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FRESHWATER LEECHES (HIRUDINEA) AS A SCREENING TOOL FOR DETECTING ORGANIC CONTAMINANTS IN THE ENVIRONMENT

by

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MANAGEMENT PERSPECTIVE

This paper represents a continuation of research aimed at evaluating leeches as screening organisms for organic contaminants in the environment. Leeches were shown to have higher bioconcentration factors for a variety of organic compounds than many other aquatic organisms. They may, therefore, be ideal "early warning indicators" for demonstrating the presence of contaminants before they are detectable in the water or other components of the environment. Leeches were also shown to eliminate chlorophenols (and there is evidence to suggest DDT and Mirex as well) more slowly than other aquatic organisms; therefore, they may provide evidence of pollution events long after the fact. This is particularly important where contamination is intermittent, either due to spills or sporadic discharges. This research supports one of the goals of the Contaminants Project, Rivers Research Branch, which is to determine the environmental occurrence of contaminants in rivers. It also supports research needs identified by the Water Quality Branch, which are to identify and develop suitable biomonitoring techniques for incorporation into existing national water quality monitoring networks.

PERSPECTIVES DE GESTION

La présente communication présente la suite des recherches visant à évaluer les sangsues comme organismes de dépistage pour les contaminants organiques dans l'environnement. Les résultats ont montré que les facteurs de bioconcentration de divers composés organiques chez les sangsues étaient plus élevés que chez beaucoup d'autres organismes aquatiques. Elles pourraient donc constituer des "indicateurs précoces" idéaux de la présence de contaminants, avant que ceux-ci ne soient décelables dans l'eau ou dans d'autres constituants de l'environnement. On a aussi constaté que les sangsues éliminaient les chlorophénols (ainsi que le DDT et le Mirex d'après certains résultats) plus lentement que d'autres organismes aquatiques; elles pourraient donc révéler des cas de pollution longtemps après le fait accompli. Cela est particulièrement important lorsque la contamination est intermittente, étant causée soit par des déversements accidentels, soit par des évacuations sporadiques. Ces recherches vont dans le sens des objectifs du Projet sur les contaminants, de la Direction de la recherche sur les cours d'eau, qui visent à évaluer la présence de contaminants dans les rivières. Elles se situent également dans le cadre des besoins en recherche définis par la Direction de la qualité des eaux, à savoir la recherche et la mise au point de méthodes appropriées de bio-surveillance pour leur incorporation éventuelle dans les réseaux nationaux existants de surveillance de la qualité de l'eau.

ABSTRACT

In earlier work, we found that leeches from an industrially polluted creek bioaccumulated chlorophenols to much higher concentrations than other resident benthic invertebrates and fish. We suggested that leeches may have significant potential as biomonitors for these and other organic contaminants in the environment. In this study, we compared the bioaccumulation and depuration of 16 organic compounds, including eight chlorophenols (CPs), lindane, DDT and four derivatives, benzothiazole (BT) and 2-(Methylthio)benzothiazole (MMBT) for three species of leeches. Dina dubia had the highest bioaccumulation capacity for most contaminants, but residues persisted longest in Erpobdella punctata. Helobdella stagnalis appeared capable of degrading some compounds. Half lives of CPs, DDT and DDT derivatives were generally longer than one month. In contrast, half lives were only 1 day for lindane, 1-2.5 days for MMBT and 7 days for BT despite very high initial tissue concentrations of the latter two compounds. Bioconcentration factors for contaminants in leeches were higher than those reported for other aquatic organisms. Half lives for lindane, DDT and DDT derivatives were consistent with the literature for other organisms, but half lives for CPs were much longer. The results suggest that leeches would be excellent biomonitors of both continuous and intermittent contamination of a waterway with CPs and DDT, as they retain these compounds for long periods after exposure. Their usefulness as a screening tool for lindane and benzothiazoles would be limited to chronically contaminated environments.

RÉSUMÉ

Lors de travaux antérieurs, nous avons observé que les sangsues d'un ruisseau pollué par l'industrie bioaccumulaient les chlorophénols jusqu'à des concentrations beaucoup plus élevées que d'autres invertébrés benthiques et poissons de l'endroit. Ces sangsues nous apparaissaient comme des bio-indicateurs offrant des possibilités très intéressantes pour ces produits et d'autres contaminants organiques dans l'environnement. Dans cette étude, nous avons comparé la bioaccumulation et l'élimination de 16 composés organiques, comprenant huit chlorophénols (CP), le lindane, le DDT et quatre dérivés, le benzothiazole (BT) et le 2-(méthylthio) benzothiazole (MMBT), chez trois espèces de sangsues. Dina dubia présentait la capacité de bioaccumulation la plus élevée pour la plupart des contaminants, mais les résidus persistaient plus longtemps chez Erpobdella punctata. Helobdella stagnalis semblait capable de dégrader certains composés. Les demi-vies des CP, du DDT et des dérivés du DDT, étaient généralement supérieures à un mois. Par contre, les demi-vies n'étaient que de 1 jour pour le lindane, 1-2.5 jours pour le MMBT et 7 jours pour le BT, en dépit des concentrations initiales très élevées des deux derniers composés dans les tissus. Les facteurs de bioconcentration des contaminants chez les sangsues étaient plus élevés que ceux signalés dans le cas d'autres organismes aquatiques. Les demi-vies pour le lindane, le DDT et les dérivés du DDT concordaient avec les valeurs trouvées dans la documentation pour d'autres organismes, mais les demi-vies des CP étaient beaucoup plus longues. Les résultats montrent que les sangsues seraient d'excellents bio-indicateurs de la contamination aussi bien continue qu'intermittente d'un cours d'eau par

les CP et le DDT, car elles retiennent ces composés pendant de longues périodes après l'exposition. Leur efficacité comme outil de dépistage pour le lindane et les benzothiazoles se limiterait à des milieux contaminés de façon chronique.

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1. INTRODUCTION

Aquatic organisms which bioaccumulate high concentrations of certain contaminants in their tissues may be used as biomonitors to determine the occurrence and levels of these contaminants in aquatic environments. This type of biomonitoring, labelled "bioanalysis" by Butler et al. (1985), offers several advantages over traditional water and sediment monitoring surveys. Because organisms concentrate contaminants from surrounding media, they can serve as early warning indicators - demonstrating the presence of contaminants long before they reach detectable levels in the water. If contaminant levels are measureable in biota, it follows that spatial and temporal trends may then be quantified. Biomonitoring also provides a direct measure of the bioavailability of contaminants which cannot be determined by water or sediment analyses alone, but which is an important aspect of risk assessment. Finally, bioanalysis may actually be more cost-effective. Organisms integrate ambient pollution conditions over

time, and are therefore more representative of environmental quality than are instantaneous samples of water or sediment. Sampling frequency can therefore be reduced without sacrificing accuracy, resulting in considerable savings in terms of time, effort and cost.

Organisms which eliminate contaminants slowly offer further benefits as biomonitors. Theoretically, the slower the elimination rate of the organism, the less frequently it must be sampled. In environments where contamination is intermittent, either due to spills or sporadic discharges, organisms with slow elimination rates will provide evidence of the pollution event long after the fact.

In earlier work (Metcalfe et al., 1984), we found that leeches from an industrially polluted creek bioaccumulated chlorophenol contaminants to much higher concentrations than other resident macroinvertebrates and fish. We suggested that leeches may have significant potential as biomonitors for these and other organic contaminants in the environment. In the present study, we report the bioaccumulation capacities and elimination rates for a variety of chlorophenols, neutral organochlorines and non-chlorinated neutral compounds for three leech species. Our results are compared with the literature on the bioaccumulation and elimination of organic contaminants by other aquatic organisms.

2. MATERIALS AND METHODS

2.1 Location of Study and Contaminants Investigated.

Canagagigue Creek is a minor tributary of the Grand River, which in turn empties into Lake Erie (Figure 1). Four major types of synthetic organic contaminants occur in the creek (Carey et al., 1983), all of which originate in the Town of Elmira, Ontario. Benzothiazoles, which are manufactured in Elmira and are used in the vulcanization of rubber goods and in automobile antifreeze, and lindane, which is used in the formulation of pesticides, both enter the creek via the municipal sewage treatment plant. Chlorophenols and DDT and its derivatives enter the creek as groundwater seepage from a disused chemical waste disposal area. Some of these buried wastes are related to the manufacture of 2,4-D and 2,4,5-T which were produced in Elmira until 1969. Sixteen individual compounds, which represented a wide range of the two major chemical properties which influence bioaccumulation (Table I), were investigated. The study site, known as CN-3 in earlier reports (e.g. Carey et al., 1983), was located approximately 1.5 km downstream from the town where leeches are abundant due to nutrient enrichment from domestic sewage and where very high concentrations of chlorophenols in resident leeches were previously reported (Metcalfe et al., 1984).

2.2 Experimental Design and Procedure

Contaminated leeches were collected from Canagagigue Creek site CN-3 on October 15, 1984. The stream temperature was 15.5°C. Specimens were harvested by hand from the undersides of rocks in the quiet areas, and with the aid of a surber sampler in the riffles. A sampling effort of eight man-hours yielded 55 Erpobdella punctata, 101 Dina dubia (both F. Erpobdellidae) and approximately 750 Helobdella stagnalis (F. Glossiphoniidae). Leeches were placed in glass jars containing creek water, and transported on ice to the laboratory where they were held overnight at 4°C to clear their gut contents. No mortality occurred. To compare the initial tissue concentrations of contaminants in leeches with exposure levels in their environment, creek water was also sampled. For the analysis of chlorophenols, lindane and DDT and its derivatives, triplicate 1L filtered water samples were collected; contaminant-free potassium hydroxide pellets were added to the samples on site to preserve them at a pH of 11.0 prior to extraction and analysis. For benzothiazole analysis, three 4.28 L filtered water samples were collected and passed through Sep-Paks (C_{18} concentration cartridges, Waters Associates) on site, then held at 4°C until they could be processed. For logistic reasons, water sampling was conducted two weeks later than biota sampling. Although concentrations of contaminants in the water are known to vary seasonally (Carey et al., 1983), changes over the two week period were not expected to be significant. Relative proportions of contaminants remain constant throughout the year.

Depuration experiments were conducted in duplicate for each species. Six 10 L glass aquaria, each covered with a tightly-fitting lid which excluded light, were used. Contaminant-free water was supplied to each aquarium at a flow rate of 500 mL/min, allowing 99% replacement in approximately 1.5 hours. Temperature ranged from 13.0 to 14.0°C for the duration of the experiment, pH was 7.9 and dissolved oxygen concentrations were at saturation levels. Several pieces of clean laboratory glassware were placed in the aquaria to provide attachment sites for the leeches.

The experiment commenced approximately 24 hr after the leeches were collected. D. dubia specimens were weighed individually, divided into two equal groups, and placed in duplicate aquaria. Care was taken to ensure a similar size distribution of animals in each duplicate. Nine very small D. dubia were not used. E. punctata specimens were distributed in the same manner using all 55 individuals. H. stagnalis specimens were equally distributed between their two aquaria in random batches of ten, as there was little size variation among individuals. Twenty leeches from each aquarium were weighed individually for comparison with the other two species. The initial live weights for each species are presented in Table II.

Leeches were allowed to depurate their body burdens of organic contaminants for a period of 27 d in the laboratory. They were not fed. Samples of leeches for contaminant analysis were taken on days 0, 1, 2, 5, 8, 13, 21 and 27, each sample consisting of 6 D. dubia, 3 E. punctata or 40 H. stagnalis from the appropriate duplicate aquarium. These numbers were determined by the minimum amount of material

required for analytical accuracy and precision (ideally .5 g, not less than .15 g). A range of organism sizes, determined visually, was selected on each occasion. Initial (Day 0) samples were taken prior to distributing the test organisms among the aquaria. The organisms in each sample were weighed individually (with the exception of H. stagnalis which were weighed in batches of 10) before being combined, wrapped in pre-cleaned aluminum foil, and stored frozen prior to analysis.

2.3 Analytical Methods

2.3.1 Leeches

2.3.1.1 Sample preparation, extraction, clean-up and fractionation.

Each frozen leech sample was ground with approximately five times its weight of previously fired (to 400°C) anhydrous sodium sulphate to a uniform consistency in a mortar. This mixture was extracted for 2.5 hr with 230 mL of residue-free dichloromethane in a Soxhlet extractor. The extract was then concentrated to 1-2 mL on a rotary evaporator at 25°C. Lipids and other high molecular weight co-extractives were removed by gel permeation chromatography using a 35 x 2.5 cm gravity flow column filled with BioBeads SX-3, with cyclohexane/dichloromethane in a 1:1 ratio as the eluant. Fraction 1 (0-110 mL), which contained the lipids, was discarded. Fraction 2 (110-240 mL), which contained the contaminants of interest, was placed in a 1 L separatory funnel with 30 mL cyclohexane and sequentially

extracted with 40, 30 and 30 mL aliquots of 0.1 M K_2CO_3. The aqueous phase containing the acid fraction was set aside for chlorophenol analysis. The solvent phase containing the neutral fraction was washed with 50 mL organic-free water, dried through anhydrous sodium sulphate, concentrated to 1-2 mL and transferred to a 10 mL Kuderna Danish tube. Two mL of iso-octane were added and the solution concentrated to 1.0 mL at 25°C under a stream of dry nitrogen. The extract was divided into two 0.5 mL portions for analysis of neutral organochlorines and non-chlorinated neutral compounds, respectively.

2.3.1.2 Analysis of chlorophenols. One mL of residue-free acetic anhydride and 10 mL of hexane were added to the acid fraction in a 125 mL Erlenmyer flask with a teflon-lined screw cap. The mixture was shaken for 1 hr, then the hexane layer was transferred to a 10 mL Kuderna Danish tube. Two mL of iso-octane were added and the solution was concentrated to 1.0 mL under dry nitrogen. The final extract was analyzed by electron capture gas chromatography using two 30 M fused silica columns (DB5 and DB17) for quantitation and confirmation. The temperature program was 70 to 270°C at 4°C/min with a 5 min hold at 270°C. The injector was held at 230°C (split 10:1) and the detector at 350°C.

2.3.1.3 Analysis of neutral organochlorines. One of the 0.5 mL portions of the neutral fraction was passed through a mini clean-up column consisting of a Pasteur pipet packed with 2 cm of 40% sulphuric acid on silica gel and 0.5 cm anhydrous sodium sulphate. Three 1 mL

rinses of residue-free hexane were then added to the column, allowing each one to penetrate the bed completely. The eluant was collected in a 10 mL Kuderna Danish tube. Two mL of iso-octane was added and the eluant was concentrated to 0.5 mL under dry nitrogen. The final volume was adjusted to 0.5 mL, then analyzed for lindane and DDT and its derivatives by electron capture gas chromatography. The temperature program was 122-138°C at 1°C/min; 138-250°C at 30°C/min; final hold at 250°C for 2 min. A minor component of total DDT in this study was o,p'-DDT. This compound co-elutes with, and therefore cannot be separated from, p,p'-DDD.

2.3.1.4 Analysis of non-chlorinated neutral compounds. The second 0.5 mL portion of the neutral fraction was analyzed for benzothiazole and its derivative MMBT by flame ionization gas chromatography using a 30 M DB5 column programmed from 90-250°C at 4°C/min. The injector (split 10/1) and detector were held at 250°C.

2.3.2 Water

2.3.2.1 Analysis of chlorophenols and neutral organochlorines. The 1 L water samples were acidified to pH 1-2 with concentrated HCl and extracted sequentially with 40, 30 and 30 mL of residue-free toluene. The toluene extract was treated identically to Fraction 2 of the leech samples from this point onward, except that the neutral fraction was not split into two portions because separate water samples had been collected for the analysis of benzothiazole derivatives.

2.3.2.2 Analysis of non-chlorinated neutral compounds. Sep-Paks containing the residues from the 4.28 L water samples were eluted with 5 mL acetone. One mL of iso-octane and sufficient methanol to produce a single phase was then added. The extract was concentrated to a final volume of 1.0 mL and analyzed by flame ionization gas chromatography as previously described for the leech samples.

3. RESULTS

3.1 Bioaccumulation Capacities of Leeches for Organic Contaminants

Leeches from Canagagigue Creek contained benzothiazoles, chlorophenols, lindane and DDT and its derivatives, although only benzothiazoles and chlorophenols were detected in creek water (Table III). D. dubia appeared to have the greatest bioconcentration capacity among the three species, as it accumulated the highest tissue concentrations for 11 of the 16 compounds. E. punctata accumulated the highest concentration for one compound (2,6-DCP) and was generally intermediate, while concentrations in H. stagnalis were lowest for nine compounds and never highest. Concentrations of 2,4,6-TCP and 2,3,6-TCP accumulated by D. dubia and E. punctata were similar, while concentrations of 2,3,4,6-TECP and PCP were approximately the same in all three species. H. stagnalis differed from the other two species in that it did not accumulate detectable residues of benzothiazole, 2,6-DCP or lindane.

In general, contaminants were bioaccumulated in proportion to their relative occurrence in the water; that is, concentrations in both leeches and water were highest for benzothiazoles, intermediate for chlorophenols and lowest for lindane and DDT and its derivatives. Among the eight chlorophenols investigated, however, this general trend was not consistent (Figure 2). The dominant chlorophenol isomer in the water was 2,4-DCP, accounting for 38% of the total chlorophenols present. It was also a dominant isomer in leeches, representing 31-46% of the total chlorophenols accumulated. However, 2,4,5-TCP accounted for higher proportions in leeches (28-44%) than in water (15%), while proportionately less 3,4-DCP was bioaccumulated (3-5%) than would be expected from its relative occurrence in water (16%). Less 2,6-DCP was also accumulated by D. dubia (2%) and H. stagnalis (0) but not by E. punctata (14%) than would be anticipated (12% in water). Pentachlorophenol, 2,3,4,6-TECP and 2,3,6-TCP were proportionately low in both leeches and water.

Bioconcentration factors (BCFs), which refer to the ratio between tissue and water concentrations, are presented for benzothiazoles and chlorophenols in Table IV. For MMBT and BT, BCFs were low, ranging from 100-400X. For chlorophenols, BCFs were higher and quite variable, ranging from 600-16,700X depending upon the species and isomer. No clear pattern relating the degree of chlorination and chemical properties of the various isomers (Table I) to their bioaccumulation potentials in leeches was evident. BCFs could not be calculated for lindane or DDT and its derivatives because these compounds were not detectable in the water.

3.2 Elimination Rates of Organic Contaminants by Leeches

Elimination rates of organic contaminants by leeches were determined using data points from each aquarium as duplicates. A single compartment model adequately described the elimination process; that is, plots of the natural logarithm (ln) of tissue concentration vs. time in days gave essentially straight lines (Figures 3, 4 and 5). Where the slope of a line was significantly different from zero at the $p \leq .05$ level, rate constants were determined and half lives were calculated (Table V).

Leeches depurated chlorophenols and DDT derivatives slowly from their systems. Half lives for chlorophenols in leech tissues were in most cases longer than one month. The elimination process for two representative isomers is shown in Figure 3. Chlorophenols persisted longest in E. punctata, where no significant depuration was observed for any compound except PCP during the 27 d experiment. All three leech species eliminated p,p'-DDD and o,p'-DDD more rapidly than p,p'-DDE and o,p'-DDE, as illustrated for the p,p' - derivatives in Figure 4. While no significant elimination was observed for p,p'-DDT in erpobdellid leeches, this compound had a half life of only 5.5 d in H. stagnalis. As was the case for chlorophenols, DDT derivatives appeared to persist longest in E. punctata.

Lindane and benzothiazole derivatives were quickly eliminated by leeches (Table V and Figure 5, respectively). The half life for lindane was a day or less; for MMBT it was 1-2.5 d and for BT, about

one week. Lindane concentrations in leech tissues were very low to begin with; however, MMBT and BT concentrations were initially very high. In fact, initial tissue concentrations of MMBT were from one to several orders of magnitude higher than any other compound, yet this contaminant was never detected in leeches after their eighth day in clean water.

3.3 Mortality and Weight Loss

The incidence of mortality during the 27 d depuration period was low (1-3%). Only one mortality occurred in each of DD1, DD2 and EP1, five in HS1, 3 in HS2 and none in EP2. Four specimens could not be accounted for in DD1, 6 in DD2, 1 in EP1 and 2 in EP2. H. stagnalis specimens remaining at the end of the experiment were not tallied.

D. dubia and E. punctata appeared to lose weight during the experiment; Table VI shows that the mean weights of sampled specimens tended to decrease over time. This evidence of weight loss is inconclusive, as we could have unintentionally sampled larger specimens earlier in the experiment. Unfortunately, we have no way of measuring weight changes in specific individuals. However, by comparing the mean weight of all specimens initially with the mean weight of all specimens at the time of sampling, we estimated the loss of biomass over the course of the experiment as about 10% for both species. This translates into a loss of approximately 1% of the original body weight/day for each individual. H. stagnalis remained in excellent condition throughout the experiment, with no indication

that the average weights of sampled specimens decreased over time (Table VI).

4. DISCUSSION

Leeches from Canagagigue Creek accumulated 16 organic contaminants in their tissues, even though only ten of these compounds were detectable in creek water. In a similar study on the occurrence of organic contaminants in rivers in southwestern New Brunswick (Metcalfe et al., 1985), 12 chlorophenol isomers were detected in resident leeches while only three were detected in river water. As the primary route of uptake for compounds as soluble as benzothiazoles, chlorophenols, lindane and even DDE (Bjerk and Brevik, 1980) would be directly from the water, leeches are clearly capable of concentrating minute amounts of these contaminants into measurable residues in their tissues.

Interspecific differences in the bioaccumulation of contaminants were apparent. D. dubia was previously shown to accumulate higher concentrations of seven chlorophenols than any other leech species in Canagagigue Creek (Metcalfe et al., 1984), and this trend apparently applies to other organic compounds as well. H. stagnalis accumulated lower concentrations of contaminants than the two erpobdellids. According to unpublished data reported by Sawyer (1974), H. stagnalis also accumulated lower concentrations of Mirex (2 ppm) than Erpobdella sp. (26 ppm) after 5 d exposure at 1.0 ppm Mirex.

Leeches generally accumulated contaminants in proportion to their relative occurrence in the water, but there were exceptions. Concentrations of BT and 2,4-DCP in erpobdellids were similar despite much higher concentrations of BT in the water. Also DDT and its derivatives were never detected in creek water, yet concentrations in leeches were higher than some of the chlorophenols. These findings can be attributed to differences in the chemical properties of the various compounds (Table I). For example, the lower BCFs observed for benzothiazoles than chlorophenols were expected, because benzothiazoles are more water-soluble and have lower K_{ow} values.

Bioconcentration factors for chlorophenols in leeches varied among the different isomers in apparent contradiction of their chemical properties (Table I). For most organochlorine compounds, increasing chlorination of the molecule results in a corresponding increase in bioconcentration. This is not necessarily the case for acidic compounds like chlorophenols, for which the percentage of the compound present in the un-ionized, biologically available, form is pH-dependent. For example, as the pKa values (at 25°) for chlorophenols range from 4.8 for PCP to 8.59 for 3,4-DCP (Jones, 1981), PCP would be more dissociated and less biologically available at neutral pH than 3,4-DCP. In support of this, Call et al. (1980) determined that 2,4,5-TCP had a greater bioconcentration potential in fish than PCP and suggested that this is because PCP has a lower pKa than TCP. Kaila and Saarikoski (1977) found 2,3,6-TCP to be more toxic than PCP to the crayfish, Astacus fluviatilis, in an aqueous medium, but less so when the compounds were injected directly into the tissues. They

believe that waterborne 2,3,6-TCP penetrates crayfish tissues more easily than PCP because of its higher pKa. Ernst and Weber (1978) reported that BCFs for chlorophenols in the polychaete, L. conchilega, increased towards the lower chlorinated isomers. Values calculated from their data are as follows: PCP-3000X, 2,3,4,6/2,3,5,6-TECP-11,000X; 2,3,4,5-TECP-17,500X; 2,4,6-TCP-20,000X; 2,4,5-TCP-24,000X. Our results for leeches also demonstrated higher BCFs for 2,4,5-TCP and 2,3,6-TCP than for 2,3,4,6-TECP and PCP, but 2,4,6-TCP deviated from this pattern. Jacob (1986) exposed the leech Haemopsis marmorata to a mixture of five chlorophenols at 10 ppb each and found, as we did, lower BCFs for 2,4,6-TCP (350X) than for 2,4,5-TCP (2280X). We observed similar BCFs for 2,3,4,6-TECP and PCP in leeches. Paasivirta et al. (1985) reported slightly higher BCFs for the 2,3,4,6-TECP than PCP in two species of freshwater fish, while Folke et al. (1983) observed the opposite in marine mussels. In both cases, the differences were small. Paasivirta et al. (1985) did not find any evidence of food chain enrichment of chlorophenols in a contaminated lake chain in Finland. However, they did note that habitat and feeding habits influenced the pattern of uptake. For example, filter-feeders accumulated the more water soluble isomers such as 2,4-DCP and 3,4,-DCP, while sediment-associated organisms accumulated the less water soluble, material-bound compounds. The pattern of chlorophenol bioconcentration we observed for leeches in Canagagigue Creek may be influenced by many factors. These include the chemical properties of the isomers (where the effects of increasing chlorination, increasing lipid solubilities and decreasing water

solubilities are offset by decreasing dissociation constants), the degree to which leeches are in contact with dissolved vs. sediment-bound compounds, and the unique metabolic capabilities of the organisms themselves.

There have been few studies which directly compare contaminant residues in leeches with those in other aquatic organisms. The only information available concerns the persistent organochlorine pesticides, DDT and Mirex. Webster (1967) reported the presence of DDT in E. punctata tissues three months after the aerial spraying of a marsh, while no residues were found in amphipods or copepods. Meeks (1968) experimentally sprayed radiolabelled DDT over a marsh and followed its uptake and depuration by all components of the food web over a period of 5 months. DDT residues in the leech, E. punctata, were higher (up to 12.6 ppm) than those in molluscs, crustaceans and insects. In fact, the only biological samples having higher concentrations of DDT than leeches were the fat of a carnivorous water snake and one species of bird. In laboratory experiments, de la Cruz and Naqvi (1973) determined that freshwater shrimp and crayfish accumulated twice as much Mirex as three species of leeches after 48 hr exposure at 2.0 ppm Mirex. In contrast, the same authors (Naqvi and de la Cruz, 1972) found that levels of Mirex in the leech, E. punctata, from a creek recently sprayed with the chemical, were higher than in 14 other invertebrates (including shrimp and crayfish) and a frog. Only water boatmen accumulated slightly higher concentrations than leeches.

It is difficult to compare the BCFs we observed for leeches with those reported for other organisms, mainly because few studies have been conducted under environmentally realistic exposure regimes such as ours. It should also be noted that BCFs generated from field studies, in which integrated values (concentrations in organisms) are compared with instantaneous values of unknown variability (concentrations in water), will be less precise than those determined under controlled laboratory conditions. Table VII presents the relevant comparisons. In all but two instances, BCFs for benzothiazoles and chlorophenols in leeches were much higher than those in other aquatic organisms. Virtanen and Hattula (1982) reported greater BCFs for 2,4,6-TCP in guppies than we observed in leeches. However, the concentrations of 2,4,6-TCP in their microcosm decreased from 0.50 to 0.05 µg/L after 36 d due to an undetermined factor despite continuous dosing at the higher concentration. The exact exposure level under these circumstances is difficult to estimate. The marine polychaete, Lanice conchilega, appears to have a BCF for chlorophenols as high as that of leeches. According to Ernst and Weber (1978) these BCFs greatly exceed those reported in the literature for fish and mussels. Metcalfe et al. (1984) noted that among 15 taxa of stream invertebrates collected from Canagagigue Creek, only oligochaetes accumulated chlorophenol residues comparable in magnitude to those in leeches. This information suggests that annelids in general may have unusually high bioconcentration capacities for chlorophenols.

We were unable to calculate BCFs for lindane in leeches because this pesticide was not detectable in creek water. However, D. dubia and

E. punctata accumulated residues of lindane (3-11 ng/g) which are similar to those reported by Schimmel et al. (1977) in pink shrimp, Penaeus duorarum (10 ng/g) exposed to 0.13 µg/L lindane in sea water. As their exposure levels were at least two orders of magnitude higher than ours, it appears that erpobdellid leeches have a higher bioconcentration potential for lindane than shrimp.

Leeches depurated DDT and its derivatives rather slowly from their systems, while lindane was eliminated very rapidly. This is consistent with the literature on the elimination rates of these compounds by other aquatic organisms (Table VIII). There is some direct evidence that leeches may eliminate DDT more slowly than other aquatic invertebrates. In a field survey, Meeks (1968) found that DDT residues were more persistent in the leech, E. punctata, than in molluscs, crustaceans or insects, exceeding 1.5 ppm 12 months after aerial spraying vs. 0.5 ppm or less in other invertebrates. H. stagnalis eliminated DDD, DDE and particularly DDT more rapidly than the two erpobdellids. This observation is not without precedent. According to an unpublished study described by Sawyer (1974), H. stagnalis from an area heavily sprayed with DDT contained significantly greater amounts of DDD and DDE than DDT, therefore appearing to dechlorinate DDT. Another unpublished study from the same source compared the uptake of Mirex by H. stagnalis and E. punctata after exposure to 1 ppm Mirex in water. H. stagnalis accumulated 5 ppm after 24 h, then tissue concentrations declined to 1 ppm after 5 d. In contrast, uptake by E. punctata was directly

proportional to exposure time, increasing from 2 after 24 h to 26 ppm after 5 d. These results suggest that H. stagnalis is capable of metabolizing Mirex while E. punctata is not. The ability of H. stagnalis to degrade organic contaminants may be a general feature of the species or Family, and could explain why certain compounds in the present study (i.e BT, 2,6-DCP, lindane) were not detected in H. stagnalis.

Chlorophenols are eliminated very rapidly by most aquatic organisms (Table IX). The long half lives we observed for these compounds in leeches are in striking contrast to those reported for fish and mussels. Elimination rates for chlorophenols did not differ markedly among leech species, although most compounds appeared to persist the longest in E. punctata. As the data for this species was the most variable, probably due to smaller sample sizes and a greater size range of specimens, it is possible that significant depuration did occur for some compounds but we were unable to detect it. There is no information available on the depuration of benzothiazoles by aquatic organisms.

D. Dubia and E. punctata lost weight during the depuration study at an estimated 1% body weight/day. In contrast, H. stagnalis did not appear to suffer any weight loss. All three species feed primarily on chironomids and oligochaetes (Barton and Metcalfe, 1986; Davies et al., 1979), although their feeding mechanisms differ. Erpobdellids, which possess a simple gut, swallow their prey whole. The more primitive glossiphonids, some of which are sanguivorous, suck the body

fluids of their prey and store them in a diverticulated crop prior to digestion. We speculate that the diverticulated gut of H. stagnalis allows it to consume larger meals and store food for longer periods of time, thus remaining in better condition than D. dubia and E. punctata when access to food is denied. Weight loss is a confounding factor in our experiment which may affect the depuration rates of compounds that are slowly eliminated by organisms which are concurrently losing weight, i.e. the depuration rates of CPs, DDT, DDD and DDE by D. dubia and E. punctata. Pruitt et al. (1977) felt that the elimination of PCP by bluegills was enhanced by the effects of starvation, due to the rapid metabolism of PCP-containing tissue. Call et al. (1980) confronted the opposite problem during a long-term experiment on the uptake and loss of three phenolic compounds by fathead minnows. Fish were fed during the experiment and gained both weight and lipid content. The elimination rates observed were believed to be slower than if the condition of the fish had remained constant, especially for compounds stored in the fat. If these theories apply to our study, we may have overestimated the depuration rates of CPs, DDT, DDD and DDE in erpobdellid leeches. Our own feeling is that the opposite could also be true. For a contaminant which is normally eliminated slowly, an accompanying weight loss would tend to concentrate the remaining residues provided that the contaminant is either transferred to other tissues, or is not stored in the tissues being metabolized.

5. CONCLUSIONS

Freshwater leeches have been shown to bioaccumulate organic contaminants to levels exceeding those reported for other aquatic organisms. For erpobdellid leeches particularly, these levels may be one to two orders of magnitude greater. Because of their high bioconcentration capacities, and because they frequently accumulate contaminants which are undetectable in the water, leeches provide an early warning system for identifying the presence of contaminants in aquatic environments.

In general, leeches accumulated contaminants in proportion to their relative occurrence in the water, and were therefore good indicators of relative water quality with respect to the tested toxic chemicals. Deviations from this pattern were observed among the chlorophenol isomers, and we attribute this in part to the unusual chemical properties of these compounds.

Leeches depurated DDT and its derivatives rather slowly from their systems, while lindane was eliminated very rapidly. This is consistent with the clearance rates of these compounds reported for other aquatic organisms. Benzothiazoles, particularly MMBT, were also eliminated rapidly despite very high initial concentrations of these compounds in leech tissues.

Chlorophenols are eliminated very rapidly by most aquatic organisms; biological half lives are generally of the order of a few hours to a few days. In contrast, half lives for these compounds in leeches were in most cases longer than one month. Because of their apparently

unique ability to retain chlorophenols for long periods after exposure, leeches are the best available screening tool for these contaminants. They would be particularly useful in environments where contamination is intermittent, either due to spills or sporadic discharges, where residues in leeches would provide evidence of the pollution event long after all traces of contamination have disappeared from the water and the tissues of other organisms.

We recommend erpobdellid leeches as potentially the most suitable for biomonitoring purposes, but each of the three species investigated had its own benefits and disadvantages. D. dubia appeared to have the highest bioconcentration capacity for most organic contaminants, but it is an uncommon, geographically restricted species which could not be widely used in comparative studies. E. punctata exhibited the slowest depuration rates, and is common and widespread in North America (Sawyer, 1974), however, its bioconcentration capacity for the compounds investigated was lower than that of D. dubia. H. stagnalis is also commonly available, and specimens did not lose weight during the 27 day depuration period. However, H. stagnalis did not accumulate certain contaminants, and appeared to degrade others. Therefore, the information generated from this species would be incomplete and difficult to interpret.

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Table I. Chemical properties of the contaminants investigated.

Compound	Abbreviation Used in Text	Solubility in Water (ppm)	Octanol/ Water Partition Coefficient (K_{ow})
Benzothiazole derivatives:			
2-(Methylthio)benzothiazole	MMBT	100[1]	1000[2]
Benzothiazole	BT	>100[1]	100[2]
Chlorophenols:			
2,6-dichlorophenol	2,6-DCP		
2,4-dichlorophenol	2,4-DCP	6200[3]	
3,4-dichlorophenol	3,4-DCP		
2,4,6-trichlorophenol	2,4,6-TCP	450[3]	4900[4]
2,3,6-trichlorophenol	2,3,6-TCP		
2,4,5-trichlorophenol	2,4,5-TCP	950[3]	5200[4]
2,3,4,6-tetrachlorophenol	2,3,4,6-TECP	200[3]	
Pentachlorophenol	PCP	10[3]	102,000[4,5]
Lindane	Lindane	7.8[6]	5200[7]
DDT and its derivatives:			
DDT	p,p'-DDT	.0055[6]	1,000,000[5,8]
DDD	p',p'-DDD; o,p'-DDD	.020[6]	1,050,000[5]
DDE	p',p'-DDE; o,p'-DDE	.014[6]	550,000[5,8]

[1]R.F. Platford (pers. comm.); [2]Brownlee et al. (1981); [3]Jones (1981); [4]Leo et al. (1971); [5]Kenaga and Goring (1978); [6]Weil et al. (1974); [7]K.L.E. Kaiser (pers. comm.); [8]Chiou et al. (1977).

Table II. Initial live weights of leeches.

Species	Aquarium	Sample Size	Mean Weight (g)	Range	s.d. as % of Mean
Dina Dubia	DD1	46	0.071	0.017-0.156	41%
	DD2	46	0.072	0.024-0.233	54%
Erpobdella punctata	EP1	28	0.191	0.042-0.517	62%
	EP2	27	0.209	0.050-0.445	56%
Helobdella stagnalis	HS1	20*	0.0068	0.0047-0.0091	19%
	HS2	20*	0.0085	0.0043-0.0132	28%

*Subsample of approximately 375 specimens per aquarium.

Table III. Concentrations of organic contaminants in water and leeches from Canagagigue Creek.

Compound	Analytical Detection Limits		Concentration In Creek Water (µg/L)*		Concentration in Leech Tissues (ng/g live weight)					
	Water (µg/L)	Leeches (ng/g)			D. dubia		E. punctata		H. stagnalis	
					DD1	DD2	EP1	EP2	HS1	HS2
MMBT	0.1	100.0	23.27	(3.59)	10900	7200	5400	4600	2200	2300
BT	0.1	100.0	3.02	(1.17)	1400	600	700	700	ND	ND
2,6-DCP	0.002	2.0	0.039	(0.006)	42	61	140	162	ND	ND
2,4-DCP	0.002	2.0	0.128	(0.013)	852	1336	335	332	202	207
3,4-DCP	0.002	2.0	0.053	(0.006)	91	146	33	31	33	32
2,4,6-TCP	0.001	1.0	0.052	(0.013)	170	263	180	188	59	61
2,3,6-TCP	0.001	1.0	0.002	(<0.001)	26	40	29	26	14	14
2,4,5-TCP	0.001	1.0	0.049	(0.013)	651	984	317	297	274	280
2,3,4,6-TECP	0.001	0.5	0.004	(0.002)	19	24	16	16	24	20
PCP	0.001	1.0	0.008	(0.005)	24	32	32	36	25	22
Lindane	0.001	0.5	ND		8	11	3	3	ND	ND
p,p'-DDT	0.001	1.0	ND		28	22	7	5	6	3
p,p'-DDD	0.001	1.0	ND		75	80	28	16	7	8
p,p'-DDE	0.001	1.0	ND		67	68	26	17	16	22
o,p'-DDD	0.001	1.0	ND		63	68	20	13	9	12
o,p'-DDE	0.002	1.0	ND		15	16	5	3	4	5

*Mean (and s.d.) of triplicate water samples.
ND = not detectable

Table IV. Average bioconcentration factors (BCFs) for benzothiazoles and chlorophenols in leeches.

Compound	Average BCFs		
	D. Dubia	E. punctata	H. stagnalis
MMBT	400X	200X	100X
BT	350X	250X	-
2,6-DCP	1300X	3900X	-
2,4-DCP	8500X	11900X	1600X
3,4-DCP	2200X	600X	600X
2,4,6-TCP	4200X	3500X	1200X
2,3,6-TCP	16500X	14000X	7000X
2,4,5-TCP	16700X	6300X	5700X
2,3,4,6-TECP	5500X	4000X	5500X
PCP	3500X	4300X	3000X

Table V. Depuration rate constants and half lives for organic contaminants in leeches.

Compound	D. dubia		E. punctata		H. stagnalis	
	Rate Constant d_{K_T} (day^{-1})	Half Life (days)	Rate Constant d_{K_T} (day^{-1})	Half Life (days)	Rate Constant d_{K_T} (day^{-1})	Half Life (days)
MMBT	.437	1.5	.281	2.5	NC	<1
BT	.073	9.5	.102	7	<DL	-
2,6-DCP	.021	34	NSD	-	<DL	-
2,4-DCP	.015	46	NSD	-	.017	41
3,4-DCP	.022	31	NSD	-	.008	87
2,4,6-TCP	.027	25	NSD	-	.020	34
2,3,6-TCP	NSD	-	NSD	-	NSD	-
2,4,5-TCP	NSD	-	NSD	-	.023	30
2,3,4,6-TECP	NSD	-	NSD	-	NSD	-
PCP	NSD	-	.025	28	NSD	-
Lindane	1.199	.5	NC	1	<DL	-
p,p'-DDT	NSD	-	NSD	-	.125	5.5
p,p'-DDD	.070	10	.033	21	.053	13
p,p'-DDE	NSD	-	NSD	-	NSD	-
o,p'-DDD	.054	13	.022	32	.115	6
o,p'-DDE	NSD	-	NSD	-	.012	58

NSD = No significant depuration observed at $p < .05$.
NC = Not calculated due to insufficient data points.
<DL = Not detectable.

Table VI. Mean weights (g) of sampled leeches and estimated loss of biomass during the experiment.

Sampling Day	D. Dubia		E. punctata		H. stagnalis	
	DD1(n=6/d)	DD2(n=6/d)	EP1(n=3/d)	EP2(n=3/d)	HS1(n=40/d)	(HS2(n=40/d)
0	.082	.073	.243	.260	.0073	.0069
1	.070	.086	.183	.163	.0071	.0078
2	.052	.075	.166	.234	.0074	.0077
5	.061	.067	.198	.214	.0075	.0075
8	.065	.062	.246	.151	.0076	.0077
13	.046	.050	.168	.199	.0075	.0078
21	.034[1]	-	.163	.194	.0072	.0071
27	-	.037[2]	.104[1]	.093[3]	.0075	.0074
Initial Mean Weight of all Specimens	.071(n=46)	.072(n=46)	.191(n=28)	.209(n=27)	-	-
Mean Weight of all Sampled Specimens	.059(n=41)	.066(n=39)	.178(n=26)	.185(n=25)	-	-
Estimated Loss of Biomass	16.9%	8.3%	6.9%	11.6%	-	-

[1]n=5; [2]n=3; [3]n=4

Table VII. Bioaccumulation of benzothiazoles and chlorophenols by leeches vs. other aquatic organisms (wet weight basis).

Compound	Leeches (this study)		Other Aquatic Organisms				
	Exposure Concentration (µg/L)	BCF	BCF	Exposure Concentration (µg/L)	Exposure Regime*, Duration	Organism	Reference
MMBT	23.27	100-400x	150x	30.40	B, 7d	Notropis cornutus (Common shiner)	Metcalfe et al. (unpublished data)
			150x			Rhinichthys cataractae (Longnose dace)	
			15x			Orconectes propinquus (crayfish)	
BT	3.02	250-350x (erpobdellids)	10x	9.68	B, 7d	N. cornutus	Metcalfe et al. (unpublished data)
			10x			R. cataractae	
			5x			O. propinquus	
2,4,6-TCP	.052	1200-4200x	3000x	.05	C, 36d	Lymnaea stagnalis (pond snail)	Virtanen and Hattula (1982)
			7000x( )			Poecilia reticulata (guppy)	
			12000x( )				
			20000x	.001	A	Lanice conchilega (marine polychaete)	Ernst & Weber (1978)
			45x	.110	A	Lota vulgaris (burbot)	Paasivirta et al. (1985)
			9x			Esox lucius (pike)	
			27x			Leuciscus rutilus (roach)	
			64x			Anodonta piscinalis (mussel)	
			73x			Simuliidae (blackfly larvae)	
			182x			Spongilla lagustris (sponge)	

Table VII. continued Bioaccumulation of benzothiazoles and chlorophenols by leeches vs. other aquatic organisms (wet weight basis).

Compound	Leeches (this study)		Other Aquatic Organisms				
	Exposure Concentration (µg/L)	BCF	BCF	Exposure Concentration (µg/L)	Exposure Regime, Duration	Organism	Reference
2,4,5-TCP	.049	5700-16700x	1900x	4.8	C,28d	Pimephales promelas (fathead minnow)	Call et al. (1980)
			1800x	49.3			
			24000x	.008	A	L. conchilega	Ernst & Weber (1978)
2,3,4,6-TECP	.004	4000-5500x	60x	.006-.008	B, 70d	Mytilus edulis (marine mussel)	Folke et al. (1983)
			11000x	.006	A	L. conchilega	Ernst & Weber (1978)
			133x	.090	A	L. vulgaris	Paasivirta et al. (1985)
			33x			E. lucius	
			211x			Leuciscus idus (Ide)	
			44x			L. rutilus	
			44x			Chironomus sp.	
			33x			S. lagustris	
PCP	.008	3000-4300x	170x	.002-.003	B, 70d	M. edulis	Folke et al. (1983)
			200x	.035	C, 115d	Salmo gairdneri (Rainbow trout)	Niimi & McFadden (1982)
			240x	.660			
			70-180x	.040	A	Sagartia troglodytes (sea anemone)	Ernst & Weber (1978)
			2600-8500x			L. conchilega	

* A = natural population; B = on site exposure; C = continuous flow, laboratory.

Table VIII. Half lives for lindane, DDT and DDD derivatives in leeches vs. other aquatic organisms.

Compound	Half Life in Leeches	Half Lives in Other Organisms		
		Organism	Half Life	Reference
Lindane	≤ 1 day	M. edulis	< 1 day	Ernst (1977)
		Leponis macrochirus (bluegill); Carassius auratus (goldfish)	< 2 days	Gakstatter & Weiss (1967)
		P. reticulata	< 1 day	Yamato et al (1983)
		Venerupis japonica (Marine short-necked clam)	< 3 days	
DDD	6-32 days	M. edulis	5 days	Ernst (1977)
DDT	5 1/2 to >>30 days	L. macrochirus; C. auratus	> 32 days	Gakstatter & Weiss (1967)

Table IX. Half lives for chlorophenols in leeches vs. other aquatic organisms.

Chlorophenol Isomer	Half Life in Leeches	Half Lives in Other Organisms*		
		Organism	Half Life	Reference
2,4,5-TCP	≥ 30 days	P. promelas	6.6-9.2 days	Call et al. (1980)
2,4,6-TCP	≥ 25 days	S. gairdneri	<10 days (liver, fat weight basis)	Landner et al. (1977)
PCP	≥ 28 days	Fundulus similis (marine killfish)	4.7 days	Trujillo et al. (1982)
		L. macrochirus	< 2 days (gills, digestive tract, liver combined)	Pruitt & Grantham (1977)
		S. gairdneri	6.2-23 hours (various organs)	Glickman et al. (1977)
		C. auratus	10 hours	Kobayashi & Akitake (1975)
		M. edulis	2-3 days	Ernst (1979)

*Whole organism - live weight basis unless otherwise indicated.

FIGURE CAPTIONS

Figure 1 Location of study.

Figure 2 Relative proportions of chlorophenol isomers in creek water and leeches.

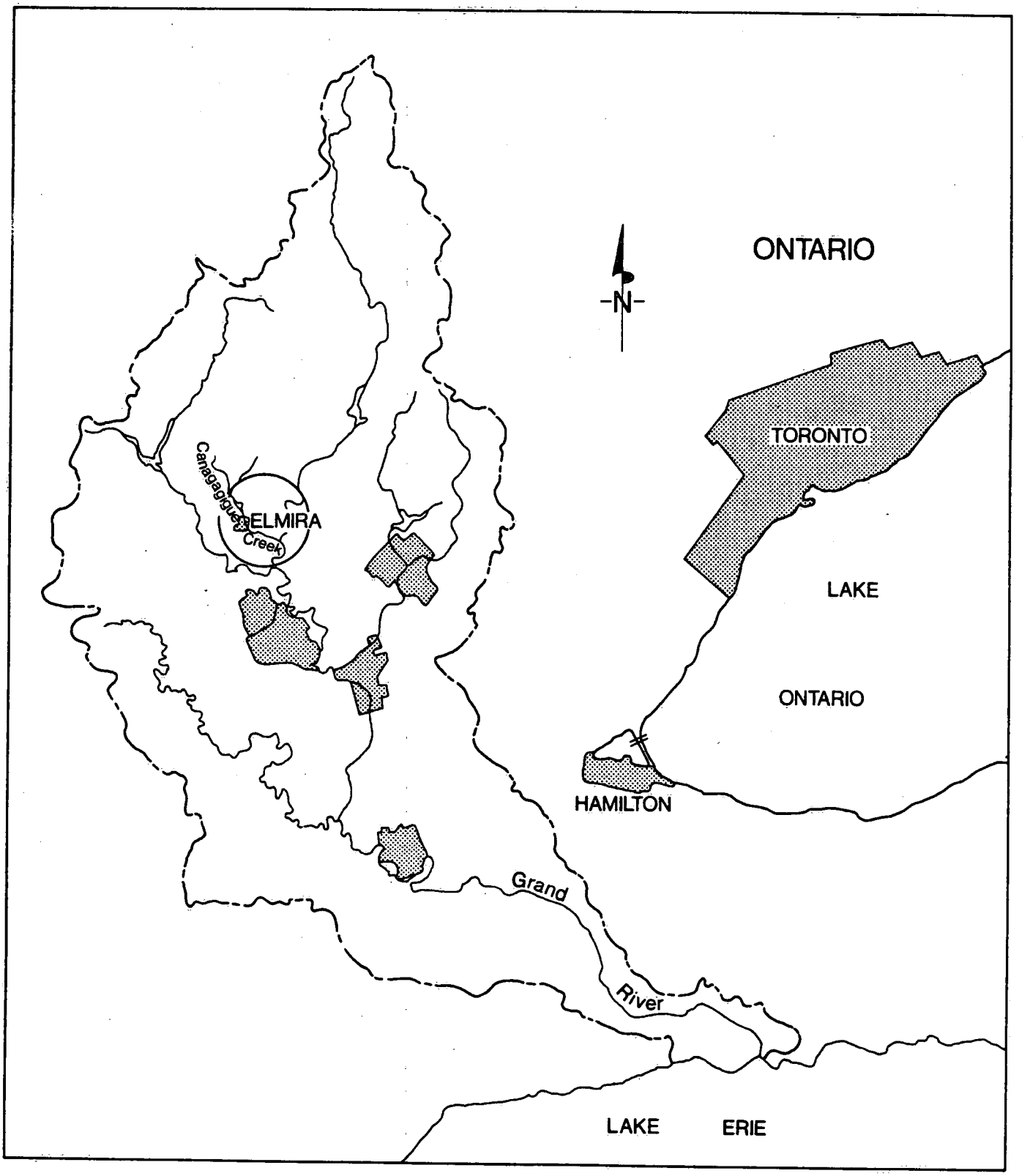
Figure 3 Depuration of chlorophenols by leeches. $C_{(o)}$ = initial tissue concentration; $t_e 1/2$ = half life; • = single data point; x = two data points with the same value.

Figure 4 Depuration of DDT derivatives by leeches

Figure 5 Depuration of benzothiazole derivatives by leeches. o = single data point and ◘ = two data points with concentrations less than the detection limit.

Running Head: LEECHES AS A SCREENING TOOL FOR ORGANIC CONTAMINANTS

Figure 1


ONTARIO
N
TORONTO
LAKE
ONTARIO
ELMIRA
Canagagigue Creek
HAMILTON
Grand
River
LAKE
ERIE

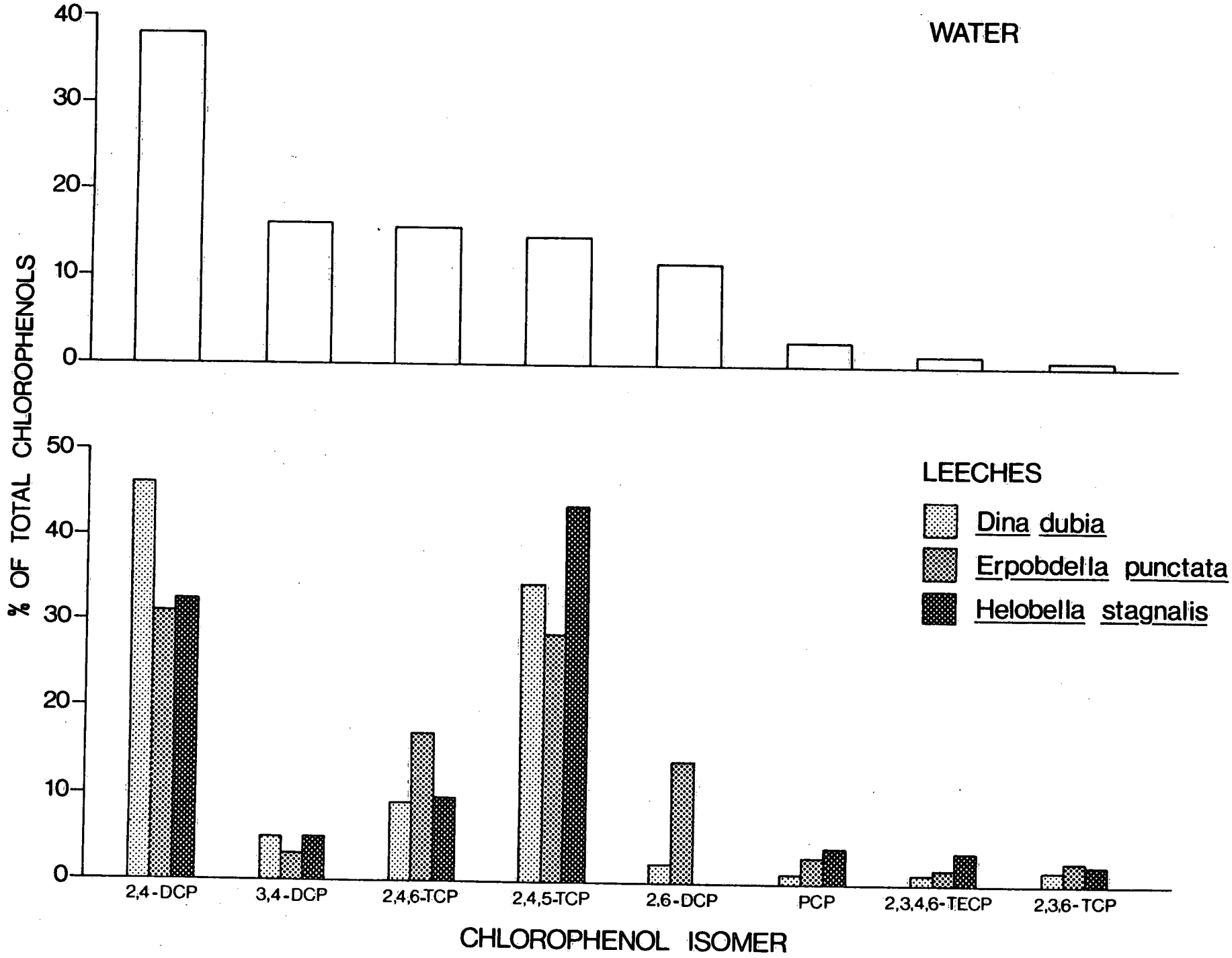

WATER
40
30
20
10
0
% OF TOTAL CHLOROPHENOLS
50
40
30
20
10
0
LEECHES
Dina dubia
Erpobdella punctata
Helobella stagnalis
2,4-DCP
3,4-DCP
2,4,6-TCP
2,4,5-TCP
2,6-DCP
PCP
2,3,4,6-TECP
2,3,6-TCP
CHLOROPHENOL ISOMER

Figure 2

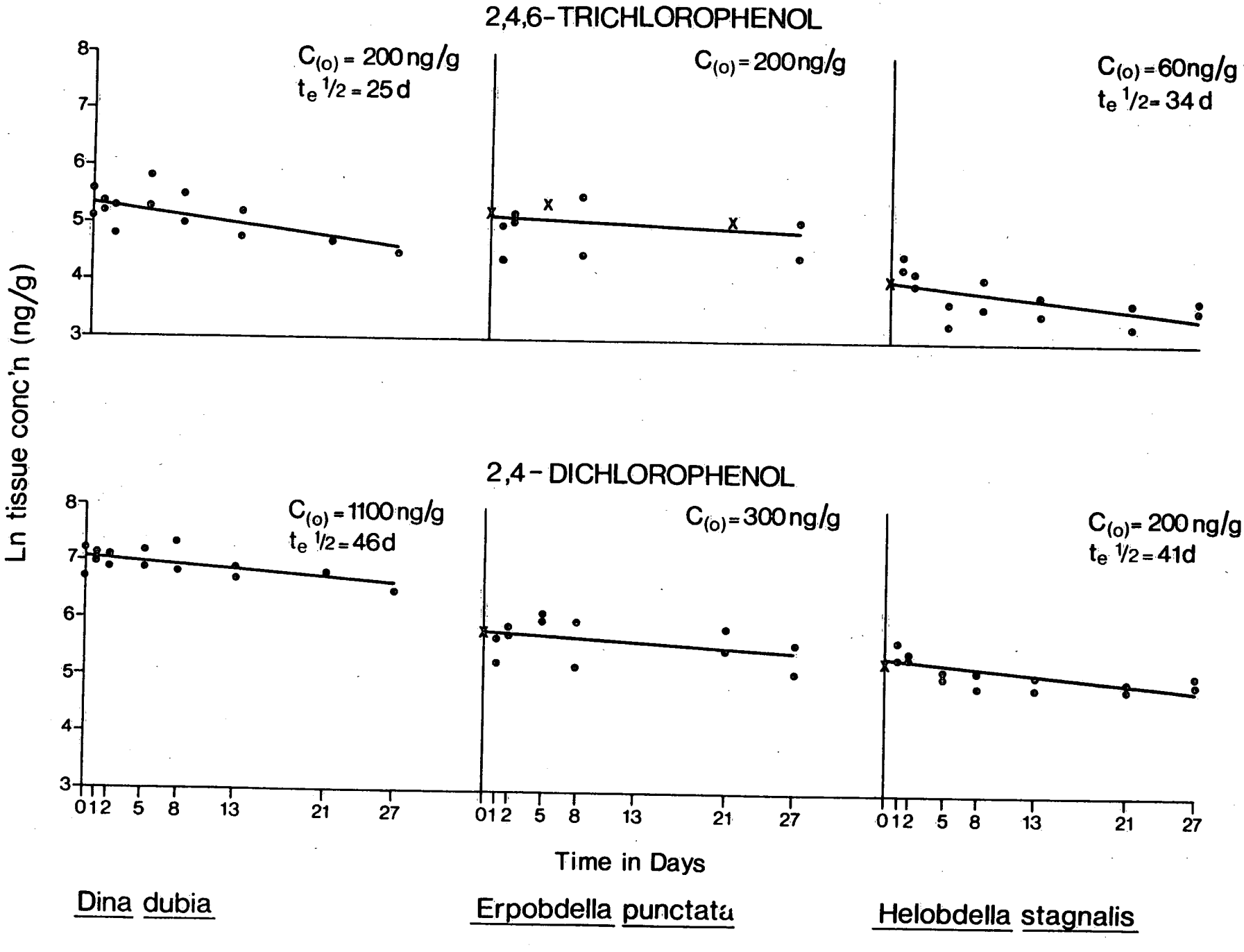

2,4,6-TRICHLOROPHENOL
$C_{(o)}$ = 200 ng/g
t_e 1/2 = 25 d
$C_{(o)}$ = 200 ng/g
$C_{(o)}$ = 60 ng/g
t_e 1/2 = 34 d
2,4 - DICHLOROPHENOL
$C_{(o)}$ = 1100 ng/g
t_e 1/2 = 46 d
$C_{(o)}$ = 300 ng/g
$C_{(o)}$ = 200 ng/g
t_e 1/2 = 41 d
Ln tissue conc'n (ng/g)
Time in Days
Dina dubia
Erpobdella punctata
Helobdella stagnalis

Figure 3

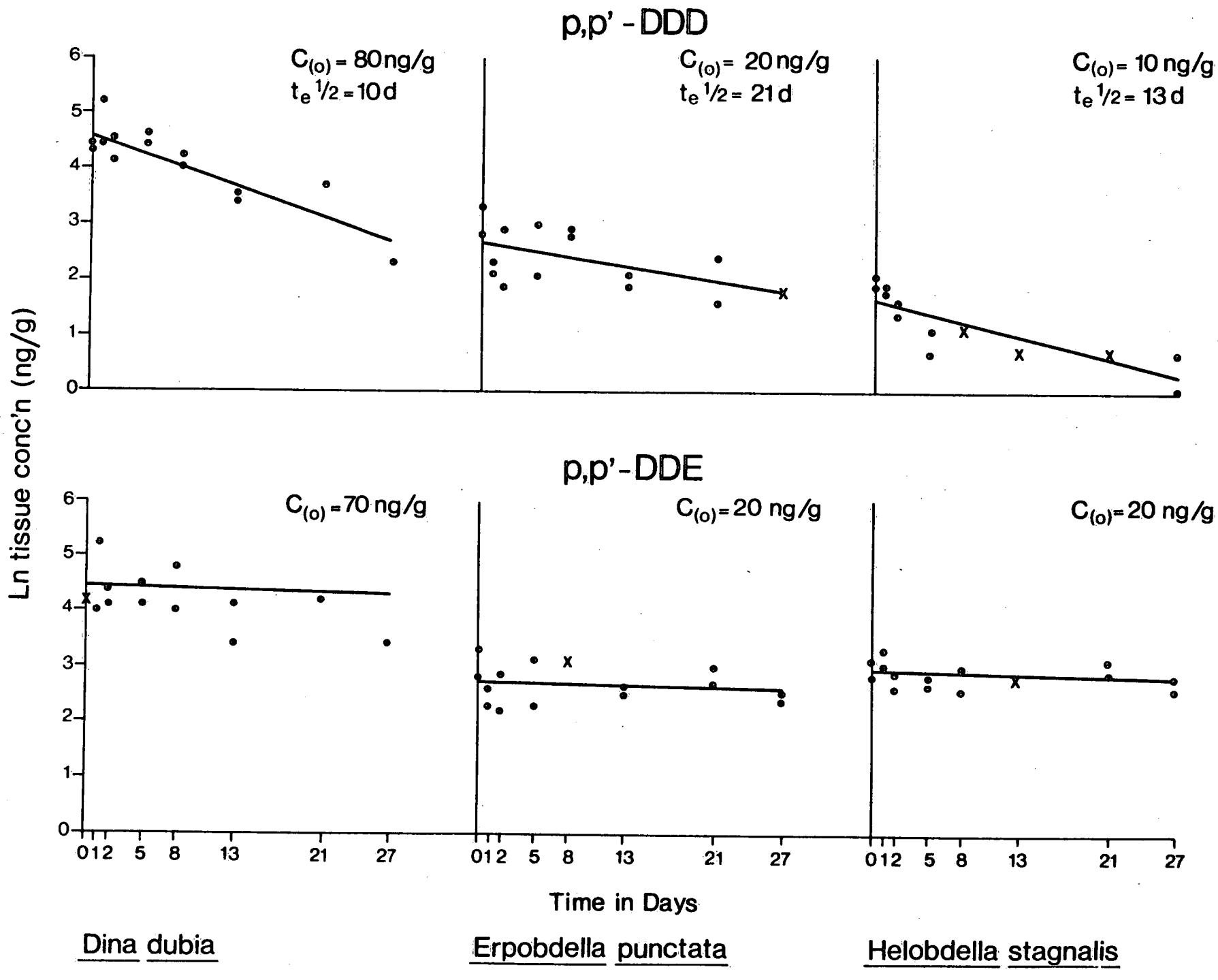

p,p' - DDD
C(o) = 80 ng/g
te 1/2 = 10 d
C(o) = 20 ng/g
te 1/2 = 21 d
C(o) = 10 ng/g
te 1/2 = 13 d
p,p' - DDE
C(o) = 70 ng/g
C(o) = 20 ng/g
C(o) = 20 ng/g
Ln tissue conc'n (ng/g)
0 1 2 3 4 5 6
0 1 2 5 8 13 21 27
Time in Days
Dina dubia
Erpobdella punctata
Helobdella stagnalis

Figure 4

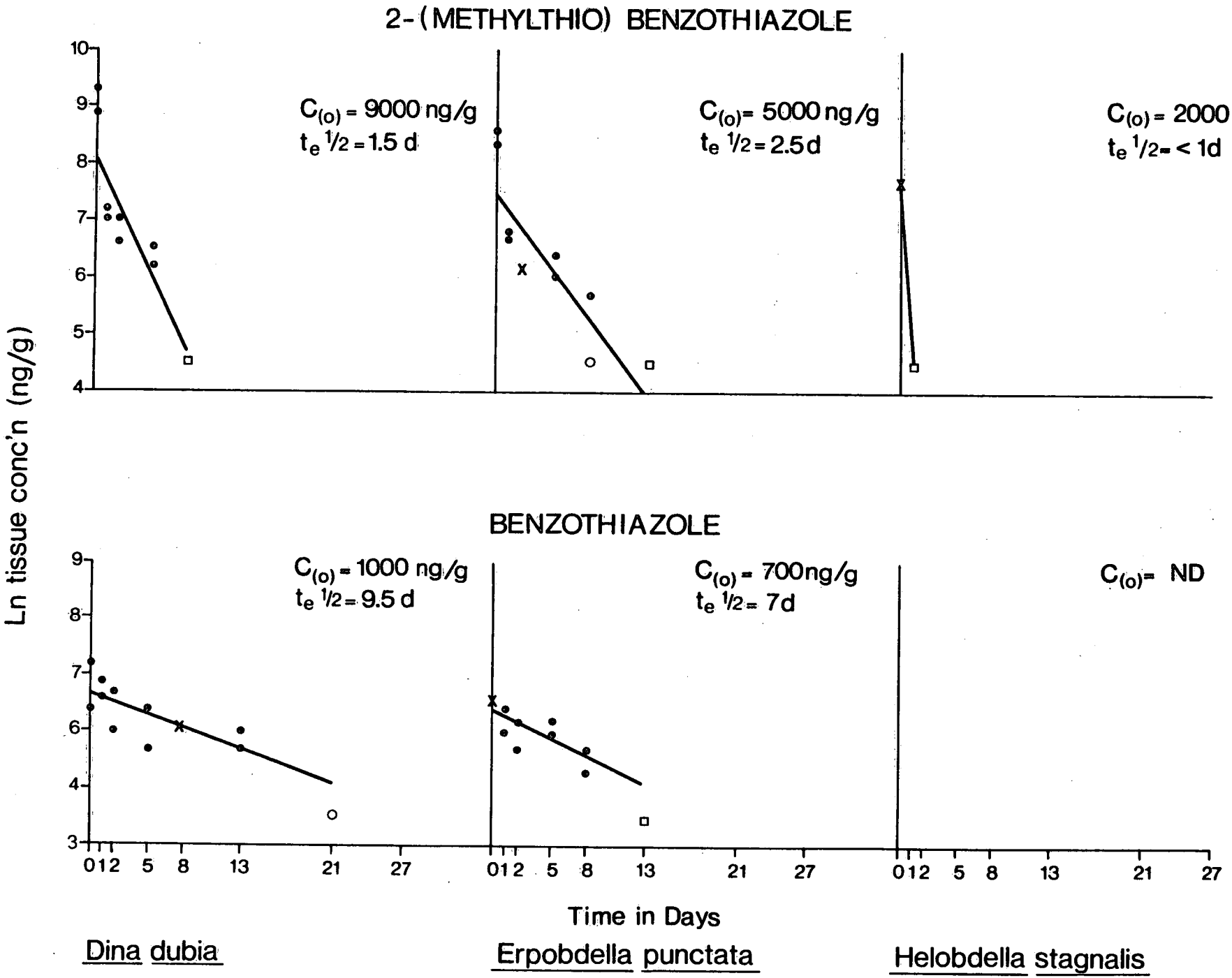

2-(METHYLTHIO) BENZOTHIAZOLE
$C_{(o)}$ = 9000 ng/g
t_e 1/2 = 1.5 d
$C_{(o)}$ = 5000 ng/g
t_e 1/2 = 2.5 d
$C_{(o)}$ = 2000 ng/g
t_e 1/2 = < 1d
BENZOTHIAZOLE
$C_{(o)}$ = 1000 ng/g
t_e 1/2 = 9.5 d
$C_{(o)}$ = 700 ng/g
t_e 1/2 = 7d
$C_{(o)}$ = ND
Ln tissue conc'n (ng/g)
Time in Days
Dina dubia
Erpobdella punctata
Helobdella stagnalis

Figure 5