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Relationship between hydrocarbon measurements and toxicity to a chironomid, fish larva and daphnid for oils and oil spill chemical treatments in laboratory freshwater marsh microcosms

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"Capsule": *Hydrocarbon measurements cannot be substituted for actual toxicity determinations.*

Abstract

This research investigated the extent to which various common hydrocarbon measures can be used to predict toxicity to freshwater aquatic organisms due to fouling by oil. Actual toxicity results, on laboratory freshwater marsh microcosms using two water-column species and a benthic species, were described earlier. The hydrocarbon measures used were TPH_g, TPH_{FID}, TPH_{MS}, TTAH (sum of 41 target aromatic hydrocarbons), principal components of 41 TAHs, and each individual TAH. In general, toxicity was more closely related to TPH_{MS} levels than to TPH_{FID} and (especially) TPH_g levels. The strongest relationships were found for TTAH levels and for the principal components of the TAHs. Regressions of toxicity on many individual TAHs were also strong, with a single group of compounds explaining as much as 59% of the variation in survival. While the various regressions were highly significant statistically and at times able to accurately predict broad differences in toxicity, the high variation in survival at a specific hydrocarbon concentration indicates that these hydrocarbon measures can not substitute for actual toxicity determinations in accurately ranking the toxicity of samples from oiled freshwater marshes.

Keywords: Oil; Dispersant; Toxicity prediction; Laboratory microcosm; Hydrocarbon levels

1. Introduction

Aquatic organisms often suffer toxic effects when exposed to oil (for example: Elmgren et al., 1983; Gundersen et al., 1996; da Silva et al., 1997; Jones et al., 1998). Toxic effects may be highly visible (e.g. the case of oil-coated waterfowl), while quantifying effects on other aquatic communities (e.g. the benthos) may require a significant amount of research. It is therefore advantageous if toxicity to aquatic organisms could be predicted from contaminant levels. A quick analysis of hydrocarbon levels following an oil spill could then result in a more direct targeting of clean-up measures. Information on the relationship between various hydrocarbon measurements and toxicity is also needed for optimizing environmental monitoring programs and for interpreting their data.

Various hydrocarbon measurements are conducted in routine monitoring programs and for assessing the impact of oil spills. Commonly used hydrocarbon measurements include: total extractable materials; total petroleum hydrocarbons (TPH); combinations of specific subgroups such as alkanes, alkynes, and aromatics; specific individual polycyclic aromatic hydrocarbons (PAHs), and the sum of various PAH subsets (e.g. Σnaphthalenes, ΣPAHs) (Swartz et al., 1995; Hutcheson et al., 1996; Barron et al., 1999; Mueller et al., 1999). Furthermore, specific variables can be obtained by different methodologies. For example, TPH can be determined by GC/MS, GC/FID, SIMS, infrared detection, UV fluorescence, or gravimetrically (Barron et al., 1999; Wong et al., 1999). Each method has advantages and disadvantages with respect to factors such as detection limit and analytical cost (Hutcheson et al., 1996).

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It is not clear which hydrocarbon measurement best predicts toxicity. There is some consensus on a few general principles. For example, shorter-chain hydrocarbons tend to be more toxic than longer-chained ones, while aliphatic hydrocarbons are relatively nontoxic (Payne et al., 1995). However, we are not aware of freshwater models that predict toxicity on the basis of specific hydrocarbon measurements. The one model that exists for estuarine and marine sediments uses concentrations of 13 PAHs, assumes that effects are additive, and weights the concentrations of the various PAHs on the basis of their toxicity to amphipods or their K_{ow} partition coefficient (Swartz et al., 1995). While this model advances our ability to predict toxicity, it is still fairly simplistic as it is based on toxicity to only one group of benthic organisms and excludes many hydrocarbons as well as interactions between components. The development of freshwater models is hampered by a shortage of data on freshwater toxicity and toxicity interactions for petroleum hydrocarbons. The lack of models using detailed hydrocarbon information to predict toxicity of oil in freshwater ecosystems means that the detail of analysis provided by the GC/MS method may be an overkill. It is possible that gravimetric or GC/FID methods prove to be powerful enough to predict toxicity. On the other hand, the GC/MS approach may eventually allow the refinement of target lists of hydrocarbons.

This paper assesses the predictive power (with respect to toxicity) of various hydrocarbon analyses. Toxicity was determined in a laboratory setting, with microcosms containing fresh marsh sediment and water. These microcosms were studied during a six month period following exposure to common hydrocarbon mixtures and chemical responses. Toxicity was studied after 1, 7, 31 and 186 days following the initial exposures, for a benthic species (the chironomid *Chironomus tentans*) and two water-column species (the water flea *Daphnia pulex* and the fish *Oryzias latipes*). Hydrocarbon content was measured gravimetrically, by GC/FID, and by GC/MS. These analyses were done for microcosm water (to relate it to toxicity in the species residing in the water-column) and for the combination of water and sediment (to relate this to toxicity in the benthic species). Statistical analyses then determined which of the hydrocarbon analyses were best suited for predicting the observed toxicity.

2. Materials and methods

2.1. Overall design and toxicity determinations

A brief summary of the general experimental design and toxicity determination is provided here; more detailed information was published previously (Bhattacharyya et al., 2003). Experiments were conducted in laboratory microcosms made with sediment collected from two freshwater marshes, with each microcosm consisting of approximately 480 ml of a sediment slurry with 320 ml well water added. Following a five month aging/acclimation period, the microcosms were subjected to one of nine different treatments (a combination of three oil treatments and three chemical treatments). The oil treatments consisted of South Louisiana crude oil, diesel fuel, or no oil (control). The chemical treatments consisted of the dispersant Corexit 9500, the cleaner Corexit 9580, or the control—no chemical added. Separate microcosms were used for each sampling point (days 1, 7, 31, and 186 after the addition of the oils and chemical treatments), since the removal of materials for toxicity testing and hydrocarbon analyses destroyed the microcosm. A total of eight replicates were used for each treatment group (four replicates for each of the two marsh soils). Oil and chemical treatments were weathered overnight and added to the microcosms based on weight, equivalent to a pre-weathering volume of 6 ml oil and/or 1.2 ml of dispersant/cleaner per 800 ml microcosm. Toxicity was determined of water (obtained by centrifugation of microcosm content, since floating marshes formed while no clear sediment/water separation occurred in the microcosms) and of the combination of sediment and water taken from the microcosms on days 1, 7, 31, and 186 after the addition of the oils and chemical treatments. The toxicity of the water was assessed on the basis of survival of newborn medaka fish and young daphnids. Sediment toxicity was assessed on the basis of survival of chironomid larva. The toxicity experiments with *D. pulex* lasted 48 h, while the others were 96 h exposures.

2.2. Hydrocarbon analyses

Gross hydrocarbon concentrations were measured four ways: Total Petroleum Hydrocarbons based on gravimetric analysis (TPH_g), TPH based on GC-FID analyses (TPH_{FID}), TPH based on GC/MS analyses (TPH_{MS}), and Target Aromatic Hydrocarbons (TAH) based on GC/MS analyses for 42 TAHs. Gravimetric analyses were done for all samples, while GC/FID and GC/MS analyses were done for a subset of samples because of the high costs of these determinations. The GC analyses were conducted on one of the four replicates of each group for all time points, with the exception of the no-oil treatments for which analyses were limited to the t1 and t186 samples. All hydrocarbon analyses were done for both the aqueous samples (in order to relate these data to toxicity to *O. latipes* and *D. pulex*), as well as for the sediment/water mixture (to relate these data to *C. tentans* toxicity).

Separate microcosms were dismantled 1, 7, 31, or 180 days after adding oils and chemicals. Half of each

microcosm was used for toxicity tests (see Bhattacharyya et al., 2003); the other half was used for hydrocarbon analyses. The water fraction (where used) was obtained by centrifugation of a subsample of the microcosm contents. Samples were generally refrigerated 1 to 5 days before hydrocarbons were extracted, although one initial set of 18 was stored 24 days. All glassware utilized in the extractions was rinsed with 5% acid and pesticide-grade dichloromethane (DCM).

Approximately 60 g of substrate (water and/or sediment) was placed in 500-ml glass flasks. Hexamethylbenzene was added to 244 (of the 576) samples to serve as an internal standard for analyses of Total Petroleum Hydrocarbons (TPH) via GC-FID. Also, internal standards for analyses via GC/MS (p-Terphenal-d14, Acenapthene-d10, and Phenanthrene-d10) were added to 122 of the samples. After adding internal standards (if any), approximately 30 ml of GC-resolve-grade DCM was added to the substrate. This volume of DCM was used because trials indicated that recovery fell below 90% if less DCM was used but recovery only slightly improved when 30 to 50 ml of DCM was used. Flasks were immediately corked with rubber stoppers covered with aluminum foil; the foil was rinsed with pesticide-grade DCM prior to use. Flasks were placed on a shaker table (180 rpm) for at least 16 h. After shaking, the DCM fraction was pipetted from underneath the water fraction and poured through a funnel containing glass wool and $NaSO_4$; this was done to remove water and particulate matter. Extracts were sealed and refrigerated until analyses.

For samples analyzed gravimetrically for TPH, the extract (8 ml on average) was poured into a DCM-rinsed, preweighed glass Petri dish and the DCM was allowed to evaporate under a fume hood. Heat was not used. The mass of the material remaining after evaporation was determined to the nearest 0.001 g. The concentration of TPH_g in the sample was calculated from the mass of the sample, the volume of DCM added to the sample, the volume of DCM evaporated, and the mass of material remaining after evaporation. Gravimetric analyses were not performed on several samples because <10 mL of DCM was recovered and analyses via GC-FID or GC-MS were also intended on those extracts and given priority. Low recovery appeared more common on samples containing the dispersant but this effect was not objectively evaluated. Gravimetric analyses were not performed on two sets of extracts ($n=36$) because they were contaminated with debris during evaporation.

Half of the extracts ($n=244$) were analyzed by GC/MS and GC-FID at the Institute for Environmental Studies at Louisiana State University. Total Petroleum Hydrocarbon concentration was calculated from the sum of peaks identified with either GC-FID ($n=122$) or GC/MS ($n=122$). An initial comparison of estimates from the two detectors on a subset of the samples indicated that the estimates were not correlated ($r=0.0188$, $n=8$, $P=0.8878$). These variables, TPH_{FID} and TPH_{MS} were therefore analyzed separately. The concentrations of 42 target aromatic hydrocarbons (see Table 2) and total target aromatic hydrocarbons (TTAH) were determined via GC-MS in 122 of the extracts.

Hydrocarbon concentrations were expressed on a wet weight basis (μg/g, with the density of both the water and sediment/water samples approaching one) rather than being normalized to organic carbon. Since all microcosms were conducted with sediment from one of two collection sites (sediment from each site was well homogenized prior to placing in the microcosms), OC-normalization would not have greatly affected the results. The organic carbon levels in the sediment were very high, averaging 77.9 and 89.5% for the two sediment types.

2.3. Statistical analyses

The extent to which toxicity (as expressed by survival in the bioassays) was related to different hydrocarbon measures was evaluated with regression techniques. Separate equations were developed for water and sediment/water mixture samples because the substantial difference in toxicity between the water-column and the benthic bioassays prevented analyzing all observations simultaneously. Furthermore, from a biological point of view it is more realistic to relate toxicity for water-column species to hydrocarbon levels in the water samples than to hydrocarbon levels in the sediment. For the two water-column species, we conducted linear regressions of survival (arcsine $\sqrt{p}$ transformed) on hydrocarbon concentrations (log transformed). For the benthic species (where mortality was often 100%), we conducted logistic regressions of survival (using two survival categories; less than 10% and $\geqslant 10\%$ survival) on hydrocarbon concentrations (log transformed). Both linear and logistic regressions were done for TPH_g, TPH_{FID}, TPH_{MS}, TTAH, 41 individual TAHs (one less than the total number analyzed, since anthracene was not detected in any of the samples), and principal components of the TAHs (see below). Because of the large number of regressions (a total of 46) conducted on each set of survival data, we used a Bonferroni adjustment when determining significance with alpha set at 0.0011 (0.05/46).

Since the various concentrations of individual TAHs were highly correlated (e.g. correlations between concentrations among the 41 TAHs measured in the water of the microcosms were as high as 0.997 and averaged 0.413), the use of a multiple regression as a way to combine the various TAHs into one analysis is inappropriate (see e.g. Philippi, 1993). We therefore conducted a principle component analysis to reduce the

large number of variables (41 individual TAHs, log transformed) to a small subset of independent variables. The 75% rule was followed, where the number of factors is chosen as the smallest number that explains at least 75% of the variance in the data. Multiple regressions were then used for determining the relationship between survival (arcsine $\sqrt{p}$ transformed) and the factor loadings for the independent factors.

All statistical analyses were conducted using StatView 5.0 (SAS Institute).

3. Results

3.1. Water-column species

Similar patterns were observed for the daphnid and medaka data, which may reflect that toxicity/hydrocarbon relationships for these two organisms were based on the same hydrocarbon concentrations (measured in water). Moreover, medaka and daphnid survival were highly correlated (Bhattacharyya et al., 2003), with $r = 0.917$ for the group means ($n = 72$) and $r = 0.853$ for the individual data ($n = 286$).

Regressions of survival on hydrocarbon measures were statistically significant for each of the broad hydrocarbon measures (Table 1). For both the daphnid and the medaka, survival was more strongly related to TPH_{MS} than to TPH_{FID} or TPH_g, with the latter showing the poorest relationship (with on average 26% of the variation in survival explained by TPH_g). While TPH_{MS} showed a closer relationship with survival than the other two TPH measures, further improvement was obtained using the sum of the individual concentrations

Table 1
Estimates of the degree to which various broad measures of hydrocarbon concentrations (in microcosms dosed with oil and chemical treatments) were related to survival in daphnids, medakas, and chironomids

	Daphnid	Medaka	Chironomid
TPH_g ($n = 213$-220)	0.285	0.232	0.181
TPH_{FID} ($n = 60$)	0.334	0.287	–
TPH_{MS} ($n = 58$-59)	0.384	0.313	–
TTAH ($n = 57$)	0.500	0.405	0.593
TAH-PCs ($n = 57$)	0.492	0.468	0.627

For daphnids and medakas, standard linear regression was used to regress survival (arcsine Σp transformed) on hydrocarbon concentrations (log transformed) in the microcosm water. However, the regressions on TAH-PCs were multiple regressions on the two principal components extracted from 41 individual PAHs by principal component analysis. For the chironomids, logistic regression was used to regress survival (grouped into categories of less than 10% and $\geqslant 10\%$ survival) on hydrocarbon concentrations (log transformed) in the sediment/water combination of the microcosms. The (logistic) regression on TAH-PCs was again a multiple regression. Shown are the amounts of variation explained by the regressions (R^2 values) where the regressions were statistically significant ($P \leqslant 0.0011$).

Table 2
List of the 42 individual target aromatic hydrocarbons analyzed and estimates of the degree to which their concentrations in microcosms dosed with oil and chemical treatments were related to survival in daphnids, medakas, and chironomids

	Daphnid	Medaka	Chironomid
Naphthalene	–	–	–
C1-Napthalenes	0.368	0.446	–
C2-Napthalenes	0.411	0.416	–
C3-Napthalenes	0.459	0.408	–
C4-Napthalenes	0.486	0.433	–
Fluorene	0.391	0.446	–
C1-Fluorenes	0.489	0.490	–
C2-Fluorenes	0.505	0.459	–
C3-Fluorenes	0.480	0.429	–
Dibenzothiophene	–	–	–
C1-Dibenzothiophenes	–	–	–
C2-Dibenzothiophenes	0.293	0.237	0.588
C3-Dibenzothiophenes	0.236	0.202	0.568
Phenanthrene	0.428	0.437	–
C1-Phenanthrenes	0.501	0.426	–
C2-Phenanthrenes	0.497	0.415	–
C3-Phenanthrenes	0.423	0.363	–
C4-Phenanthrenes	0.274	0.233	0.481
Naphthobenzthiophene	–	–	–
C1-Naphthobenzthiophenes	–	–	–
C2-Naphthobenzthiophenes	–	–	–
C3-Naphthobenzthiophenes	–	–	–
Fluoranthene	–	–	–
Pyrene	0.346	0.246	–
C1-Pyrenes	0.288	0.207	–
C2-Pyrenes	–	–	–
C3-Pyrenes	–	–	–
C4-Pyrenes	–	–	–
Benzo[a]anthracene	–	–	–
Chrysene	–	–	–
C1-Chrysenes	–	–	–
C2-Chrysenes	–	–	–
C3-Chrysenes	–	–	–
C4-Chrysenes	–	–	–
Benzo[b,k]fluoranthene [a]	–	–	–
Benzo[e]pyrene	–	–	–
Benzo[a]pyrene	–	–	–
Perylene	–	–	–
Indeno[1,2,3-cd]pyrene	–	–	–[b]
Benzo[g,h,i]perylene	–	–	–[b]
Dibenzo[a,h]anthracene	–	–	–[b]

For daphnids and medakas, standard linear regression was used to regress survival (arcsine $\sqrt{p}$ transformed) on hydrocarbon concentrations (log transformed) in the microcosm water. For the chironomids, logistic regression was used to regress survival (grouped into categories of less than 10% and $\geqslant 10\%$ survival) on hydrocarbon concentrations (log transformed) in the sediment/water combination of the microcosms. Sample size was 57 for all regressions. Shown are the amounts of variation explained by the regression (R^2 value) where the regression was statistically significant ($P \leqslant 0.0011$)

Samples were analyzed for anthracene but this compound was not detected in any sample.

[a] Benzo[b]fluoranthene and benzo[k]fluoranthrene were combined (to simplify integration).

[b] Logistic regression could not be completed (due to singular error matrix).

of the target aromatic hydrocarbons, with the regressions on TTAH for the daphnid and the medaka explaining respectively 50 and 40% of the variation in survival (Table 1). The principal component analysis on the concentrations of the individual target aromatic hydrocarbons yielded two factors that explained 78% of the total variance in hydrocarbon concentrations. The first factor was closely related to concentrations of the dibenzothiophenes, and to a lesser extend to levels of napthadibenzothiophenes, napthalenes, pyrenes and chrysenes (data not shown). The second factor was closely related to the fluorenes and phenanthrenes. While this method reduced the 41 highly correlated variables to two uncorrelated major variables, the multiple regressions using these two variables had coefficients of determination of just under 50%; similar to those obtained with the regressions on the sum of all the target aromatic hydrocarbons (Table 1).

For the gross hydrocarbon measure that showed generally the best relationship with survival (TTAH), the individual data-points and the regressions were plotted for a visual representation of the degree to which survival corresponded to TTAH concentrations (Fig. 1). These graphs show that while there was indeed a highly significant relationship between these two variables, TTAH concentrations were not good predictors for toxicity, especially at intermediate concentrations where survival ranged from almost zero to 100% at the same TTAH level (Fig. 1).

Many of the individual target aromatic hydrocarbons showed a strong relationship with survival of both the daphnid and the chironomid species, with an absence of clearly different patterns among the two water-column species. The best relationships were observed for alkylated naphthalenes, fluorene, alkylated fluorenes, phenanthrene and alkylated phenanthrenes, with coefficients of determination generally in the range of 0.4–0.5 (Table 2). Statistically significant but weaker regressions (R^2 values in the 0.2–0.3 range) were observed for the C2- and C3-dibenzothiophenes, pyrene and C1-pyrenes. No significant regressions were observed for naphtalene, dibenzothiophene, napthobenzothiophenes, fluoranthene or the TAHs beyond C1-pyrene.

Graphs showing the individual data-points and the fitted regression line were drawn for the TAH that showed on average the closest correlation with survival (C4-naphthalenes). The regressions of survival on the concentration of this TAH group show that there was in general a close relationship between these variables, but also a large amount of variation not explained by the regression (Fig. 2).

3.2. Benthic species

Regressions of chironomid survival on hydrocarbon concentrations were statistically significant for three of the broad hydrocarbon measures (Table 1). The strength of the relationship with TPH_g was fairly low,

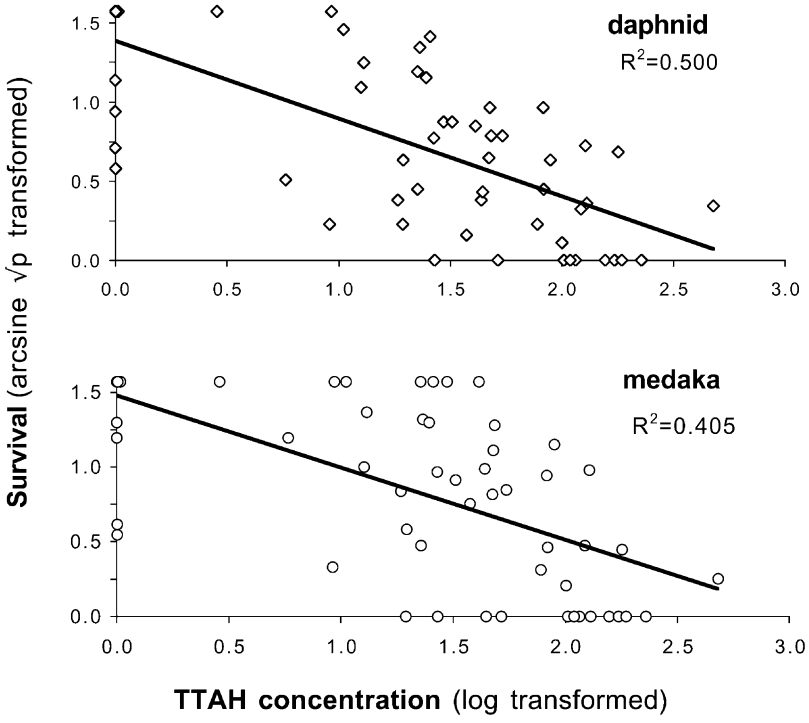

Fig. 1. Relationship between survival (of *Daphnia pulex* and the medaka *Oryzias latipes*) and the sum of the concentrations of 41 target aromatic hydrocarbons (TTAH) in microcosm water. Survival was arcsine $\sqrt{p}$ transformed (100% survival corresponds to a transformed value of 1.57) while TTAH concentrations were log-transformed. Sample size was 57 for each species.

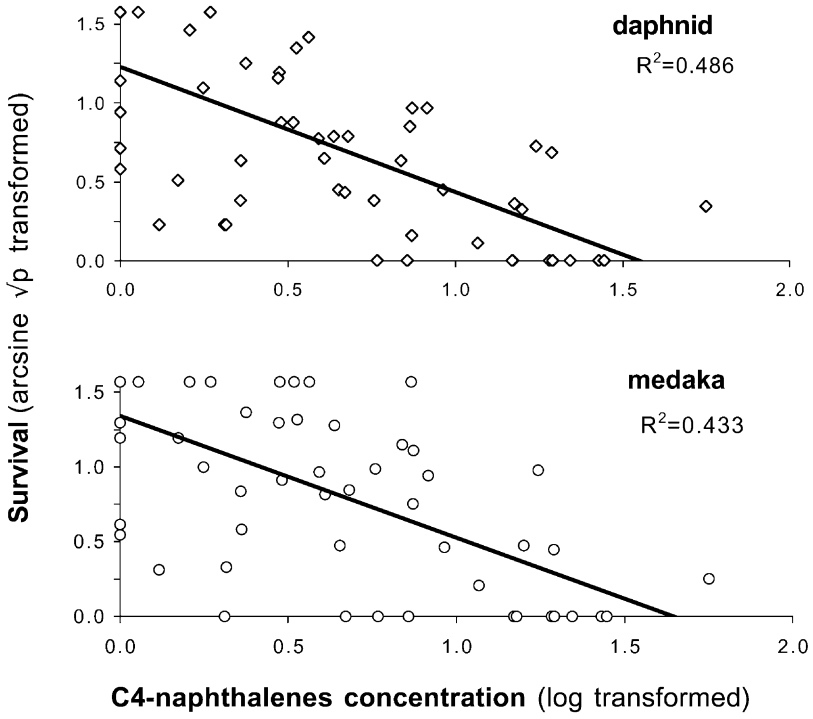

Fig. 2. Relationship between survival (of *Daphnia pulex* and the medaka *Oryzias latipes*) and the concentration of C4-naphthalenes in microcosm water. Survival was arcsine $\sqrt{p}$ transformed (100% survival corresponds to a transformed value of 1.57) while the concentration of C4-naphthalenes was log-transformed. Sample size was 57 for each species.

with the regression explaining only 18% of the variation in survival. In contrast, the regression on the TTAH measure explained substantially more (59%) of the variation in survival. In the procedure where the 41 target compounds were reduced to a small number of independent factors, again only two factors were needed to explain more than 75% of the variance in hydrocarbon concentrations (data not shown). The first factor (explaining 45% of the variance) was closely related to the dibenzothiophenes, naphthobenzothiophenes, alkylated pyrenes and the various chrysenes, while the second factor (explaining 33% of the variance) was closely related to the fluorenes, phenanthrenes and the parent pyrene compound. This procedure resulted in a strong relationship with survival, with the regression explaining 63% of the variation in survival (Table 1).

For the logistic regressions of chironomid survival on concentrations of individual target aromatic hydrocarbons (Table 2), statistically significant relationships ($P<0.0011$) were found for only a few of the hydrocarbons (3 out of 41). For these, two alkylated dibenzothiophenes and an alkylated phenanthrene, R^2 values ranged from 0.48 to 0.59.

To illustrate the predictive power of the logistic regressions, the frequency of correct classifications were quantified for those regressions that were statistically significant (Table 3). With the two survival categories used (less than 10% and $\geqslant 10\%$ survival), the regressions correctly classified survival on the basis of hydrocarbon levels for 91% of the toxicity data on average. Accuracy was higher for predicting low survival ($<10\%$) than it was for predicting higher survival values. Among the different hydrocarbon measures for which the logistic regression was significant, the predictive power was lower for TPH_g than the other measures. The regression on TPH_g concentration was especially poor in accurately predicting survival in the $\geqslant 10\%$ category (Table 3).

Table 3
Accuracy (expressed as % correct classification) for the statistically significant logistic regressions in predicting chironomid survival (grouped into categories of less than 10% and $\geqslant 10\%$ survival) from various hydrocarbon concentrations (log transformed) in the sediment/water combination of the microcosms. $N=57$, except where indicated otherwise

	$<10\%$ (%)	$\geqslant 10\%$ (%)	Overall (%)
TPH_g ($n=213$)	94.3	30.8	82.6
TTAH	93.9	87.5	93.0
TAH-PCs	93.9	87.5	93.0
C2-Ddibenzothiophenes	93.9	87.5	93.0
C3-Dibenzothiophenes	93.9	87.5	93.0
C4-Phenanthrenes	91.8	87.5	91.2

4. Discussion

Our results may appear to indicate that much stronger relationships between toxicity and hydrocarbon measures existed for the water-column species *D. pulex* and *O. latipes* than for the benthic *C. tentans*. However, this may well be a consequence of the different statistical methodologies used. Due to the high mortalities among the chironomids (majority of datapoints with 100% mortality), regular regression analysis was not appropriate. In the logistic regression analysis that was used, specific survival values were placed into two categories and this loss of information reduced the power for detecting significant relationships.

The patterns among the three bioassay species in the strength of regressions for the individual target aromatic hydrocarbons indicate that the sediment-dwelling species was affected by different PAHs than the two water-column species. For example, the concentrations of the alkylated naphthalenes and fluorenes showed a strong relationship with toxicity to the daphnid and medaka, but not to the chironomid. The difference between the two groups of species is not surprising, since the various PAHs will distribute differently between water and sediment and since the two groups of organisms are expected to differ in their PAH uptake routes. The chironomid ingests sediments and is therefore likely to accumulate hydrocarbons both from interstitial water and from the sediment. It is expected that the dissolved fraction of PAHs will play a relatively more important role in the bioaccumulation of PAHs by the water-column species. For an oil spill assessment, this means that the analysis of petroleum hydrocarbons in one compartment within the aquatic environment is unlikely to provide a complete picture of the risk to both water-column and benthic organisms.

The strength of the relationships between concentration of individual PAHs in oiled microcosms and toxicity do not provide a direct estimate of each PAH's contribution to toxicity, since the concentrations of the various PAHs were highly correlated (which explains why the amount of variation in survival explained by all the components sums up to well over 100%; e.g. 690% for the daphnid). However, a high R^2 value for a specific PAH is indicative of this compound having a larger contribution to the overall toxicity than one with a low R^2 value. We are not aware of other analyses of the strength of relationships between concentrations of individual PAHs in oil and toxicity to freshwater organisms. The compounds with the highest R^2 value with chironomid toxicity in our study (C4-phenanthrenes, C2- and C3-dibenzothiophenes) do not include those compounds identified as making the largest contribution to amphipod toxicity in PAH-contaminated marine and estuarine sediments (Swartz et al., 1995). The latter included the benzo[b,k]fluoranthenes, pyrene and fluoranthene. One problem with this comparison is that the amphipod analysis was limited to parent compounds, while our study did include the various groups of alkylated PAHs (for which little toxicity information exists). A study using Microtox® to test for toxicity of oil-contaminated estuarine sediments (Mueller et al., 1999) did not detect a significant relationship for any of the individual PAHs tested. However, the sediment toxicity in the latter study approached the detection limit of the Microtox® assay. In the present study, the occurrence of large differences among individual PAHs in their contribution to toxicity means that using one of them as a reference compound for all aromatics (as was done in e.g. Hutcheson et al., 1996) might well lead to incorrect toxicity evaluations.

Among the gross measures of petroleum hydrocarbon levels, the best relationships with toxicity to the water-column species were obtained for TPH_{MS}, followed by TPH_{FID} and by TPH_g. The latter explained only about 25% of the variance in survival. A relatively poor fit of toxicity with TPH_g levels was also obtained in other studies (Mueller et al., 1999; Wong et al., 1999). While the relationship between toxicity to the benthic species and gross hydrocarbon measures was statistically significant for TPH_g but not for TPH_{MS} or TPH_{FID}, the difference in sample size prevents one from interpreting the differences in significance as evidence of differences in the strength of association.

The sum of all the target aromatic hydrocarbons (TTAH) showed a strong relationship with toxicity, explaining on average 50% of the variation in survival. This results contrasts with results obtained in the Mueller et al. (1999) study with oil-contaminated estuarine sediment, where no relationship between toxicity and TTAH was observed. It is not clear if that difference is indicative of inherent differences between freshwater and estuarine environments, or related to the detection limit issue of the Mueller et al. (1999) study.

Using a more complicated procedure than just adding up the concentrations of the 41 target aromatic hydrocarbons (using a multiple regression with two independent variables obtained by principal component analysis of the target aromatic hydrocarbon data-set) had basically a similar result, with the hydrocarbon data explaining on average 53% of the variability in survival. Adding up the concentrations of all TAHs is of course a rather crude approach, since it is based on the simplistic and unrealistic assumptions that the toxicity of each component is equivalent and that there are no interactions in toxicity between any of the components. The ΣPAH model (Swartz et al., 1995) is in principle an improvement, since it includes information on the relative toxicity of the various PAHs. However, an equivalent model has not yet been developed for freshwater organisms, while the ΣPAH model assumes that toxicities are additive and ignores alkylated PAHs. It is well

known that the alkylated PAHs contribute to oil toxicity (see e.g. McAuliffe, 1987). The use of ΣPAH or TTAH as a predictor of toxicity assumes that aromatic hydrocarbons are a major determinant of oil toxicity; which may not hold true for some oils (Barron et al., 1999).

For several TAHs (especially alkylated naphthalenes, fluorenes and phenanthrenes), regressions of survival on concentrations of individual TAHs had coefficients of determination that were equivalent to those of the TTAH measure. This means that measuring just one PAH may provide as much information on toxicity as can be obtained by measuring a large number of PAHs. This relationship between concentration of one PAH in an oil-contaminated sample and that sample's toxicity is mostly correlative, since many other PAHs in that sample contribute to the toxicity. Moreover, components other than PAHs, such as alkanes, may be causing toxicity (Peterson, 1994). The phenomenon that an individual contaminant in a complex mixture (especially when concentrations of components are highly correlated) shows a strong relationship with toxicity but is responsible for only a small proportion of the toxicity, has been referred to as "the mixture paradox" (Swartz, 1999). This is not really a problem, as long as it is kept in mind that the relationship between a single PAH in a complex mixture and that mixture's toxicity is not necessarily a causal relationship.

The relationships between toxicity and the hydrocarbon measures used in this study were at best able to explain about 60% of the variation in survival. The importance of unidentified factors became more evident when the regressions were plotted together with the individual data-points. This large amount of unexplained variability has also been observed in other studies (e.g. Swartz, 1999) and means that while the hydrocarbon measures used here do provide information on potential toxicity, these measures can not accurately rank toxicity of samples and can only provide a general guideline. The logistic regression analyses on the chironomid data showed that a gross prediction of toxicity (distinguishing between very low survival and survival of 10% and higher) on the basis of hydrocarbon levels, could be obtained relatively accurately. Instead of using chemical data to predict toxicity to water-column and benthic animals, it may potentially be possible to estimate toxicity on the basis of effects on other ecosystem components such as vascular plant and microbial communities. However, observations that vascular plants are fairly tolerant of crude oil (e.g. Lin and Mendelssohn, 1996), and that microbial communities are temporarily stimulated by crude oils (Nyman, 1999) preclude using indices of vegetative health and microbial activity as a basis of ranking animal toxicity among sites. It therefore appears that current insights do not allow us to precisely quantify impacts at oil-contaminated freshwater sites without doing the actual toxicity measurements. Efforts to increase the number of compounds identified and quantified, combined with additional experiments relating toxicity to chemical concentrations and the toxicity interactions between the various compounds, should eventually provide the information needed to predict toxicity on the basis of chemical analyses.

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