 6. Pulp and paper effluent toxicity in relation to waste treatment application.

Treatment*	No. of effluents	Toxic unit/effluents T**	A**	M**	Total toxic units per effluents
0	21	9.9	89		138.9
	19			40	
1	21	3.5			
	19		20		51.5
	16			28	
2	6	1.0		1.1	4.5
	5		2.4		
	Total	14.4	111.4	69.1	

* 0 = no treatment; 1 = primary treatment; 2 = secondary treatment.
** T = trout; A = algae; M = Microtox.

We also found in course of the evaluation that differences in toxicity showed up more clearly with sublethal indicators (algae and Microtox) than with the trout bioassay.

Of the effluents studied, two had a standard treatment system regarded as the best practicable technology (Environment Canada, 1984b). Neither the trout nor the Microtox assay detected any toxicity in the first such effluent, and the algal assay showed only very slight toxicity (EC50 of 56% and 76% for two series of effluent samples). Even better, none of the three indicators detected any toxicity in the second of the two effluents.

Final effluents that received the same treatment and originated in the same industrial process had a toxicity which changed relatively little when sampled on different dates (table 7). That is indicative of a steady level of toxicity on such effluents and is in line with the findings of Loch and McLeod (1974).

Table 7. Toxicity variability for two final effluents sampled at different times (between May 1981 and July 1982).

Effluent code	Process*	Treatment*	LC50 or EC50** at different sampling times Trout	Algae	Microtox
A	2	2	>100, 90, 88, 88,	15, 6, 69, 84, 62,	>100, >100, >100
B	2	1	28, 49, 45	5, 2, 2	6, 6, 2

* See process codes given in table 5.
* See treatment codes given in table 6.
* Effective concentrations are expressed in v/v%.
* Unavailable.

In short, the bioassays and indicators used showed themselves to be sensitive and reliable instruments for measuring the (sub) lethal toxicity of liquid waste from the pulp and paper industry. They may therefore be recommended for future evaluations of this kind, since the use of such complementary bioassays is conducive to a better appreciation of the ecotoxicity of waste at different trophic levels. Using bioassays in combination is increasingly recommended as part of an integrated ecotoxicological approach in an attempt to better understand the potential hazards that complex industrial wastes represent for receiving waters (Cairns, 1983; Environment Canada 1984a; Millemann et al, 1984; Blaise et al, 1985).

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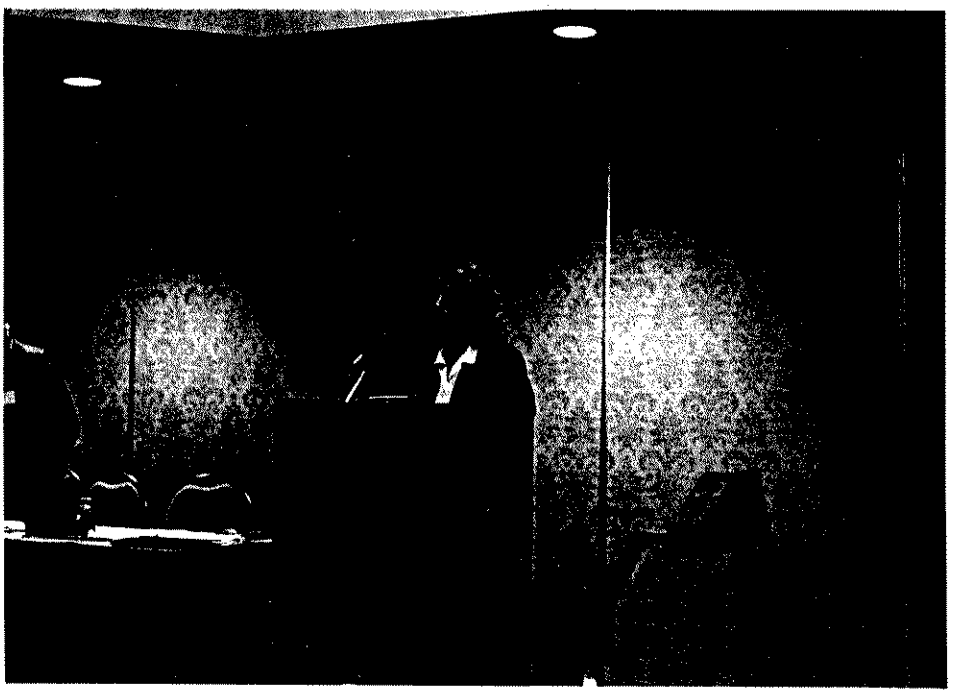
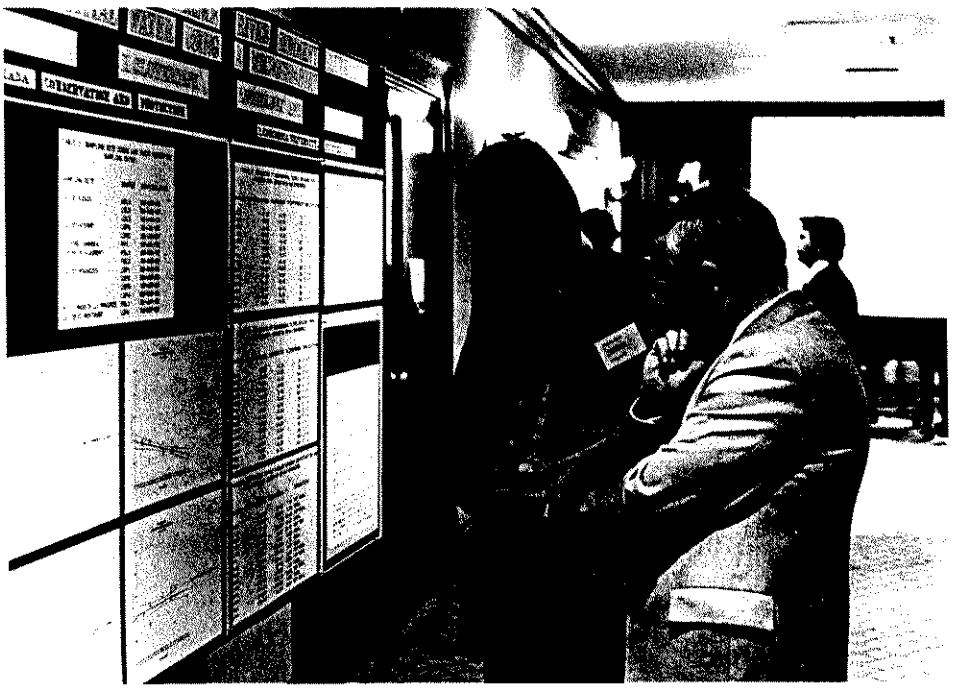
PICTURES/PHOTOGRAPHIES

Left: Dr. J.H. McCormick (EPA), chairperson of second session "Ecotoxicology of acid precipitations." Right: K. Wilkinson (INRS), speaker on aluminum toxicity.



Dr. F. Denizeau (Université du Québec), speaker on cell cultures.

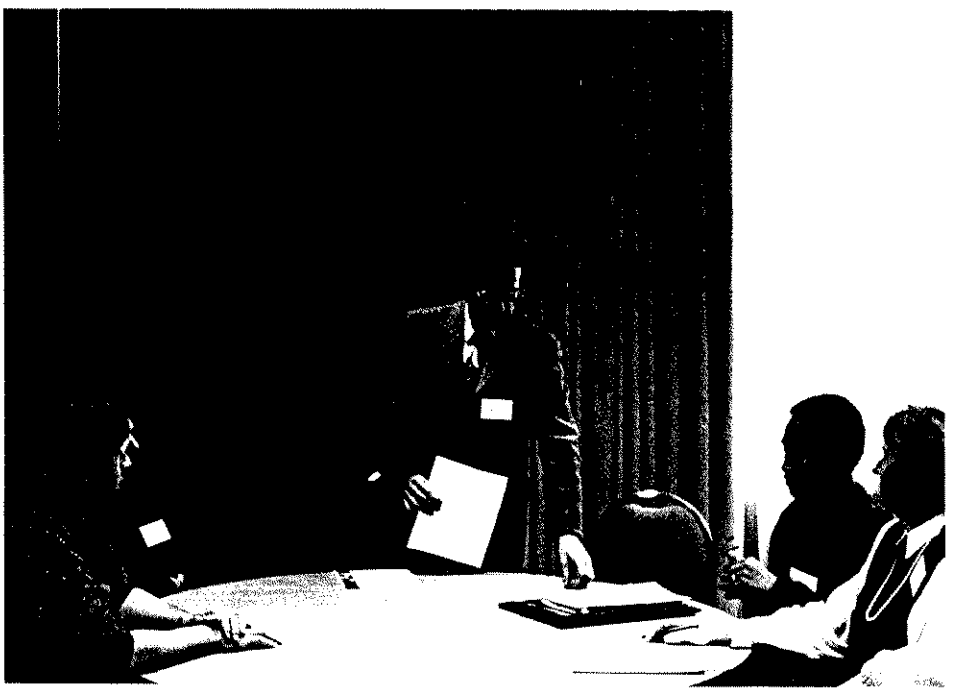


Left: H. Sloterdijk (Environment Canada). Right: Dr. J.M. Sala (ALCAN)

Left: S. Thorn (Environment Canada). Right: D. St. Laurent (Université du Québec

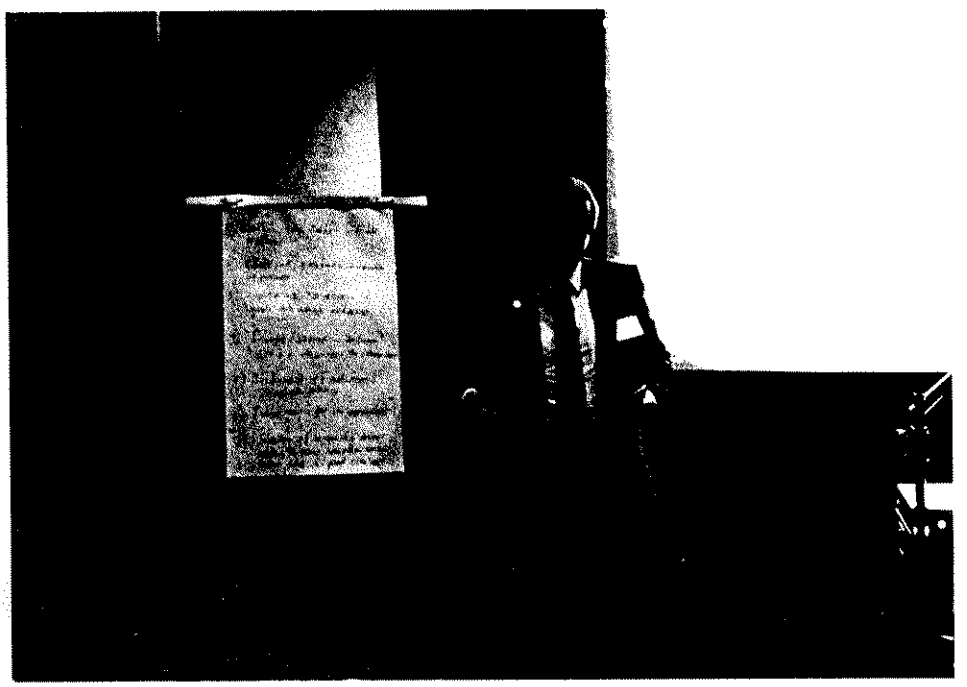
SPECIAL SESSIONS

N. Bermingham (Environment Canada), chairperson of the second special session.



C. Blaise (Environment Canada), moderator of a group.

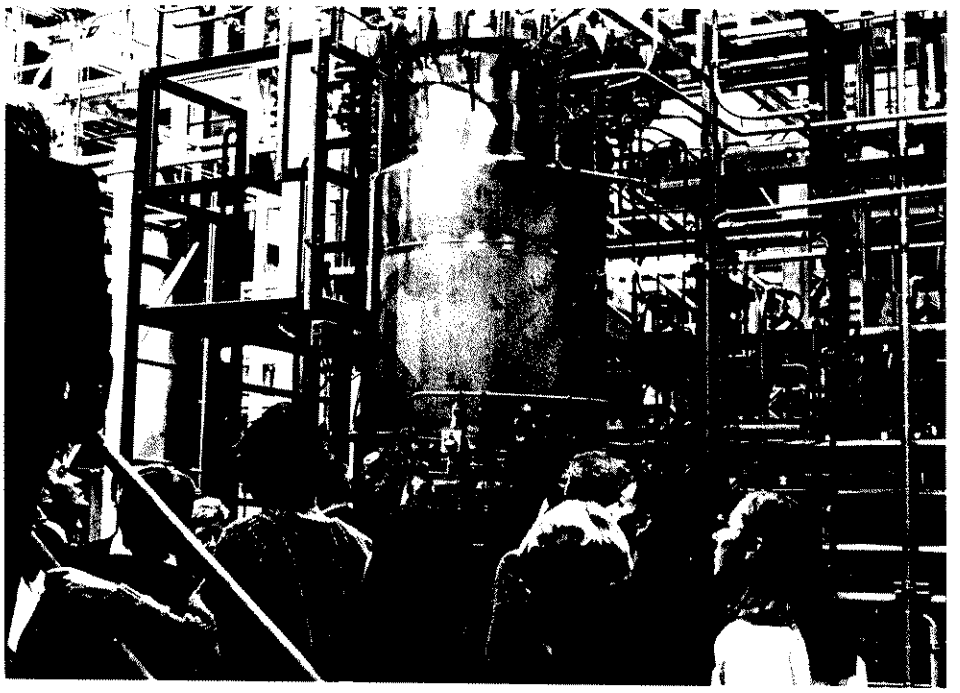
Dr. P. Hodson (Fisheries and Oceans), moderator of a group.



R. Parker (Environment Canada), moderator of a group.

Guided Tour of Biotechnology Research Institute

Dr. B. Coupal, general director of BRI and a group of guests.



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COMEDIC BANQUET "FESTIN DES GOUVERNEURS"

Dr. and Mrs. R. Van Coillie, banquet governor, welcomes guests.

Bilingual comedians

COMEDIC BANQUET (continued)

Dr. A. Niimi and Dr. R. Gray enjoying the evening.

Dr. G. Westlake and others discusses the success of the Workshop.

LIST OF AUTHORS/LISTE DES AUTEURS