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EFFECTS OF DISSOLVED ORGANIC CARBON ON ACCUMULATION AND ACUTE TOXICITY OF FENVALERATE, DELTAMETHRIN AND CYHALOTHRIN TO DAPHNIA MAGNA (STRAUS)

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ABSTRACT

The effects of dissolved organic carbon (DOC) in the form of Aldrich humic acid on the accumulation and acute toxicities of three synthetic pyrethroids, fenvalerate, deltamethrin and cyhalothrin, to Daphnia magna in laboratory experiments were investigated. Concentrations of DOC as low as 2.6 $mg.L^{-1}$, 3.2 $mg.L^{-1}$ and 3.1 $mg.L^{-1}$ for deltamethrin, fenvalerate and cyhalothrin respectively resulted in a significant decrease in bioaccumulation. Acute toxicities of all three pyrethroids were found to decrease as DOC concentrations increased e.g., at a DOC concentration of 15.5 $mg.L^{-1}$, the acute toxicity of fenvalerate was reduced by a factor of 17. The percentages of deltamethrin and fenvalerate bound to DOC increased as DOC concentrations increased after 2-h and 24-h contact times. At low concentrations of DOC (e.g., 1.7 $mg.L^{-1}$) as much as 40% of fenvalerate and 20% of deltamethrin were found sorbed to the dissolved material. After 24 h contact times, 76.4 and 80.8% of fenvalerate and deltamethrin respectively were bound to DOC. Reverse-phase partition coefficents (K_{rp}) for both fenvalerate and deltamethrin were found to vary with DOC concentrations and were in the range 1.0 to 4.8 X 10^5 $L.kg^{-1}$ for fenvalerate and 0.9 to 5.6 X 10^5 for deltamethrin.

RÉSUMÉ

Nous avons étudié en laboratoire les effets du carbone organique dissous (COD), sous forme d'acide humique d'Aldrich, sur l'accumulation de trois pyréthroïdes synthétiques, soit le fenvalérate, la deltaméthrine et la cyhalothrine dans _Daphnia magna_. Nous avons aussi vérifié si le COD modifiait la toxicité aiguë de ces substances envers cet organisme. Nous avons observé une réduction de la bio-accumulation de la deltaméthrine, du fenvalérate et de la cyhalothrine quand on les mettait en présence de COD dont les concentrations étaient aussi faibles que 2,6 mg.L^{-1}, 3,2 mg.L^{-1} et 3,1 mg.L^{-1}, respectivement. Les toxicités aiguës des trois pyréthroïdes diminuaient avec l'augmentation des concentrations en COD. Par exemple, la toxicité aiguë du fenvalérate était 17 fois moins grande quand la concentration en COD était de 15,5 mg.L^{-1}. Les pourcentages de deltaméthrine et de fenvélate liés au COD variaient en raison directe des concentrations de COD 2 heures et 24 heures après avoir mis ces pyréthroïdes en présence de COD. Avec de faibles concentrations en COD (1,7 mg.L^{-1}, par exemple), jusqu'à 40 % du fenvalérate et 20 % de la deltaméthrine étaient sorbés au matériel dissous. Après 24 heures, 76,4 et 80,8 % du fenvalérate et de la deltaméthrine, respectivement, étaient liés au COD. La détermination des coefficients de partage en phase inversée (Kδξ) pour le fenvalérate et le deltaméthrine a montré qu'ils variaient en fonction de la concentration du COD. Le coefficient du fenvalérate variait de 1,0 à 4,8 x 10^{5} L.kg^{-1} et celui de la deltaméthrine variait de 0,9 à 5,6 x 10^{5}.

MANAGEMENT PERSPECTIVE

In recent years, several studies have demonstrated that dissolved organic carbon (DOC), especially humic acid, can form stable complexes with hydrophobic organic compounds and inorganic trace metals thus decreasing their bioavailability to aquatic biota. It has been suggested that the binding of such contaminants may result in a contaminant-DOC complex which is too large or too polar to penetrate biological membranes. A reduction in the bioavailability of a toxicant to aquatic biota should also result in a reduction in the potential dose experienced by the organism and a decrease in toxicity but this aspect has been studied rarely. Synthetic pyrethroid insecticides such as deltamethrin, fenvalerate and permethrin are hydrophobic organic pesticides which are used extensively in large-acreage crops and are known to be extremely toxic to aquatic organisms in laboratory studies. Partitioning of these pesticides into dissolved organic material may explain partially why the toxicity of these chemicals is reduced under field conditions. This paper describes the effects of DOC on bioaccumulation and acute toxicity of three synthetic pyrethroids to Daphnia magna. It includes research on a new synthetic pyrethroid, cyhalothrin, which is currently undergoing evaluation by Agriculture Canada.

PERSPECTIVE - GESTION

Ces dernières années, plusieurs études ont démontré que le carbone organique dissous (COD), particulièrement l'acide humique, peut réduire la biodisponibilité des métaux inorganiques présents à l'état de traces et des composés organiques hydrophobes dans le biote aquatique en formant avec ces substances des complexes stables. Certains auteurs pensent que la fixation de ces contaminants peut résulter en la formation de complexes contaminant-COD dont la grande taille ou la trop forte polarité les empêchent de traverser les membranes biologiques. La réduction de la biodisponibilité d'une substance toxique dans le biote aquatique devrait réduire le degré d'exposition des organismes à cette substance, et donc donner lieu à une réduction de sa toxicité, mais cette question a rarement été étudiée. Les pyréthroïdes synthétiques utilisées comme insecticides comme le fenvalérate et la perméthrine sont des pesticides organiques hydrophobes largement utilisés dans les grandes cultures. Des études en laboratoire ont montré que ces substances sont extrêmement toxiques pour les organismes aquatiques. La fixation de ces pesticides sur le matériel organique dissous pourrait en partie être responsable de la réduction de la toxicité de ces substances chimiques sur le terrain. Cet article décrit les effets du COD sur la bio-accumulation de trois pyréthroïdes synthétiques dans <u>Daphnia magna</u> de même que ses effets sur leur toxicité aiguë envers cet organisme. L'un de ces pyréthroïdes est la cyhalothrine, une nouvelle substance synthétique qu'Agriculture Canada est en train d'évaluer.

INTRODUCTION

Dissolved organic carbon (DOC), by definition, consists of colloidal plus dissolved substances that pass through 0.3 to 0.5 μm filters (Wetzel 1983; McCarthy and Jimenez, 1985). In surface waters, concentrations of DOC range normally from 1 to 10 $mg.L^{-1}$ with peaks of >30 $mg.L^{-1}$ in swamps and bogs. Chemically, DOC consists of low-molecular-weight compounds such as carbohydrates, fatty acids and peptides, etc. as well as high-molecular-weight (100->100,000 daltons) humic and fulvic materials (Leversee et al. 1983; Aho 1986). In recent years, several studies have demonstrated that DOC, especially humic acid, can form stable complexes with hydrophobic organic compounds and inorganic trace metals thus enhancing their apparent solubilities i.e., the relatively water-insoluble hydrophobic compound is held in solution by partitioning into the more water-soluble DOC (Hassett and Anderson, 1979; Carter and Suffet, 1982; Stackhouse and Benson, 1989). Further studies have revealed that such partitioning into DOC decreases the bioavailability of contaminants such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to aquatic biota (McCarthy and Jimenez 1985; Carlberg et al., 1986; Landrum et al., 1987; Servos and Muir, 1989; Servos et al., 1989; Kukkonen et al., 1989). Reductions in bioavailability may significantly influence the bioaccumulation of potentially hazardous chemicals discharged into humic water systems but the influence of

DOC on toxicity has rarely been studied (but see Kukkonen and Oikari, 1987).

Synthetic pyrethroid insecticides such as permethrin, deltamethrin, cypermethrin and fenvalerate, are hydrophobic organic pesticides used to control insect pests in large-acreage crops such as cereals, potatoes, tobacco, cotton and fruit. The contamination of surface waters in agricultural areas by synthetic pyrethroids through overspray, drift or runoff is of concern because these compounds have been shown to be extremely toxic to aquatic organisms, especially crustaceans, in laboratory studies (Smith and Stratton, 1986; Anderson, 1989). However, under field conditions, the impact of these chemicals is generally less than expected on the basis of laboratory data. Muir et al. (1985a) suggest that this is due to rapid partitioning of the pyrethroids into suspended particles, plants, sediment and air as well as hydrolysis and isomerization of these chemicals (Maguire et al., 1989). The complexing of synthetic pyrethroids with DOC is also likely to occur in natural waters due to the high octanol-water partition coefficients of these compounds (log K_{OW}'s range from 6.2-6.8) (Briggs et al., 1982). Such partitioning may reduce the toxicities of these chemicals to aquatic biota (Muir et al., 1985b) but little information is available. The objective of this study is to evaluate the effects of DOC in the form of Aldrich humic acid on the bioavailability and acute toxicity of three synthetic pyrethoids to the cladoceran, *Daphnia magna* under laboratory conditions. The pyrethroids were deltamethrin ([(S)-α-cyano-3-phenoxy-benzyl(1R,3R)-cis-2,2-dimethyl-3-(2,2-dibromovinyl) cyclopropanecarboxylate] and fenvalerate [(RS)-α-cyano-3-phenoxy-benzyl (RS)-2-(4-chlorophenyl-3-methyl-butyrate], two insecticides currently registered in Canada for broad spectrum use under the trade names Decis[R] and Belmark[R] respectively. The third pyrethroid, cyhalothrin [(RS)-α-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-tri-fluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate], is a new experimental pyrethroid with greater insecticidal properties than the other currently registered chemicals (personal communication, Chipman Chemicals Inc., Stoney Creek, Ontario). This product is being developed for use in Canada under the trade name Karate[R].

Material and Methods

The position of the radiolabel, the specific activity and the purity for each pyrethroid used in this study were as follows: [^{14}C]-cyhalothrin labelled at the C_1-position of the cyclopropane ring (sp. act. 1.95 $GBq.mmol^{-1}$; purity 97.9%); [^{14}C]-fenvalerate labelled at the carbonyl group of the acid moiety (sp. act. 0.16 $GBq.mmol^{-1}$; purity 96.4%); [^{14}C]-deltamethrin labelled at the benzyl carbon (sp. act 2.19 $GBq.mmol^{-1}$; purity > 95%). Technical cyhalothrin (96.5%), technical deltamethrin (99.9%) and an emulsifiable concentrate of fenvalerate (300 $g.L^{-1}$ a.i.) were used to determine acute toxicities (24-h and 48-h EC_{50}s). Stock solutions of each pesticide were prepared by dilution of the chemicals in pesticide grade acetone (Caledon Laboratories, Georgetown, Ontario).

Concentrations of radiolabelled pesticides were determined by placing 100 μL aliquots of each stock solution in triplicate scintillation vials with 15 mL OCS scintillation fluor (Amersham Searle Corp.). The vials were counted for 10 min. in a Packard Tri-Carb Series 4000 liquid scintillation counter (LSC) with automatic correction for background and quenching using the external standards ratio method. Concentrations were calculated using the specific activities for each radiolabelled chemical.

Concentrations of pesticides in the acute toxicity experiments were prepared by serial dilutions of stock solutions ranging from 0.025 to 25 $\mu g.L^{-1}$ and were nominal.

Dissolved organic carbon was added in the form of Aldrich humic acid (HA) (Lot # 3601 KE; Aldrich Chemical Co., Milwaukee, WI). A solution of HA was prepared by dissolving 500 mg in 1 L of MilliQ deionized water. The crude solution was centrifuged at 10,000 X g for 30 min., the supernatant filtered (0.3 μm Gelman Type A/E filters) and the final stock solution added to the test waters to give a range of DOC concentrations (1.0 to 12.0 $mg.L^{-1}$). Actual dissolved organic carbon concentrations were determined using a Beckman Model 915B Total Carbon Analyzer from water samples which were acidified with 60 μL concentrated HCl and bubbled with N_2 gas for 5 min. All pesticides and humic acid combinations were allowed a 2 h contact time before the addition of animals to allow equilibrium between pyrethroids and dissolved organic matter. A longer contact time was not appropriate due to the known rapid degradation of synthetic pyrethroids especially deltamethrin in water (Muir et al., 1985a; Maguire et al. (1989)).

Daphnia magna were obtained from a culture maintained at the Canada Centre for Inland Waters, Burlington, Ontario. Gravid adult females were acclimated to experimental conditions (20 to 21 °C; 16hL:8hD) in 10 L aquaria in filtered Lake Ontario water and fed ad libitum with the algae, Scenedesmus spp. For accumulation experiments, 5 to 7 day old animals were placed in filtered, Lake Ontario water for 2 h without food to permit clearance of their guts and then transferred to filtered water with various concentrations of DOC added as Aldrich humic acid and one of the three synthetic pyrethroids. Experiments were conducted in 300 mL BOD bottles, each containing 5 animals and each concentration of pesticide and humic acid was replicated three times. The bottles were placed on a rotating wheel for 24 h in a temperature- and light-controlled incubator (Cuhel and Lean, 1987). Dissolved oxygen and pH were measured at the beginning and end of each experiment and ranged from 6.8 to 7.3 $mg.L^{-1}$ and 6.9 to 7.3 respectively. At the end of the exposure period, 5 mL aliquots of water were removed from each bottle and placed in a scintillation vial with 15 mL Aquasol II (New England Nuclear) for LSC. Animals were collected by pouring the contents of each bottle through Nitex sieves (185 µm) and rinsing the animals with 100 mL deionized water. With the use of fine forceps and a stereoscope, the animals were dried on glass fiber filters (2.1 cm GF/A) and transferred to preweighed aluminum dishes for determination of wet weight on a Mettler ME 30 microbalance. After weighing, animals were transferred to a scintillation vial and 2 mL of the tissue solubilizer NCS (Amersham Searle Corp.) was added. The vials were heated overnight at 48°C in an incubator to allow digestion of animal tissues then returned to room temperature. Glacial acetic acid (0.74 µL) was added to reduce colour quenching followed by 15 mL of OCS scintillation fluor. Each vial was shaken vigourously, dark adapted for 24 h and the radioactivity in the animals determined by LSC. Accumulation of each pesticide in the animals was calculated as nmol pesticide.g wet weight^{-1} (DPM.g daphnid^{-1} ÷ specific activity of each chemical). The relationship between accumulation of each chemical by Daphnia and the presence of DOC concentration was determined by regression on log transformed data.

Acute lethalities of the three pyrethroids were determined by exposing groups of 5 D. magna to 200 mL of water containing test concentrations in 250 mL glass beakers. Each concentration was replicated 3 times and the animals were handled and disturbed as little as possible during bioassays (any damaged animals were not utilized). Percent mortality in terms of immobilization (the inability of test animals to swim during a 10 sec. period of observation after the water in each beaker was gently swirled) was determined at 24-h and 48-h and EC_{50} values were calculated by probit analysis.

Separate experiments using identical glassware, water and humic acid as those used in accumulation experiments were conducted to determine the proportion of deltamethrin and fenvalerate associated with DOC. Percent sorption of cyhalothrin to DOC was not determined due to accidental loss of the radiolabelled sample. Each pesticide (final concentrations - 0.86 $\mu g.L^{-1}$ deltamethrin; 4.28 $\mu g.L^{-1}$ fenvalerate) was added to water with increasing concentrations of DOC (2.0 to 19.0 $mg.L^{-1}$). After contact times of 2-h and 24-h, triplicate 4 mL samples were taken from each bottle, diluted with scintillation fluor and assayed by LSC to determine total pesticide concentrations. An additional 4 mL from each bottle was passed through a reversed-phase cartridge (C_{18} Sep Pak; Waters Scientific, Milford, MA) and the eluant assayed by LSC. Landrum _et al_., 1984 have demonstrated that this reverse-phase separation method allows hydrophobic chemicals, free in solution or weakly bound to DOC, to remain on the cartridge whereas any chemical sorbed to DOC passes through the cartridge. Linear regressions of the percent sorption of each pesticide to DOC vs. DOC concentration were performed and a partition coefficient (K_{rp}) in $L.kg^{-1}$ was calculated by dividing the bound concentration ($ng.L^{1}$) of each pesticide per kilogram of DOC by the freely dissolved concentration ($ng.L^{-1}$) of the chemical.

The determination of the amount of fenvalerate and deltamethrin associated with DOC allowed the calculation of bioconcentration factors (BCFs) for _Daphnia_ in accumulation experiments using a) concentrations of pesticides determined experimentally (includes both bound and freely dissolved pesticides) and b) concentrations of pesticides predicted by reverse-phase methodology to be freely dissolved and thus truly bioavailable. BCFs calculated after a 24-h exposure period were determined as follows:

$$BCF = C_a/C_w \qquad (1)$$

where C_a and C_w are the concentrations of pesticide in the animals (ng. g^{-1} wet weight) and in the water (ng.mL^{-1}) respectively. The free portion (f) of each pyrethroid was calculated according to the method of Kukkonen et al., 1989 as follows:

$$f = 1/(1 + K_{rp} \cdot DOC) \qquad (2)$$

where DOC is the dissolved organic carbon concentration (kg C.L^{-1}) in the sample.

The fitting of regression lines, probit analysis and all statistical analyses were accomplished using Parastat, a program developed and assembled for the IBM personal computer by T. James, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada.

RESULTS

The accumulation of synthetic pyrethroids by D. magna from water containing Aldrich humic acid was reduced by an increase in dissolved organic carbon concentrations (Fig. 1). The equations indicate that the relationship between accumulation and DOC is logarithmic. The decreases in the accumulation of synthetic pyrethroids by cladocerans occurred despite an apparent increase in the percentage of each chemical in the water as DOC concentration increased (Tables 1, 2 and 3). However, measurements of total radioactivity in water samples taken during accumulation experiments did not differentiate between the amount of pesticide complexed to DOC and the amount freely dissolved in the water. Relatively low concentrations of DOC were sufficient to significantly reduce the accumulation of pyrethroids by D. magna. For example, accumulation of deltamethrin was significantly reduced at 2.6 $mg.L^{-1}$ DOC; concentrations of 3.2 $mg.L^{-1}$ and 3.1 $mg.L^{-1}$ DOC resulted in a significant reduction of fenvalerate and cyhalothrin respectively within the animals. The percent sorption of pesticides to the glassware also decreased as concentrations of DOC increased, although results were not always significant and were somewhat variable. Sorption to the glass bottles was greatest for deltamethrin and ranged from 9.3 to 22.5%; sorption to glass for fenvalerate and cyhalothrin was less at 1.5 to 8.6% and 1.6 to 6.8% respectively. Recovery of total applied radioactivity was 91.8 to 100.8% for cyhalothrin and 80.6 to 97.5% for fenvalerate and deltamethrin. Reductions in recovery likely can be attributed to binding of the chemicals to the glass pipettes used for subsampling and some volatilization of the chemicals.

The acute toxicities (24-h and 48-h EC_{50}'s) of all three synthetic pyrethroids were found to decrease as DOC concentrations increased (Table 4). DOC had the greatest influence in the first 24 hours of exposure on the toxicity of fenvalerate where a concentration of 15.5 $mg.L^{-1}$ resulted in a 17 X reduction in toxicity. After 48 h of exposure, a DOC concentration of 10.1 $mg.L^{-1}$ similarily reduced the toxicity of deltamethrin from 0.05 $\mu g.L^{-1}$ to 0.85 $\mu g.^{-1}$.

Reverse-phase separation methodology confirmed that the percentages of deltamethrin and fenvalerate associated with DOC increased as DOC concentrations increased (Fig. 2). There were no significant differences ($P \leq 0.05$) between the slopes of the lines of regression between different contact times and between chemicals. At low concentrations of DOC (e.g., 1.7 $mg.L^{-1}$) as much as 40% of fenvalerate and 20% of deltamethrin were found associated with the dissolved material. At higher concentrations of DOC and a longer contact time, 76.4% and 80.8% of fenvalerate and deltamethrin respectively were bound to DOC.

The reverse-phase partition coefficients (K_{rp}) for fenvalerate tended to decrease slightly as DOC concentrations increased (Fig. 3) but there was no significant linear relationship between the two parameters ($P \leq 0.05$). In addition, time of contact did not have any significant effects on the regressions. In contrast, K_{rp}'s for deltamethrin increased slightly as DOC concentrations increased after a 24 h contact time and values were slightly lower than those for fenvalerate.

Comparisons of bioconcentration factors (BCFs) in accumulation experiments with D. magna using total concentrations of pesticides in water samples (i.e., both sorbed and freely dissolved) vs. estimated concentrations of truly dissolved chemicals are presented in Fig. 4. BCFs for both fenvalerate and deltamethrin were increased slightly when concentrations of freely dissolved pesticides were used but the overall trend was a decrease in BCFs as DOC concentrations increased.

DISCUSSION

A reduction in the accumulation of synthetic pyrethroids by D. magna in the presence of increasing concentrations of DOC (as Aldrich humic acid) agrees with other reported results for fish and aquatic invertebrates exposed to hydrophobic contaminants (see McCarthy et al., 1985; Landrum et al., 1985; Servos and Muir, 1989). Leversee et al., 1983 and Kukkonen et al., 1989 found that accumulations of benzo(a)pyrene (BaP) and dehydroabietic acid (DHAA) were reduced by 25-80% in D. magna in the presence of Aldrich humic acid and natural aquatic humus. The decrease in accumulation of synthetic pyrethroids by cladocerans in the present study occurred despite an apparent increase in the percentage of each chemical in the water as DOC concentrations increased. Other studies have shown that hydrophobic contaminants can form stable complexes with dissolved colloidal material resulting in an enhancement of the apparent solubility of the contaminant (i.e., the contaminant takes on the solubility characteristics of the DOC) (Hassett and Anderson, 1979; Carter and Suffet, 1982; Wijayaratne and Means, 1984; Saint-Fort and Visser, 1988). Reverse-phase methodology with deltamethrin and fenvalerate confirmed that only a portion of each pesticide is truly dissolved in water with the remainder complexed to the DOC. Although the association of cyhalothrin with DOC was not examined in this study, it should behave in a similar manner because its K_{ow} is very close to those of fenvalerate and deltamethrin.

Relatively low concentrations of DOC (e.g., 1.7 to 3.2 $mg.L^{-1}$) were sufficient to reduce the accumulation of pyrethroids by Daphnia significantly. Spacie et al., 1984 have shown that as little as 1 $mg.L^{-1}$ Aldrich humic acid reduced the rate of uptake of BaP by bluegills by 67% and levels of 1-2 $mg.L^{-1}$ DOC have reduced the bioaccumulation of PAH's in Daphnia (Leversee et al., 1983). The binding of synthetic pyrethroids to DOC macromolecules, as with other contaminants, may result in either a DOC-pesticide complex which is too large or too polar to penetrate across biological membranes. As suggested by Landrum et al., 1985, the contaminant does not have sufficient time to diffuse out of the complex and move by passive diffusion across respiratory membranes or integument and is therefore unavailable for uptake by biota. The result is that Daphnia are only able to accumulate the pesticide which is truly free in solution.

A decrease in the bioavailability of a toxicant to aquatic biota should result in a reduction in the potential dose experienced by the organism and therefore a decrease in the toxicity. In the present study, the acute toxicities (24-h and 48-h EC_{50}'s) of all three synthetic pyrethroids were found to decrease as DOC concentrations increased. Kukkonen and Oikari (1987) also reported that the LC_{50} (48-h) for 2,3,6-trichlorophenol (CP-3) to D. magna was 34 % greater in water containing natural aquatic humus (DOC = 23.5 $mg.L^{-1}$) than in the control water (DOC = 0.1 $mg.L^{-1}$) with no prior contact time between CP-3 and humus. However, after a 13 d incubation period for humus and contaminant, a reverse response was observed i.e., acute toxicity in the humic water was more than doubled compared to the control water. Surface-associated chemical and microbial processes in the contaminant-DOC complex may have allowed production of a more toxic metabolite in the above study but this aspect requires further study. The results suggest that the presence of DOC in water may ameliorate or enhance the toxicity of hydrophobic contaminants under field conditions and may help to explain why laboratory and field toxicity data are not always well correlated.

The partitioning of hydrophobic contaminants to DOC can be described by the partition coefficient, K_{rp}. In addition to the hydrophobicity of the contaminant (measured as the octanol-water partition coefficient), the affinity for binding to DOC is known to be dependent on the organic content, size, polarity and molecular configuration of the sorbent (Chiou et al., 1986). The K_{rp}'s determined in this study for deltamethrin and fenvalerate are in the same range as those calculated for other hydrophobic contaminants with similar log K_{ow}'s. For example, Landrum et al., 1984 found that the K_{rp} for p, p'-DDT (log K_{ow} = 6.19) sorbed to Aldrich HA was 2.8 X 10^5, a value that compares favourably to that of fenvalerate with a log K_{ow} of 6.4 (1.0-4.8 X 10^5). The affinity for binding to DOC has been shown to decrease slightly as concentrations of DOC increase (this study for fenvalerate; McCarthy and Jimenez, 1985; Landrum et al., 1985). The slight increase in K_{rp}'s in the present study for deltamethrin with increasing concentrations of DOC is unusual but may be explained by degradation of the chemical. In contrast to fenvalerate, deltamethrin is known to undergo photolytic degradation in water in hours (Maguire et al., 1989) and DOC has been shown to change the rates of photolysis of many organic compounds (Mudambi and Hassett, 1988). It is therefore possible that different concentrations of DOC resulted in differing rates of degradation of parent deltamethrin over the course of the reverse-phase experiment (24 h) resulting in variable K_{rp}'s with increased DOC concentrations.

The seasonality, source and molecular composition of DOC can also be expected to affect the binding efficiency of contaminants to organic material (Morehead et al., 1986; Johnson et al., 1987) and therefore the effects of this material on toxicity. Aldrich humic acid appears to be richer in complexing sites than humic material from natural waters and several studies have suggested that the use of this chemical may overestimate the sorption of hydrophobic compounds. For example, Landrum et al., 1984 found the K_{rp} for PAH's and DDT for natural vs. Aldrich humic acid were one order of magnitude lower for natural DOC. In addition, Morehead et al., 1986 found that the partition coefficients for PAH's in different natural waters ranged over several orders of magnitude. If the differences in the acute toxicities of the synthetic pyrethroids in this study are exaggerated due to the use of Aldrich humic acid, then it is very likely that very little difference would be noted in natural waters with normal ranges of aquatic humus.

Very few studies have measured the true dissolved concentration of a contaminant in water and included it in the determination of bioconcentration factors (see Servos et al., 1989). Failure to measure the free concentration of a contaminant in water will underestimate BCF. In the present study, a comparison of BCFs for both fenvalerate and deltamethrin using the true dissolved concentrations vs. total (bound + dissolved) concentrations in the accumulation experiments resulted in a slight increase in BCFs as expected but the overall trend was still a decrease in BCFs as DOC concentrations increased. Servos et al. (1989) reported similar results in their study on the effect of DOC on the bioavailability of dioxins to rainbow trout and suggest that metabolic transformations, solubility factors, steric hindrances or experimental errors may lead to an overestimation of the free water concentration of these chemicals and therefore an underestimation of the BCF. It is possible that the presence of DOC in water may affect the rate of diffusion of freely dissolved contaminants through biological membrances but this aspect requires further study.

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Table 1. The % total [^{14}C]-deltamethrin (0.21 $\mu g.L^{-1}$)[a] in water, sorbed to glass and accumulated by Daphnia magna in the presence of Aldrich humic acid (measured as DOC, $mg.L^{-1}$) after 24 h exposure (± S.D.).

DOC $mg.L^{-1}$	Water	Glass	Animals	Total
1.3	61.5[b]±1.6	16.9[b]±6.9	2.6[b]±0.4	81.1±7.8
2.4	61.1[b]±5.8	16.8[b]±4.8	2.7[b]±0.4	80.6±3.7
2.6	59.9[b]±6.4	22.5[b]±8.6	1.7[c]±0.3	84.1±1.9
4.0	72.5[c]±7.8	17.6[c]±3.1	1.1[cd]±0.3	91.3±9.7
4.8	79.2[c]±6.8	12.9[c]±1.6	0.8[d]±0.3	92.9±6.7
7.2	78.0[c]±6.9	18.9[b]±3.4	0.7[d]±0.1	97.5±9.8
12.0	80.5[c]±2.5	9.3[c]±2.9	0.4[d]±0.1	90.2±4.8

[a]Total DPM applied 16129 ± 311

[b-d]Mean values followed by the same letter are not significantly different ($P < 0.001$)

Table 2. The % of total [^{14}C]-fenvalerate (2.33 $\mu g.L^{-1}$)[a] in water, sorbed to glass and accumulated by <u>Daphnia</u> <u>magna</u> in the presence of Aldrich humic acid (measured as DOC, $mg.L^{-1}$) after 24 h exposure (± S.D.).

DOC $mg.L^{-1}$	Water	Glass	Animals	Total
1.4	74.1^{b}±2.6	8.6^{b}±2.6	3.3^{b}±0.3	86.0±1.0
1.5	77.7^{b}±1.8	6.8^{bc}±2.2	2.8^{b}±0.3	87.3±3.6
2.0	80.7^{b}±8.9	4.9^{cd}±0.4	2.3^{bc}±0.5	83.1±4.6
3.2	83.8^{b}±5.4	1.5^{d}±0.2	1.6^{d}±0.1	86.9±5.2
4.5	82.7^{b}±1.1	2.7^{d}±1.3	1.0^{e}±0.1	86.4±2.0
6.0	79.8^{b}±2.1	4.2^{bc}±0.9	0.8^{e}±0.1	84.7±1.3
13.1	86.5^{b}±2.5	3.4^{bc}±0.4	0.5^{e}±0.1	90.4±2.6

[a] Total DPM applied 16317 ± 166

[b-e] Mean values followed by the same letter are not significantly different ($P \leq 0.001$)

Table 3. The % total [^{14}C]-cyhalothrin (0.86 $\mu g.L^{-1}$)[a] in water, sorbed to glass and accumulated by *Daphnia magna* in the presence of Aldrich humic acid (measured as DOC, $mg.L^{-1}$) after 24 h exposure (± S.D.).

DOC $mg.L^{-1}$	Water	Glass	Animals	Total
1.3	83.3^{b}±8.1	4.7^{bc}±0.8	3.8^{b}±0.4	91.8±8.6
1.5	93.2^{bc}±11.6	4.4^{bc}±0.6	4.2^{bc}±0.6	101.8±10.1
2.3	85.5^{b}±6.1	6.8^{b}±1.3	2.7^{cd}±0.8	95.0±7.3
3.1	95.3^{c}±0.6	2.9^{c}±0.2	2.0^{de}±0.3	100.1±0.4
4.7	95.6^{c}±1.7	3.5^{bc}±1.5	1.3^{def}0.2	100.5±0.8
6.1	97.9^{c}±5.1	2.0^{c}±0.2	1.0^{ef}±0.3	100.8±4.9
12.8	98.2^{c}±3.4	1.6^{c}±0.1	0.5^{f}±0.2	100.3±3.5

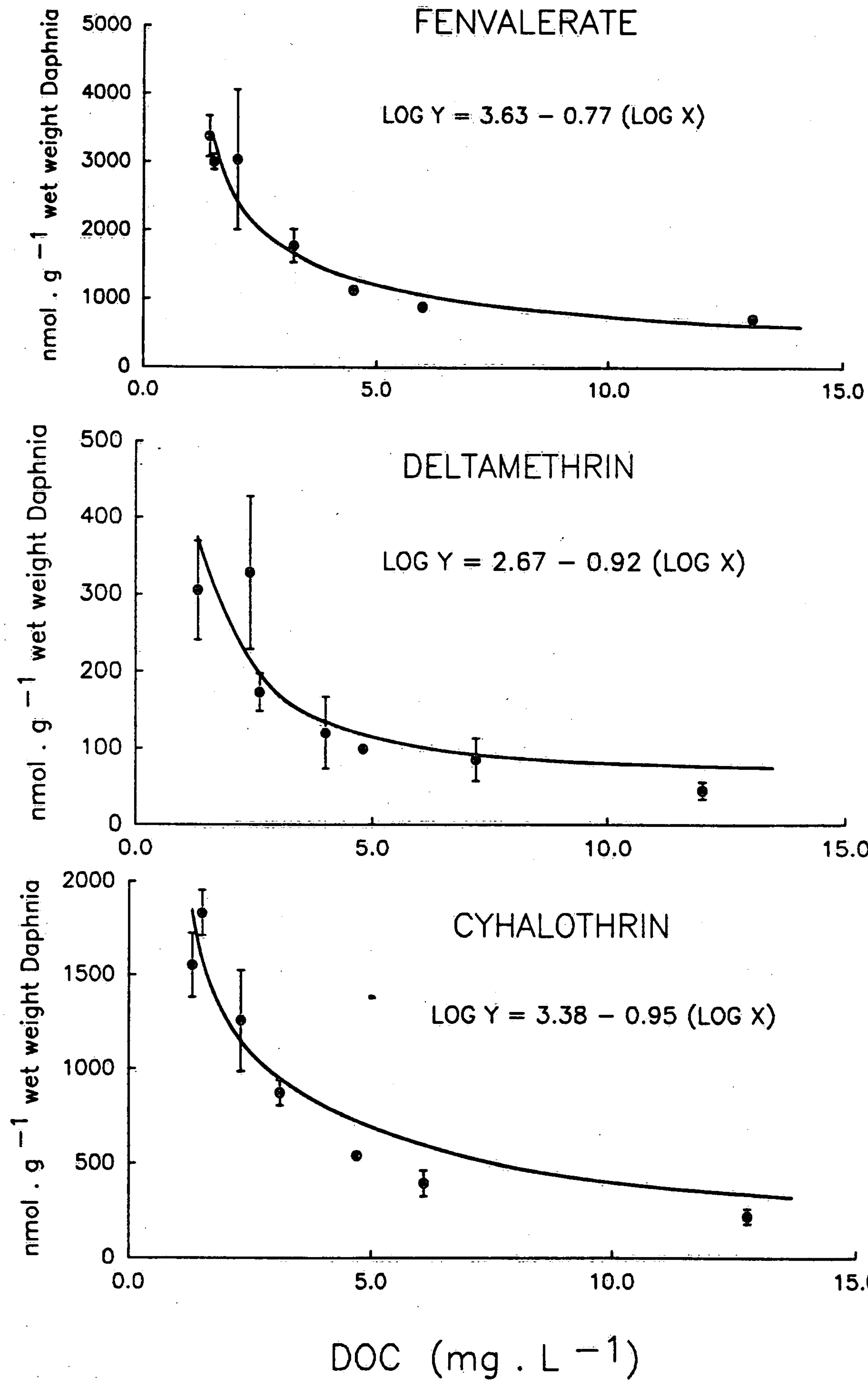
[a] Total DPM applied 67015 ± 1834

[b-f] Mean values followed by the same letter are not significantly different ($P \leq 0.001$)

Table 4. Acute toxicities of cyhalothrin, fenvalerate and deltamethrin to *Daphnia magna* in various Aldrich humic acid concentrations (DOC, $mg.L^{-1}$).

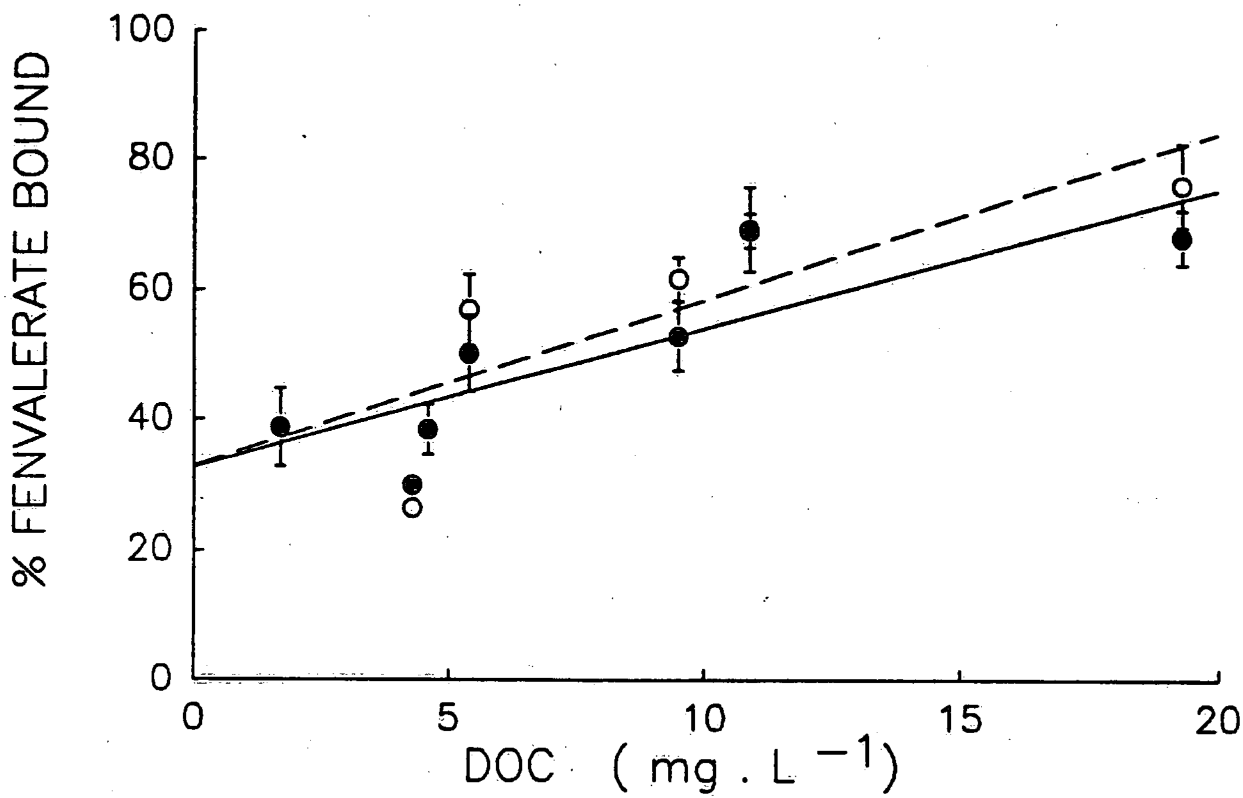
Chemical	DOC $mg.L^{-1}$	24 h EC_{50}	48 h EC_{50}
Cyhalothrin	1.3	0.61 (0.37-1.03)	0.19 (0.12-0.29)
	6.7	2.91 (1.98-4.29)	0.18 (0.11-0.29)
	9.7	2.86 (1.54-5.35)	0.33 (0.23-0.49)
Fenvalerate	3.3	1.14 (0.73-1.79)	0.43 (0.29-0.64)
	8.6	11.04 (7.67-15.91)	0.67 (0.43-1.04)
	15.5	19.50 (9.73-39.04)	3.67 (2.28-6.00)
Deltamethrin	2.2	0.64 (0.25-1.63)	0.05 (0.03-0.09)
	4.5	3.42 (1.81-6.47)	1.01 (0.63-1.61)
	10.1	4.65 (2.51-8.64)	0.85 (0.53-1.34)

Fig. 1. Accumulation of three synthetic pyrethroids by *D. magna* in the presence of increased concentrations of DOC (mg/L) as Aldrich HA.

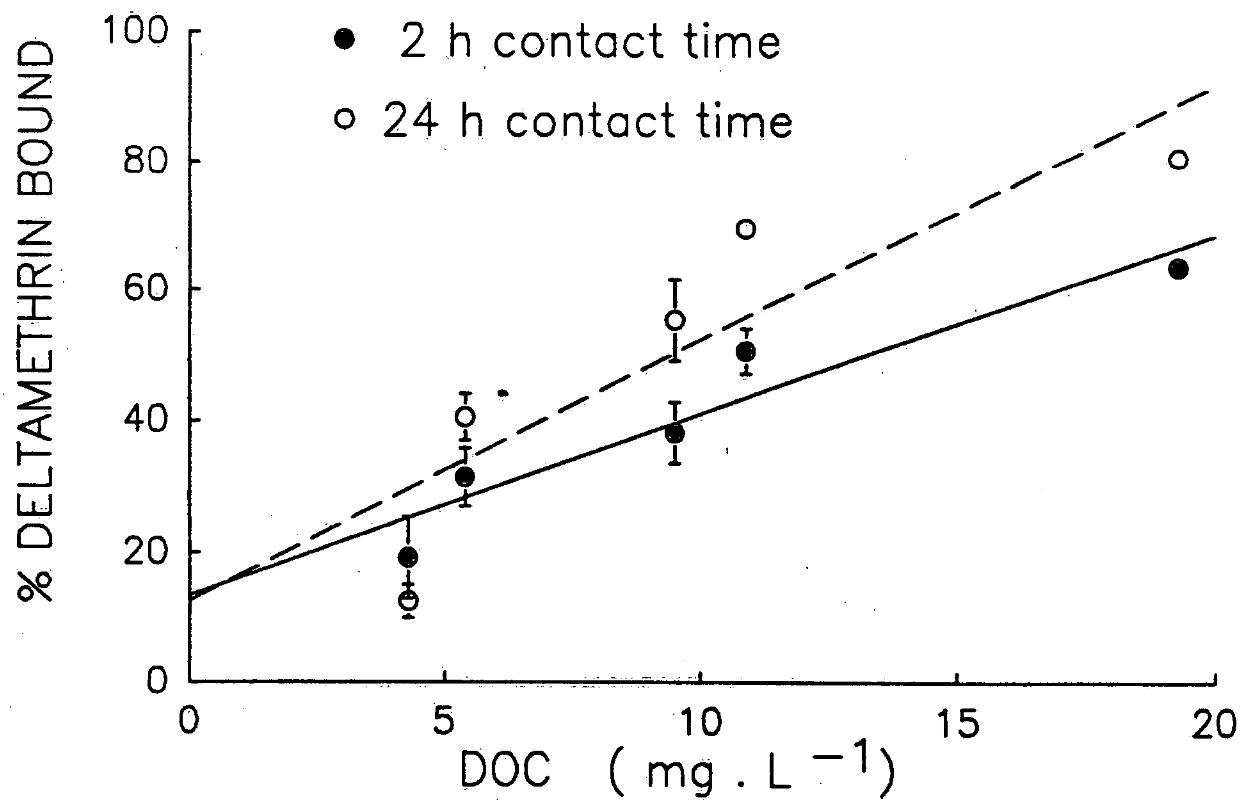


FENVALERATE
LOG Y = 3.63 – 0.77 (LOG X)
DELTAMETHRIN
LOG Y = 2.67 – 0.92 (LOG X)
CYHALOTHRIN
LOG Y = 3.38 – 0.95 (LOG X)
nmol . g −1 wet weight Daphnia
DOC (mg . L −1)

Fig. 2. Percent binding of fenvalerate and deltamethrin to DOC (mg/L) after 2 h and 24 h contact time.



% FENVALERATE BOUND
100
80
60
40
20
0
0
5
10
15
20
DOC (mg . L −1)



% DELTAMETHRIN BOUND
100
80
60
40
20
0
0
5
10
15
20
DOC (mg . L −1)
2 h contact time
24 h contact time

Fig. 3. Effect of DOC concentrations (mg/L) on partition coefficient (K_{rp}) for fenvalerate and deltamethrin.

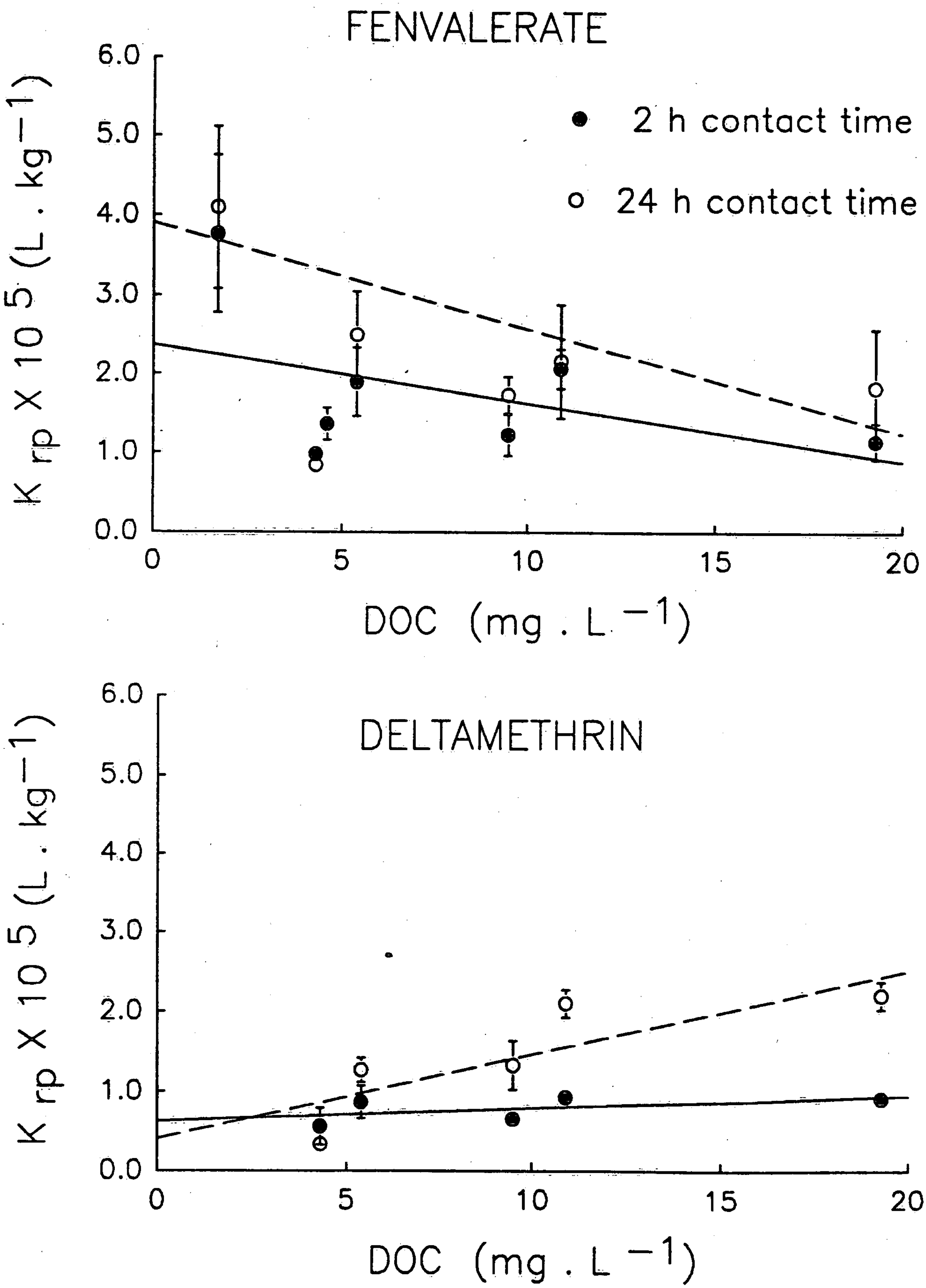
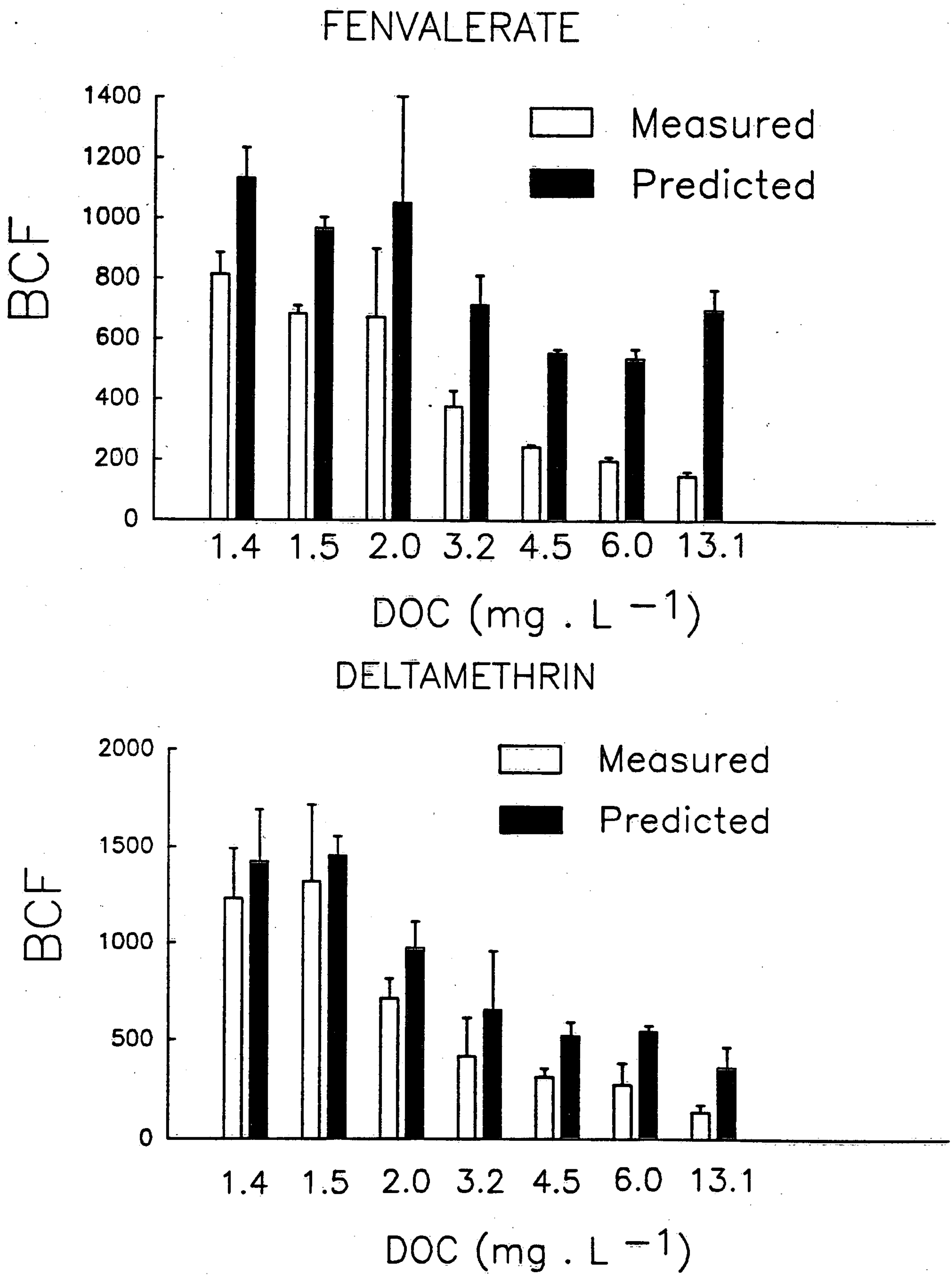

FENVALERATE
2 h contact time
24 h contact time
K rp X 10 5 (L . kg−1)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
5
10
15
20
DOC (mg . L −1)
DELTAMETHRIN
K rp X 10 5 (L . kg−1)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
5
10
15
20
DOC (mg . L −1)

Fig. 4. Comparison of BCF for fenvalerate and deltamethrin in *Daphnia* exposed to differing levels of DOC (mg/L)
a) measured - C_w includes both sorbed and freely dissolved pesticide
b) predicted - C_w = only freely dissolved



FENVALERATE
1400
1200
1000
800
600
400
200
0
BCF
Measured
Predicted
1.4
1.5
2.0
3.2
4.5
6.0
13.1
DOC (mg . L −1)
DELTAMETHRIN
2000
1500
1000
500
0
BCF
Measured
Predicted
1.4
1.5
2.0
3.2
4.5
6.0
13.1
DOC (mg . L −1)