PREDICTING THE ACUTE AND CHRONIC EFFECTS OF PAHs USING THE TARGET LIPID MODEL OF NARCOTIC TOXICITY: IS A NEW PARADIGM NEEDED?

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Introduction

The narcosis target lipid model (NTLM) (Di Toro et al. 2000) was developed to predict the acute and chronic aquatic toxicity of chemicals that act via narcosis to aquatic organisms, namely Type I narcotics such as alcohols, aliphatic and monocyclic hydrocarbons and monocyclic and polycyclic aromatic hydrocarbons (PAHs). The majority of the data focused on mortality as the acute endpoint. An acute-to-chronic ratio (ACR) was then applied to convert the acute endpoint to a chronic endpoint. The ACRs were based on chronic effects of mortality, reproduction and growth.
In a recent paper by Ott et al (2002) a new paradigm for oil toxicity is offered that claims oil is toxic to fish at total PAHs levels as low as 1 µg/L. At this concentration sub-lethal effects similar to blue sacs disease (BSD) symptoms (i.e. edemas, hemorrhaging, heart abnormalities) are observed. This "new paradigm" for aquatic toxicity from oil is not new at all. The NTLM included FCVs for individual PAHs, which ranged from 528 µg/L for acenaphthylene and 0.48 µg/L for dibenzo(a,h)anthracene. These FCVs are the concentrations of the individual PAHs that would be predicted to cause long-term effects. The predicted toxicity varies as a function of log (K_{ow}) such that toxicity increases with increasing log (K_{ow}). The PAHs that have a log(K_{ow}) greater than 6.0, have FCVs in the range of 1 µg/L.

The main difference between the new paradigm and the NTLM is the normalization of the aquatic toxicity of PAHs in mixtures. In the Ott et al. paradigm, the aquatic toxicity of individual PAHs is assumed to be the same. The chronic aquatic toxicity due to total PAHs is 1 µg/L, regardless of which PAHs are present. This is incorrect because it assumes that the aquatic toxicity from the individual PAHs is similar. The toxicity of the sample depends on which PAHs are present. Converting the concentrations to toxic units normalizes the differences in toxicity. A concentration of 1 TU from total PAHs implies toxicity. A concentration of 1 µg/L of total PAHs is not necessarily toxic.

This research focuses on applying the NTLM to predict the acute and chronic effects from PAHs both from single chemical exposure and from mixtures of PAHs such as those that would result from an oil spill. The chronic effects investigated include the more common effects of mortality, reproduction and growth as well as BSD symptoms. Data analysis indicates that the NTLM is protective of the organism from BSD symptoms. In addition, it will be shown that the NTLM correctly predicts effects from the heavier PAHs at the low ppb level. No new paradigm is needed to explain the observed chronic toxicity of PAH mixtures.

Methodology

Toxicity Prediction- Narcosis Target Lipid Model

The water-effect concentrations, $C^*_{w,i}$, for each PAH are computed from the NTLM that accounts for variations in toxicity due to species sensitivities and chemical differences. The model is for Type I narcotic chemicals and is based on the inverse relationship between toxicity in water-only exposures, as defined by the LC50 (mmol/L) and the log K_{ow}.

$$\log(LC50) = \log(C_i^*) + \Delta c - m\log(K_{OW}) \quad (1)$$

where C_i^* is the critical target lipid body burden (CTLBB) (µmol/g octanol = µmol/g lipid), the concentration in the organism that causes mortality to 50% of the population, *m* is the universal narcosis slope (-0.936), and Δc is the chemical class correction factor, where Δc = -0.352 for PAHs since they were found to be slightly more toxic than baseline narcotics. Note that these coefficients reflect a recent update to the NTLM.

Toxic units are incorporated to assess the toxicity of PAH mixtures. In water, a toxic unit (TU) is defined as

$$TU_{W,i} = C_{W,i} / C^*_{W,i} \quad (2)$$

where $C_{w,i}$ is the measured concentration of chemical *i* in the water and $C^*_{w,i}$ is the effect concentration for chemical *i*. Typically, $C^*_{w,i}$ is the LC50 for chemical *i*. The molar concentrations are used in computing toxic units to normalize molecular weight differences between compounds in the mixture. For each chemical in the mixture, the individual toxic units are computed using Equation 2. The individual toxic units are then summed to compute the toxicity of the mixture

$$TU = \sum_i TU_{W,i} \quad (3)$$

Effects are predicted TU > =1

An acute to chronic ratio (ACR) is used to convert the acute value into a chronic effect concentration.

$$LC50_{chronic} = LC50_{acute} / ACR \quad (4)$$

Uncertainty in Toxicity Predictions - Uncertainty in Narcosis Target Lipid Model

Methodology presented by McGrath et al. (2004) was incorporated to compute 95% confidence limits in the species-specific water effect concentrations (Equation 1), which depend on the variance in the universal narcosis slope, CTLBB and ACR

$$\log(LC50_{5,chronic}) = E\{m\log(K_{OW})\} + E\{\log(C_L^*)\} - E\{\log(ACR)\} - k_Z\sqrt{V\{m\log(K_{OW})^2\} + V\{\log C_L^*\} + V\{\log(ACR)\}} \quad (5)$$

$$\log(LC50_{95,chronic}) = E\{m\log(K_{OW})\} + E\{\log(C_L^*)\} - E\{\log(ACR)\} + k_Z\sqrt{V\{m\log(K_{OW})^2\} + V\{\log C_L^*\} + V\{\log(ACR)\}} \quad (6)$$

where the E and V terms represent respectively the mean and variance of the slope, CTLBB and ACR, and k_z is the 95% confidence sample size-dependent extrapolation factor. The ACR terms are only considered when computing chronic effect concentrations

Model Validation: Single PAH-Acute Exposures

The NTLM is applied to predict the acute effects, meaning lethality, from exposure to single PAHs. Acute toxicity data were considered for species for which CTLBB were available. There are a total of 148 data points from 20 different species. Comparison of the predicted and observed LC50s for PAHs are shown below (Figure 1). For the data shown, 96 percent of the predicted values (140 out of 146) fall within a factor of 5 of the observed values

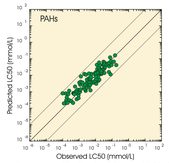

Figure 1. Comparison of Predicted and Observed LC50 for PAHs

Model Validation: PAH Mixtures-Acute Exposures

There is a significant amount of published data relating toxicity and exposure to oils, which is really exposure to a mixture of PAHs. However, the majority of those data are not useful in the TLM application because the authors report the toxicity as total PAH or total petroleum hydrocarbons on a mass basis, i.e., mg/L. For use in the TLM, the concentrations of the individual components or individual PAHs in the oil are needed for computing toxic units (TUs) (Equation 2). Concentration data were available for two sources where the acute toxicity from exposure to PAH mixtures was determined.

Source 1: *The toxicity of water-soluble fractions (WSFs) prepared from neat (unweathered) and naturally weathered Exxon Valdez crude oil was obtained from William Stubblefield (ENSR, unpublished data). WSFs were prepared from 10:1 (water: oil) solutions. Each WSF was analyzed for BTEX, biphenyl, 19 parent PAHs and 21 alkylated homologs of parent PAHs. Fathead minnow (Pimephales promelas) bioassays (48-hr) were conducted on dilutions of the WSF.*

Source 2: *Barata et al. (2005) tested the toxicity of a mixture of 9 PAHs (naphthalene, 1-methylnaphthalene, 1,2-dimethylnaphthalene, phenanthrene, pyrene, fluorene, 1-methylphenanthrene, dibenzothiophene and fluoranthene). Mortality of the adult copepod, Oithona davisae, was measured after 48 hours of exposure.*

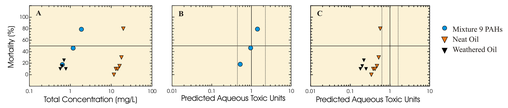

Figure 2. The observed mortality as a function of the total concentration (mg TMC/L) in each treatment is shown above (panel A). The observed mortality normalized to toxic units is presented for *O. davisae* (panel B) and *P. promelas* (panel C). The solid lines indicate a TU equal to 1, where 50 % effects are expected. The dashed lines are the 5th and 95th percentiles based on variations in species-specific CTLBB (Equations 5 and 6, without ACR terms).

Model Validation: Single PAH-Chronic Exposures *(growth, reproduction, mortality)*

The mechanism for acute effects from PAHs is most likely narcosis. The mechanism(s) for chronic effects, is unknown. To derive chronic effects, the TLM incorporates an ACR. In computing the ACRs, the chronic effects that are considered are only those that the EPA deems will impact an organism's ability to survive (growth effects, reproduction effects and long-term mortality). To determine if the application of ACRs is appropriate for PAHs, the distributions of ACRs for non-PAH hydrocarbons (e.g. benzene, cyclohexane, pentane, etc.) and PAHs were compared. The data are provided in Table 1. The ACRs probability distributions for non-PAH hydrocarbons and PAHs are very similar. Since the ACRs are similar for hydrocarbons and PAHs the mechanism(s) for chronic effects for both hydrocarbons and PAHs is also similar. Therefore, using ACR to compute chronic effect levels is appropriate for PAHs.

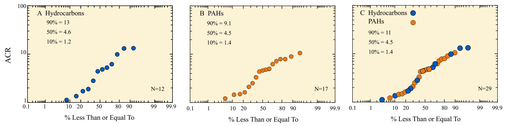

Figure 3. ACR Probability Distributions for Hydrocarbons (A), PAHs (B) and Combined Hydrocarbons and PAHs (C)

Table 1. **Database of acute and chronic values used in development of ACRs.**

Species	Chemical	Acute LC50/EC50 (µg/L)	Chronic Endpoint	Chronic (µg/L)	ACR	Source
Cyprinodon variegatus	Acenaphthene	3,100	survival	710	4.36	Ward et al., 1981
Daphnia magna	Fluoranthene	117	growth	24.5	4.78	Spehar et al., 1999
	Phenanthrene	117	survival and reproduction	96.4	1.21	Call et al., 1986
	Biphenyl	362		322	1.12	Gersich et al., 1989
	Methylterbutylether	472,000	not specified	42,000	11.2	Mancini et al., 2002
Mysidopsis bahia	Acenaphthene	466	reproduction	286	1.63	Horne et al., 1983
	Acenaphthene	460	reproduction	64.0	7.19	Thursby et al., 1989
	Fluoranthene	30.5	survival and reproduction	14.4	2.11	Spehar et al., 1999
	Fluoranthene	40.0	survival and reproduction	15.9	2.52	U.S. EPA, 1978
	Phenanthrene	27.1	survival	8.13	3.33	Kuhn and Lussier, 1987
	Pyrene	28.3	reproduction	4.53	6.24	Champlin and Poucher, 1992
	Methylterbutylether	187,000	not specified	36,000	5.2	Mancini et al., 2002
Onchorhyncus mykiss	Phenanthrene	50.0	survival and reproduction	6.32	7.91	Call et al., 1986
Paratarytarsus species	Acenaphthene	2,040	survival, growth and reproduction	411	4.96	Northwestern Aquatic Sciences, 1982
	Acenaphthene	2,040	survival and growth	227	8.97	Northwestern Aquatic Sciences, 1982; Thursby 1991
Pimephales promelas	Acenaphthene	608	growth	405	1.50	Cairns and Nebeker, 1982; Thursby 1991
	Acenaphthene	608	growth	419	1.45	Cairns and Nebeker, 1982
	Naphthalene	7,900	survival and growth	620	12.7	DeGraeve et al., 1982
	Fluoranthene	69.0	survival and growth	15.0	4.60	Spehar et al., 1999
	Methylterbutylether	980,000	not specified	289,400	3.39	Mancini et al., 2002
Brachionus calyciflorus	Xylene	248,500		40,300	6.17	Snell and Moffat, 1992
Ceriodaphnia dubia	Toluene	3,750		2,780	1.35	Niederlehner et al., 1998
	Ethylbenzene	3,200		1,680	1.90	Niederlehner et al., 1998
Selenastrum capricornutum	Benzene	100,000	biomass growth	8,300	12	Mayer et al., 2001
	Cyclohexane	9,317	biomass growth	952	9.8	Exxon Biomedical Sciences, 1998
	Methylterbutylether	491,000	biomass growth	103,000	4.8	European Commission, 2000
	Pentane	10,700	biomass growth	2,040	5.2	Exxon Biomedical Sciences, 1997
Skeletonema costatum	Ethylbenzene	7,700		4,500	1.7	Boeri, R. 1987
Onchorhyncus mykiss	Naphthalene	1,200	growth	380	3.2	Moles and Rice, 1983

Model Validation: Single PAH Chronic Exposure - Other Sub-lethal Effects

There is a significant amount of literature that suggests early life-stage (ELS) exposure to PAHs causes blue sacs disease like symptoms, such as yolk sac edemas, pericardial edemas, and spinal deformities. These sub-lethal effects were not included in the development of the ACRs and as such, the ACRs may not be protective of these types of effects. To determine if the TLM is protective of these types of effects, relevant literature was reviewed. Data were only included if the reported concentrations were below solubility (Table 2). That the "new paradigm" of 1µg/L total PAH causing ELS sub-lethal effects is not universally applicable. Toxicity is dependent on species and the chemical.

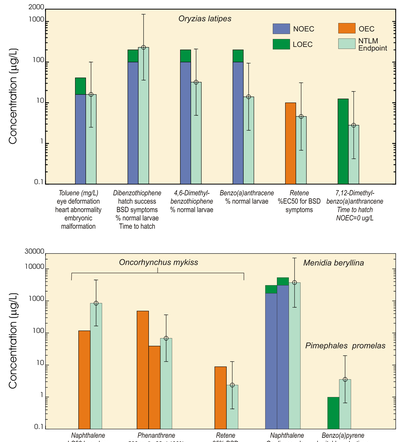

Figure 4. Comparison of NOEC/LOEC/OEC from ELS exposures for single PAH and toluene to TLM predicted chronic effect concentrations. The symbols represent the average predicted concentration. The bars represent the 5th and 95th percentiles and are based on variation in CTLBB for a species and variation in ACR. The statistics for the ACR were determined using only ACR for PAH and MAH. For all cases, the TLM was protective of these types of sub-lethal endpoints.

Table 2. Comparison of narcosis target lipid model chronic endpoints to NOECs, LOECs, OECs (sub-lethal effects) from early stage tests for single PAH exposures

Species	Life Stage	Chemical	Experiment Type	Measurement Type	Effects	Observed Concentration	NTLM Effect Concentration (µg/L)	HC5 (µg/L)	HC95 (µg/L)	Reference
Oryzias Latipes (Japanese medaka)	embryos and fry	Toluene	Static	Measured	deformation of eyes embryonic malformation heart abnormally	LOEC = 41 mg/L; NOEC = 16 mg/L	16 mg/L	2.5 mg/L	100 mg/L	Stoss and Haines, 1979
	embryo	Retene	PCD (partition controlled delivery) - constant	Measured	17 day EC50 for BSD	10 µg/L	4.6	0.68	31	Kiparissis et al 2003
	ELS - embryos	dibenzothiophene	Static renewal	Nominal	Hatching success; time to hatch; BSD symptoms; % normal	LOEC = 200 µ NOEC = 100 µ	230	36	1500	Rhodes et al 2005
	ELS - embryos	benzo(a) anthracene	Static renewa l	Nominal	18 d % normal	LOEC = 200 µ NOEC = 100 µ	14	2.1	94	Rhodes et al 2005
	ELS - embryos	dimethyld benzothiophene	Static renewal	Nominal	18 d - % normal	LOEC = 200 µ NOEC = 100 µ	32	4.9	210	Rhodes et al 2005
	ELS - embryos	7,12-dimethylbenz (a)anthracene	Static renewal	Nominal	18 d - time to hatch	LOEC = 12.5 µg/L; NOEC = 0 µg/L	2.8	0.42	19	Rhodes et al 2005
Pimephales promelas (fathead minnow)	larvae	Benzo(a)pyrene	Static renewal	Nominal	heritable reductions in larvae survival LOEC	1 µg/L	3.6	0.66	20	White et al 1999
Oncorhynchus mykiss (Rainbow trout)	embryo - larval	Naphthalene	Flow -through	Measured	27 day LC50; note that pro sity deformed larvae counted as dead	120 µg/L	880	170	4700	Black et al 1983

Model Validation: PAH Mixtures Chronic Exposures - Sub-lethal Effects

Two studies linking ELS exposure to PAH mixtures and sub-lethal effects are presented.

Study 1: Rhodes et al. (2005) determined the effects of PAH mixtures on embryonic development. In this study, *O. latipes* was exposed for 18-d to three mixtures of PAHs. One mixture contained three parent PAHs, phenanthrene, dibenzothiophene and benzo(a)anthracene. Another mixture contained three dimethylated PAHs, 3,6-dimethylphenanthrene, 4,6-dimethyldibenzothiophene and 7,12-dimethylbenzo(a)anthracene. The last mixture was an oil sands extract. On this extract, the concentrations of 16 PAHs and their alkylated homologs were measured. NOEC and LOEC were reported when effects were observed. The oil sands extract was the only mixture that induced BSD symptoms.

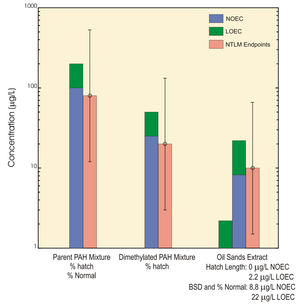

Figure 5. A comparison of the observed and predicted effect concentrations for the three mixtures is shown below. For the TLM, the symbols represent the predicted concentration. The bars represent the 5th and 95th percentiles and are based on variation in CTLBB for a species and variation in ACR. The statistics for the ACR were determined using only ACR for PAH and MAH. For all cases, the TLM was protective of these types of sub-lethal endpoints.

Study 2: Carls et al. (1999) investigated the sensitivity of Pacific Herring eggs (*Clupea pallasi*) to seawater contacted with weathered Alaskan North Slope crude oil. Two 16 day treatments were conducted. The first 16 days is referred to as the less weathered oil (LWO) exposure. The second 16 days is referred to as the more weathered oil exposure (MWO). For this study, the concentration of PAHs varied with time. The concentrations of biphenyl and 38 PAHs were measured throughout the exposure period. In addition to larvae and egg death, a variety of sub-lethal responses were monitored.

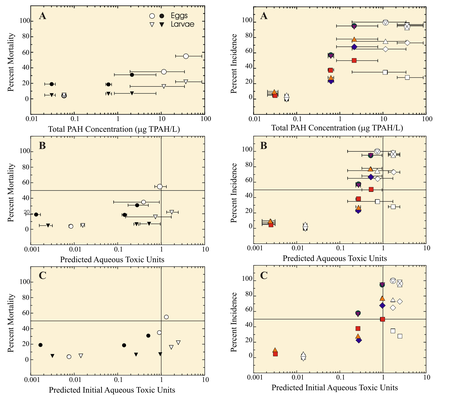

Figure 6. Comparison of percent incidence of various sublethal biological responses of herring eggs and larvae as a function of total PAH concentration (µg TPAH/L) (A) and normalized to predicted aqueous toxic units (B) for all treatments in Carls et al. 1999. The bottom panel (C) shows the percent mortality as a function of the predicted aqueous toxic units using the initial concentration data. The open and filled symbols are for data from the less weathered oil and more weathered oil tests, respectively (○ ● yolk sac edema; ▽▼ reduced jaw size; □■ pericardinal edema; ◇◆ spinal defects; △▲ non-effective swimmers). The symbols represent the average concentration over the exposure period. The bars represent the minimum and maximum concentrations.

Conclusions

- The NTLM can be applied to predict the acute toxicity of PAHs in single exposures and as mixtures
- The application of an ACR to convert acute effect concentrations to chronic effect concentrations is appropriate for PAHs
- The NTLM is protective of sub-lethal effects resulting from early life stage exposure to PAHs
- The "new paradigm" that chronic aquatic toxicity due to total PAHs is 1 µg/L is incorrect because it assumes that the aquatic toxicity from the individual PAHs is similar. The toxicity of the sample depends on which PAHs are present.
- Converting the mass concentrations to toxic units normalizes the differences in toxicity. A concentration of 1 TU from total PAHs implies toxicity. A concentration of 1 µg/L of total PAHs is not necessarily toxic. Toxicity is dependent on species and chemical.

References available on request (jmcgrath@hydroqual.com)

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