PHOTOTOXIC TARGET LIPID MODEL OF SINGLE POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract: A phototoxic target lipid model (PTLM) is developed to predict phototoxicity of individual polycyclic aromatic hydrocarbons (PAHs) measured either as median lethal concentration (LC50) or median lethal time (LT50) for a 50% toxic response. The model is able to account for the differences in the physical/chemical properties of PAHs, test species sensitivities, and variations in light source characteristics, intensity, and length of exposure. The PTLM is based on the narcotic target lipid model (NTLM) of PAHs. Both models rely on the assumption that mortality occurs when the toxicant concentration in the target lipid of the organism reaches a threshold concentration. The PTLM is applied to observed LC50s and LT50s for 20 individual PAHs, 15 test species—including arthropods, fishes, amphibians, annelids, mollusks, and algae—exposed to simulated solar and various UV light sources, for exposure times varying from less than 1 h to 100 h, a total of 333 observations. The LC50 concentrations range from less than 0.1 µg/L to greater than 106 µg/L. The model has 2 fitting parameters that are constant and apply to all PAHs and organisms. The root mean square errors of prediction for log(LC50) and log(LT50) are 0.473 and 0.382, respectively. The results indicate that the PTLM can predict the phototoxicity of single PAHs over a wide range of exposure conditions and to organisms with a wide range of sensitivities. Environ Toxicol Chem 2016;9999:1–12. © 2016 SETAC

Keywords: PAHs Phototoxicity modeling Phototoxic target lipid model Toxic unit addition Photon absorption

INTRODUCTION

Phototoxicity is the toxicity exhibited by a chemical in the presence of certain wavelengths of light. Phototoxic polycyclic aromatic hydrocarbons (PAHs) absorb light in the ultraviolet (UV) region and, to some extent, in the visible (VIS) region. The effect of light absorption on the toxicity of PAHs has been reported since the late 1920s [1]. Subsequently, the toxic effect of PAHs sensitizers in mammalian models in the presence of UV light was recognized [2,3], resulting in the study of the occurrence, mechanism of action, and predictability of phototoxicity of PAHs to various organisms. Arfsten et al. [4], Diamond [5], and Giesy et al. [6] present comprehensive reviews describing toxicological interactions between PAHs and light.

There are few studies that predict the phototoxicity of PAHs to aquatic organisms. Morgan and Warshawsky [7] investigated the photodynamic immobilization of Artemia salina nauplii coexposed to 41 PAHs and monochromatic UV light. The rate of immobilization was determined to be proportional to the amount of light absorbed by the compound with a proportionality constant \( \phi_i \) for a compound \( i \), which was termed the “quantum yield for immobilization.” The relative photodynamic activity was defined as the ratio of \( \phi_i \) to that of a reference compound \( \phi_0 \) (benzo[c]acridine). Newsted and Giesy [8] and Oris and Giesy [9] investigated the photo-enhanced toxicity of PAHs and their relative potencies to Daphnia magna and Pimephales promelas larvae, respectively. Potency \( \Phi \) was defined as a rate of mortality per unit time per unit of absorbed irradiance [8,9]. The relative potency factor (RPF) for each PAH was defined as the ratio of its potency factor to the potency factor for the reference compounds benzo[b]anthracene [8] and benzo[a]pyrene [9]. This was similar to the method used by Morgan and Warshawsky [7] to calculate relative photodynamic activity, except that the estimated internal PAH concentrations were used rather than the ambient water concentrations. Mekenyan et al. [10] reanalyzed the data presented by Newsted and Giesy [8] and found that PAHs exhibiting photo-enhanced toxicity to \( D. \ magn\) a exhibited a HOMO-LUMO (highest occupied molecular orbital energy–lowest unoccupied molecular orbital energy) window of 7.2 ± 0.4 eV and proposed using the HOMO-LUMO gap as an indicator of PAH phototoxicity in \( D. \ magn\) a.

Veith et al. [11] studied the effects of substituents on the HOMO–LUMO gap of PAHs. Alkyl and hydroxyl substituents did not change the HOMO–LUMO gap of PAHs significantly, whereas nitro, alkane, and chloro substitution on PAHs had considerable effects on the HOMO–LUMO gap. A window of 7.75 ± 0.25 eV was suggested by Veith et al. [11] for the substituted PAHs as their phototoxicity indicator.

Oris and Giesy [12] developed a model for the phototoxicity of anthracene to Lepomis spp. at varying light intensities and aqueous concentrations using the Bunsen–Roscoe law of reciprocity. The Bunsen–Roscoe law states that for a fixed concentration of the sensitizer—in this case, anthracene—a biological effect is proportional to the product of light intensity and reaction time [13]. Regression analysis of the experimentally determined log(median lethal time [LT50] × UV intensity) versus the log of the corresponding anthracene aqueous

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concentration did not produce the common slope and intercept that was expected. The applicability of the Bunsen–Roscoe law to PAHs phototoxicity was rejected as a result of the variability of slopes and intercepts. Ankley et al. [14] applied the Bunsen–Roscoe law to *Lampris variatus* co-exposed to UV light and fluoranthene, anthracene, or pyrene [15]. The experimentally determined log(LT50) values were proportional to the log(initial residue × UV intensity) using measured tissue residues of PAHs in the organism, contrary to the aqueous concentration used in the model by Oris and Giesy [12]. Ankley et al. [14,15] reported considerable variations among the slopes and intercepts for the 3 PAHs tested, whereas Bunsen–Roscoe law predicted common slope of −1.0. The Bunsen–Roscoe law was also applied to predict additive phototoxicity of binary mixtures of fluoranthene, anthracene, and pyrene by Erickson et al. [16]. Similar to Ankley et al. [14,15], the observed slopes of log(LT50) versus log(initial residue × UV intensity) deviated from −1.0.

Krylov et al. [17] and Huang et al. [18] developed a quantitative structure–activity relationship (QSAR) model to explain the toxicity of 16 PAHs to *Lemna gibba* under simulated solar radiation on the basis of photosensitization reactions and direct toxicity of the photomodification products. To estimate the component of toxicity attributable to photosensitization reactions, the toxicity of the parent PAHs was used. The toxicity of the photomodified PAHs was determined by irradiating the test solution prior to incubation. A photosensitization factor and a photomodification factor were calculated for each PAH investigated [18]. The sums of the photosensitization and photomodification factors were correlated to a measure of relative toxicity that was calculated from the growth rate inhibition induced by exposure to 2 mg/L of each PAH. However, this concentration was above the water solubility of the PAHs of interest. The possible shortcomings of predictions based on the measurements in supersaturated solutions and mechanisms to cope with issues of PAHs water solubility and the associated toxicity are discussed in the section PAHs’ aqueous solubility.

Grote et al. [19] developed a predictive phototoxicity model for the green alga *Scenedesmus vacuolatus* by modifying the model previously developed by Ankley et al. [14]. The tissue concentration was replaced by the median effective dose (ED50), which is the concentration in the tissue corresponding to 50% mortality. Another modification was replacing the incident UV intensity with a measure of UV absorption (*J*), which had been introduced by Krylov et al. [17]. The model linearly correlated log(ED50) to log(*J*) for different PAHs. The average slope was −0.51 ± 0.09 (varying from −0.412 to −0.678). However, the intercepts varied significantly for each PAH, ranging from −0.363 to −2.152. A relative phototoxic efficacy was defined to accommodate the varying intercepts and to describe the distinct behavior of different compounds. The factors quantifying the efficacy for each compound were computed individually such that the factors when multiplied by *J* caused the varying intercepts to converge to a common value. The reported relative phototoxic efficacy for each compound was calculated by dividing the factor fitted for the compound by that of the reference compound benzo[g,h,i]fluoranthene. The relative phototoxic efficacy values found by Grote et al. [19] were different from those reported for *A. salina* [7], *D. magna* [8], or *P. promelas* [9]. This observation suggests that the relative potency values are organism dependent and the models developed previously are applicable only to the specific organisms tested.

Sellin Jeffries et al. [20] also developed a model based on the Bunsen–Roscoe law. The experimental data from the literature for various PAHs, test organisms, and lighting conditions were combined to correlate (LT50)−1 with the product of the UVA light intensity (*I* _UVA_) and total phototoxic PAH equivalents

\[
t_{p\text{PAHeq}} = \sum_i ([\text{PAH}]_i \times \text{RPA}_i)
\]

where [PAH]_i is the whole-body concentration of PAH_i (µM/g) and RPA_i is the photodynamic activity of PAH_i relative to anthracene

\[
\text{RPA}_i = \frac{(\text{LT50})^{-1}_i}{(\text{LT50}_{\text{anthracene}})}
\]

The resulting relative photodynamic activities varied by species and irradiance spectra of the light sources used to obtain the LT50s. Therefore, relative photodynamic activities were not found to be a consistent metric for characterizing the phototoxicity potential of individual PAHs.

The objective of the present study is to present a predictive phototoxicity model based on the target lipid model of PAH toxicity [21] that accounts for all of these factors without including an empirical relative photodynamic activity or similar normalization and that can predict both median lethal concentrations (LC50s) and LT50s. The model is applied to data for 20 PAHs, 15 organisms, and different light exposure conditions. In particular, the model can predict both the LC50 and LT50 within the same framework.

**PHOTOToxicity MODELing FOR I ndividual P AHs**

**Bioconcentration of PAHs**

Early observations of PAH phototoxicity established that chemical concentrations inside the organism have a pivotal role in determining the extent of phototoxicity [3,22,23]. Several experiments demonstrated that irradiation of the exposure media prior to addition of the organisms did not increase the observed toxicity, whereas increasing the length of the PAH uptake period prior to UV irradiation did increase the toxicity, suggesting that photovaculated toxicity occurred within the organism rather than in the exposure media [3,22,23]. Accordingly, the subsequent models of the phototoxic process used the concentration of PAHs in the organism tissue as a measure of the dose [7,8,14,15,19,24]. This requires a method for determining or estimating the concentration of PAHs in the organism.

The narcotic target lipid model (NTLM) [21] employs the lipid-water partitioning equation to compute the organism concentration

\[
C_{LN} = K_{LW} \times C_{W}
\]

where *C* _LN_ is the concentration of the chemical in the lipid fraction of the organism (mol chemical/kg lipid), *K* _LW_ is the lipid–water partition coefficient (L/kg lipid), and *C* _W_ is the aqueous concentration of the chemical (mol chemical/L).

**Baseline narcosis**

The phototoxicity model is based on the NTLM of baseline narcosis. When the chemical concentration in water produces 50% mortality (NLC50) in the absence of exposure to light—the “N” is used to denote narcotic toxicity (i.e., the toxicity without exposure to light)—the chemical concentration in the organism
target lipid is equal to the narcotic critical target lipid body burden (CTLBB), \( C_{LN} \),

\[
C_{LN} = K_{LW} \text{NLC50}
\]

or

\[
\log(\text{NLC50}) = \log(C_{LN}) - \log(K_{LW})
\]

where the superscript asterisk (*) denotes the concentration at which the toxic endpoint occurs. The NTLM [21,25] predicts narcotic toxicity using Equation 6, in which the lipid-water partition coefficient is replaced with an estimation equation

\[
\log(\text{NLC50}) = -0.936[\log(K_{OW})] + \log(C_{LN}) + \Delta c
\]

where \( K_{OW} \) is the octanol-water partition coefficient (L/kg octanol), \( C_{LN} \) is the narcotic CTLBB (mmol chemical/kg octanol), and \( \Delta c \) accounts for a chemical class correction (log[mmol/L]) required for using \( \log(K_{OW}) \) to predict \( \log(K_{LW}) \) as discussed below.

There are 2 critical assumptions that underlie the NTLM. First, the \( \log(K_{LW}) \), estimated using the slope of the \( \log(\text{NLC50}) \) versus \( \log(K_{OW}) \) regression, is constant for all species; that is, the target lipid where the toxic effect occurs has the same target \( \log(K_{LW}) \) in all aquatic organisms. Second, the species sensitivity as determined by the CTLBB concentration is specific to the organism but is the same for any narcotic chemical. This follows from the experimentally demonstrated additivity of narcotic toxic units [21].

These assumptions were validated by comparisons of observed and predicted NLC50s for 156 chemicals—including halogenated and nonhalogenated aliphatic and aromatic hydrocarbons, PAHs, alcohols, ethers, furans, and ketones—and for 47 species—including fish, amphibians, arthropods, mollusks, polychaetes, coelenterates, and protozoans [21,25]. The CTLBB has been determined for 47 aquatic [25] and 5 algal species [26].

It has been demonstrated that PAHs are toxic via the narcotic mode of action [25] and their toxicity in the dark can be predicted using the NTLM. The chemical class correction, \( \Delta c \), was determined to be \(-0.352\) for PAHs [25]. It was later found that this correction does not imply that PAHs are more toxic than baseline narcotics, but rather that the chemical class correction, \( \Delta c \), corrects the relationship between the \( K_{LW} \) (Equation 3) and \( K_{OW} \) in Equation 6 for the various classes of chemicals [27].

**Light absorption**

Upon exposure to light, PAHs inside the organism absorb light from the UVB spectrum, UVA spectrum, and a portion of the visible spectrum. The amount of absorbed light at each wavelength depends on the product of the absorption spectrum of the PAH and the irradiance spectrum of the light [17]. Figure 1A presents the molar absorption coefficient spectra for 4 PAHs (fluoranthene, anthracene, phenanthrene, and chrysene) and the solar radiation spectrum. The extent to which there is an overlap of the molar absorption coefficient and solar radiation spectra is shown in Figure 1B, which presents the action spectrum = molar absorption coefficient spectrum \( \times \) the irradiance spectrum, which is related to the quantity of the light that is absorbed.

The dependence of PAH toxicity on the amount of light absorbed is shown in Figure 2, which presents the toxicity to

![Figure 1](image1.png)

**Figure 1.** (A) Molar absorption coefficient spectra, \( \varepsilon (\lambda) \) [55], of 4 polycyclic aromatic hydrocarbons (fluoranthene, anthracene, phenanthrene, and chrysene) and solar radiation spectrum, \( I (\lambda) \) [59], on the primary and secondary axes, respectively, versus wavelength \( \lambda \). (B) Action spectra = product of molar absorption coefficient and solar radiation spectra. The area under each curve is related to the quantity of light energy absorbed by each polycyclic aromatic hydrocarbon.

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absence of light can be as large as 2 orders of magnitude. Interestingly, even though phenanthrene and anthracene are isomers and exert essentially equal narcotic toxicity, the difference in their light absorption properties (i.e., spectral molar absorption coefficient) leads to a considerable gap between their observed phototoxicity, as shown in Figure 2.

The total amount of absorbed light is quantified by the integral of the product of the spectra of the incident light intensity and the molar absorption coefficient of a compound. The most useful form of this integral expresses the amount of light energy absorbed in terms of moles of photons absorbed per mole of PAH

\[ P_{abs} = \int_{\lambda_1}^{\lambda_2} I_0(\lambda) \left( \frac{\lambda}{N_A h c} \right) T_{exp} \varepsilon(\lambda) d\lambda \]  

where \( P_{abs} \) denotes number of photons absorbed by the PAH (mole photon/mole PAH), \( \lambda \) is the wavelength (nm), \( I_0(\lambda) \) indicates the incident light intensity (W/m²/nm) at wavelength \( \lambda \), \( N_A \) is the Avogadro number \( (6.022 \times 10^{23}) \), \( h \) is the Planck’s constant \( (6.626 \times 10^{-34} \text{ J} \cdot \text{s}) \), \( c \) is the speed of light \( (2.998 \times 10^8 \text{ m/s}) \), \( T_{exp} \) is the time of exposure to the light (s), and \( \varepsilon(\lambda) \) is the molar absorption coefficient (L/mol/cm) of the PAH at wavelength \( \lambda \) (nm). The term \( \lambda/N_A h c \) converts the incident light \( I_0(\lambda) \) in units of W/m²/nm to moles photons/m²/s, which, when multiplied by the exposure time \( T_{exp} \) (s), yields the total moles of incident photons per cubic meter absorbed at the end of the exposure time. It is important to note that the dose of light energy can be expressed as moles of photons absorbed per mole of PAH rather than the less useful energy units. The dimensional analysis of \( P_{abs} \) can be found in the Supplemental Data (section SI3). \( P_{abs} \) plays a central role in the PTLM. Interestingly, there is an empirical relationship between \( P_{abs} \) and the HOMO-LUMO energy of the dataset employed in the PTLM formation. It is observed that \( P_{abs} \) decreases outside of the HOMO-LUMO energy window \( (7.2 \pm 0.4 \text{ eV}) \) (10]). See Supplemental, Data, section SI6 and Figure S9.

Reactive excited species formation

As discussed in the section Bioconcentration of PAHs, it was observed that photo-enhanced toxicity occurred within the organism, rather than in the exposure media [3,22,23], where the light absorbed by PAHs initiates photochemical reactions. Additionally, fish and shrimp exposure to UV light only (at 8 W/m² upward and 24 W/m² UVA) resulted in greater than 90% survival for the organisms [28], indicating no direct effects of UV radiation on phototoxicity. Briefly, as currently understood, the phototoxic action pathway includes 2 types of reaction mechanisms: photosensitization and photomodification. Photosensitization is initiated when a PAH absorbs a photon, which elevates the PAH to an excited singlet state. The excited singlet-state PAH can undergo an intersystem crossing to form the excited triplet state. The triplet PAH can transfer energy to triplet oxygen and decay back to the ground state PAH. Energy transfer to triplet oxygen can lead to reactive oxygen species (ROS) formation [29,30]. Reactive oxygen species has been accepted as the agent responsible for the oxidative damage and lipid peroxidation in the organisms [29,31]. The second mechanism, photomodification, results in the formation of new toxic products, usually via oxidation of the PAHs [8,17,30,32,33]. Studies have suggested that photomodification can play a significant role in PAH phototoxicity [17,18].

Previous models of PAHs phototoxicity were primarily based on photosensitization reactions [7,8,10] with the toxic species assumed to be ROS. However, direct experimental evidence suggesting that the formation of ROS is the sole cause of phototoxicity has not been reported. Because either ROS (photosensitization) or photomodified PAHs (photomodification) present in the lipid can cause toxicity, the toxic reactant will be denoted as reactive toxic species (RTS).

PTLM FORMULATION

RTS formation in target lipid

Assuming that the organism lipid is uniformly irradiated, the concentration of photons absorbed by the PAH in the lipid fraction of the organism \( (C_{LP}; \text{ mol photon/kg lipid}) \) can be computed as the product of the ratio of moles of photons absorbed per mole of PAH \( (P_{abs}; \text{ mol photon/mol PAH}) \) (Equation 7) and the PAH concentration in target lipid \( (C_{LN}; \text{ mol PAH/kg lipid}) \)

\[ C_{LP} = P_{abs} C_{LN} \]  

The concentration of RTS produced can be computed by specifying the quantum yield of RTS formation, \( \Phi_{RTS} \), which is defined as the ratio of moles of RTS produced in the lipid fraction per mole of photons absorbed

\[ \Phi_{RTS} = \frac{C_{LRTS}}{C_{LP}} \]  

Therefore, the concentration of RTS in the lipid fraction (mol RTS/kg lipid) is

\[ C_{LRTS} = \Phi_{RTS} C_{LP} \]  

Substituting the concentration of photons absorbed in the lipid fraction \( (C_{LP}) \) with the PAH target lipid concentration using Equation 8 yields

\[ C_{LRTS} = \Phi_{RTS} P_{abs} C_{LP} \]  

Finally, using Equation 3 to relate the target lipid concentration to the aqueous concentration yields

\[ C_{LRTS} = \Phi_{RTS} P_{abs} K_{LW} C_{W} \]  

This equation is analogous to Equation 3, the basis for the target lipid model of narcosis toxicity. This analogy will be pursued in the formulation of the phototoxic target lipid model.

PHOTOTOXIC TARGET LIPID MODEL

RTS effect

The PAH body burdens predicted from the target lipid model were found to be comparable to the measured concentrations in extracted lipid [21]. The observation suggested that there is a correlation between concentrations of PAHs in the lipid tissues and the observed adverse effect. The target lipid model is based on the assumption that mortality occurs when the chemical concentration in the target lipid reaches a threshold concentration [21,25]. An analogy can be drawn between the target lipid narcotic and the target lipid phototoxic modes of action. At 50% mortality, the
relationship between the narcotic LC50 and the critical target lipid body burden is given by Equation 4. Similarly, at 50% mortality, the concentration of RTS in target lipid is the RTS critical target lipid body burden ($C_{LRTS}$). The corresponding aqueous LC50 concentration of the PAH that produces the critical concentration of RTS in the lipid is denoted by RLC50, where R denotes the toxicity attributable to the RTS. Consequently, Equation 12 yields

$$C_{LRTS} = \Phi_{RTS} P_{abs} K_{LW} RLC50$$

where $C_{LRTS}$ is the RTS critical lipid body burden (mol RTS/kg lipid) and RLC50 is the aqueous concentration of the PAH (mol PAH/L).

The connection between the narcotic target lipid model and the phototoxic reactive species is made using Equation 2 for $K_{LW}$ in Equation 13 to yield

$$C_{LRTS} = \Phi_{RTS} P_{abs} \left( \frac{C_{LN}}{NLC50} \right) RLC50$$

Equation 14 can be rearranged to define the ratio of the phototoxic reactive species LC50 (RLC50) relative to the narcotic LC50 (NLC50)

$$\frac{RLC50}{NLC50} = (\Phi_{RTS} P_{abs})^{-1} \left( \frac{C_{LRTS}}{C_{LN}} \right)$$

The expression $\frac{C_{LRTS}}{C_{LN}}$ represents the sensitivity of the organisms to RTS toxicity relative to narcosis toxicity. It is convenient to define the ratio as

$$R^* = \frac{C_{LRTS}}{C_{LN}}$$

where $R^*$ is the ratio of critical body burdens (mol RTS/mol PAH). Therefore, Equation 16 becomes

$$\frac{RLC50}{NLC50} = \frac{R^*}{\Phi_{RTS} P_{abs}}$$

Equation 17 states that the ratio of the phototoxic reactive species LC50 to the narcotic LC50 is inversely related to the RTS production in lipid ($\Phi_{RTS} P_{abs}$) and directly related to the ratio of critical body burdens ($R^*$). This is the basic equation used in the PTLM for predicting the toxicity attributable to RTS (RLC50).

**RTS and narcotic PAHs**

The toxic components in phototoxicity are both the unexcited PAH and RTS since both are present in the target lipid. The toxicity of individual components in a mixture depends on the mode of action of each component. If the mode of action is the same, then toxic units (TU) can be used to compute the resulting toxicity [34,35]. The TU is defined as the ratio of the measured concentration of a chemical in a medium (e.g., water) to the corresponding effect concentration in that medium (e.g., LC50) [24,35]. Therefore, the toxic unit for the RTS-related toxicity ($TUR$) and the narcotic toxicity ($TUN$) are

$$TUR = \frac{C_{PAH,W}}{RLC50}$$

and

$$TUN = \frac{C_{PAH,W}}{NLC50}$$

where $C_{PAH,W}$ is the aqueous PAH concentration (mol PAH/L), $TUR$ and $TUN$ are the toxic units associated with the reactive species toxicity and the narcotic toxicity, respectively. Toxic unit additivity is assumed because the toxic mechanism for both the reactive species and narcosis occur in the lipid of the cell membrane and cause alteration of lipid properties [36–39]. Therefore, the total phototoxic toxic unit, $TUP$, is the sum of the reactive species and narcotic toxic units

$$TUP = TUR + TUN$$

It remains to compute the PAH aqueous concentration that causes 50% mortality ($C_{PAH,W}^{PC}$) which is equal to the phototoxic LC50, indicated as PLC50. It is that concentration at which the total toxic unit concentration is unity [24]. That is, at 50% mortality:

$$C_{PAH,W}^{PC} = PLC50$$

and

$$TUR + TUN = 1$$

Combining Equations 18 through 22 yields

$$PLC50^{-1} = (RLC50)^{-1} + (NLC50)^{-1}$$

Rearranging Equation 23 yields

$$PLC50 = \frac{1}{1 + (RLC50/NLC50)^{-1}}$$

Combining Equations 17 and 24 yields

$$PLC50 = \frac{1}{1 + (\Phi_{RTS} P_{abs}) R^*}$$

Equation 25 is the final form of the PTLM. It contains 3 terms: the quantity of photons absorbed (Equation 7), the quantum yield that determines the quantity of RTS produced by excited PAH (Equation 9), and the ratio of the critical body burden for phototoxicity to narcosis toxicity (Equation 16). It states that when no photons absorption takes place ($P_{abs} = 0$), PLC50 is equal to NLC50; that is, no phototoxic effect is observed and the PAH acts as a narcotic. However, if $P_{abs}$ is greater than 0, then PLC50 is less than NLC50 and phototoxicity is occurring. What remains to determine is whether Equation 25 in fact can predict both the observed ratios of PLC50 to NLC50, as well as PLC50 itself.

**PLC50 and PLT50 data**

A phototoxic database for individual PAHs was compiled from the available literature sources [8,9,12,14,15,28,33,40–54]. A total of 15 freshwater and marine species—including arthropods (insect and crustacean), fishes, amphipians, annelids, invertebrates, mollusks, and algae—are represented. There are 20 PAHs and various light sources and light exposure durations. The species and chemicals used in the PTLM development are
presented in Table 1. A complete list of the 333 individual observations is provided in Supplemental Data, Table S1. The data were used as reported.

The toxic endpoint was reported as either the LC50 at a fixed length of exposure (48 h or 96 h of exposure) or as the LT50 at a fixed PAH concentration, based on either survival or inhibition to growth, indicated in Supplemental Data, Table S1. The measurement protocol (measured or nominal chemistry) is also presented in Supplemental Data, Table S1. The PLTM can accommodate both types of data. Supplemental Data, Table S2 provides the endpoint type and the light regime (periodic or continuous) for each observation. For PLC50s, the total time of light exposure is $T_{\text{exp}}$ in Equation 7 (e.g., $T_{\text{exp}} = 32$ h) for a 48-h LC50 with a 16:8-h light:dark photoperiod). For the phototoxic time-to-death data (PLT50s), the reported PLT50 values are the total light exposure time ($T_{\text{exp}} = \text{PLT50}$), which is the model prediction, whereas the corresponding aqueous concentrations at which this time to death occurs is the PLC50, for which the exposure PAH concentration is substituted. To emphasize the type of the phototoxic data being considered, the PLC50s associated with time to death data, $T_{\text{exp}}$, is denoted by PLT50 in Equation 7; that is

$$P_{\text{abs}} = \int_{\lambda_1}^{\lambda_2} I_0(\lambda)(\lambda/N_{\text{ase}})(\text{PLT50})\varepsilon(\lambda)\,d\lambda \quad \text{(26)}$$

### Table 1. List of organisms and polycyclic aromatic hydrocarbons (PAHs) used in the phototoxic target lipid model (PLTM) modeling

<table>
<thead>
<tr>
<th>Full Name</th>
<th>Abbreviation</th>
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<tr>
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<td>M. liliana</td>
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<td>S. capricornatum</td>
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<td>D. magna</td>
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<td>M. lateralis</td>
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<td>A. salina</td>
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<td>Palaemonetes pugio</td>
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<th>Compounds</th>
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<td>BbFU</td>
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<tr>
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<td>Benzo[a]fluoranthene</td>
<td>BaF</td>
</tr>
<tr>
<td>Benzo[e]fluorene</td>
<td>BeF</td>
</tr>
</tbody>
</table>

### NLC50 data

To predict the PLC50 or PLT50, the NLC50 concentration is required (Equation 25). It is predicted using the NTLM without recourse to the phototoxic data. Thus, the NLC50 is a pure prediction. Also, because the observed NLC50, usually as a dark control, was not provided for the majority of studies, using the NTLM for all data allows a consistent approach. Therefore, NTLM predictions are preferred over the experimental NLC50s. The $K_{\text{OW}}$ values and CTLBBs used for the NTLM predictions are provided in Supplemental Data, Tables S3 and S4, respectively.

If the CTLBB was not available for a species in the database, the LC50 in the dark control experiment reported by the authors was used. This was the case for only a single observation associated with Macomona liliana [49]. With this only exception, the NLC50 concentration is predicted using the NTLM.

### PAHs’ aqueous solubility

For chemicals for which the reported PLC50 exceeded the aqueous solubility of the compound ($S$), it is assumed that the concentrations at which organisms were exposed is the aqueous solubility. That is, if PLC50 is greater than $S$, then, for those cases, PLC50 is equal to $S$. The PAH solubilities are calculated using

$$\log(S) = -1.4141[\log(K_{\text{OW}})] + 7.102 \quad \text{(27)}$$

where $S$ is the solid solubility ($\mu\text{mol/L}$) [25]. The $K_{\text{OW}}$ values used to calculate water solubilities are included in Supplemental Data, Table S3.

### Molar absorption coefficient spectra

The absorbance spectra, quantified by the molar absorption coefficient, $\varepsilon(\lambda)$, were obtained from the literature. The absorbance is measured in an organic solvent since the PAHs are not soluble enough in water. Most spectra were measured in cyclohexane [55]. Fluorene, benzo[a]fluorene, benzo[b]-fluorene, perylene, benzanthrene, and acenaphthene [56] were measured in ethanol and the solvent for the benzo[b]anthracene spectrum was benzene [56]. The $\varepsilon(\lambda)$ data are presented at the intervals of 1 nm in Supplemental Data, Tables S5a and S5b for the PAHs used in the PLTM model development.

A comparison of the effect of the solvent used to measure the light absorption by PAHs, presented in Supplemental Data, section S12 (Supplemental Data, Figure S1), indicates no significant differences. This suggests that using $\varepsilon(\lambda)$ measured in organic solvents for which $\varepsilon(\lambda)$ is available in the literature will provide acceptable estimates of $P_{\text{abs}}$. The integral (Equation 7) was calculated as a summation of the product of $I(\lambda)$ and $\varepsilon(\lambda)$ at the intervals of 1 nm.

### Light irradiance spectra

For each phototoxicity observation, the associated irradiance spectrum of the light source $I(\lambda)$ was extracted from the reference when provided by the authors. Otherwise, it was acquired from the spectrum published by the manufacturer of the light source. A total of 10 spectra were included in the database. The light source corresponding to each observation is presented in Supplemental Data, Table S2. Additionally, Supplemental Data, Tables S6a and S6b present $I(\lambda)$ at intervals of 1 nm for the light sources. It is possible the actual light intensities exposed to the organisms are likely to undergo some
attenuation in the exposure chambers. It is assumed that this is not a significant loss of incident radiation.

**RTS production**

The dose (Equation 13) requires the quantum yield: the ratio of toxic species produced per photon absorbed, $\Phi_{RTS}$. This quantity is dependent on the PAH’s properties. It has previously been proposed to use a quantum yield of toxicity [7–9,19] to describe the distinct behavior of different PAHs. In these studies, $\Phi_{RTS}$ is understood to be analogous to the quantum yield defined in photochemistry (i.e., the capacity of a compound to elucidate a specific effect relative to the absorbed dose of radiation). Depending on the bioassay and the test species used, different magnitudes of quantum yields were found for the quantum yields of single PAHs [7–9,19].

To proceed, it is assumed that $\Phi_{RTS}$ is constant for all PAHs considered below. The suitability of this assumption is tested and discussed below in the statistical analyses section.

**Estimation of PTLM parameters**

The parameter $R^*$ is defined as the ratio of the RTS critical body burden to the narcotic critical body burden ($R^* = \frac{C_{LRTS}}{C_{LN}}$).

A fundamental assumption in the NTLM is that $C_{LN}$ is the same for any narcotic chemical. It quantifies the species sensitivity to narcotic toxicity. Since the site of action of phototoxicity is assumed to be the target lipid, it is also assumed that $C_{LRTS}$ is the same for any phototoxic chemical. Since both $C_{LN}$ and $C_{LRTS}$ are the same for all PAHs, so also is $R^*$. This assumption is also tested below in the Statistical analysis section within the Results and Discussion.

At this stage of development, the PTLM has 2 constants: $R^*$ and $\Phi_{RTS}$. However, it has been found that the slope of log(PLC50) versus log(irradiance intensity) is less than unity [19]. Therefore, a third parameter, $a$, is needed for Equation 25

\[
\frac{\text{PLC50}}{\text{NLC50}} = \frac{1}{1 + \left(\frac{\text{PLC50}}{\Phi_{RTS}} \right)^a} \quad (28)
\]

where the notation PLC50 indicates that both types of phototoxicity data, PLC50s from phototoxic LC50 and LT50 endpoints, are included in the modeling framework. Because both parameters $R^*$ and $\Phi_{RTS}$ are assumed to be constant across the chemicals and species, so is their ratio ($R^*/\Phi_{RTS}$), and they can be combined into a single constant, $R^*$, expressed in mol RTS/mol PAH, which results in Equation 29

\[
\frac{\text{PLC50}}{\text{NLC50}} = \frac{1}{1 + \left(\frac{\text{PLC50}}{R^*} \right)} \quad (29)
\]

For each observation with species $k$ and PAH $j$, the PLC50$_{k,j}$ for that species--PAH pair is computed as

\[
\frac{\text{PLC50}_{k,j}}{\text{NLC50}_{k,j}} = \frac{1}{1 + \left(\frac{\text{PLC50}_{k,j}}{R^*} \right)} \quad (30)
\]

where $k = 1, \ldots, N_k$ and $j = 1, \ldots, N_c$, corresponding to the $N_k = 15$ species and $N_c = 20$ PAHs, respectively. The species dependency of the PTLM is introduced by the NLC50$_{k,j}$, whereas the PAH, light source, and exposure time are accounted for by $P_{abs,j}$. The model parameters $a$ and $R^*$ and their standard errors are computed using the R program nonlinear regression package: nls [57].

**RESULTS AND DISCUSSION**

The model (Equation 30) is fitted using the experimental data summarized in Supplemental Data, Table S1. In total, 333 data points comprising 20 PAHs and 15 species are compiled in the dataset. The PTLM model parameters $a$ and $R^*$, as well as the respective standard errors, are tabulated in Table 2.

The PTLM model is capable of predicting both LC50 and LT50 data. This greatly expands the quantity of data that can be analyzed using the PTLM. For notational simplicity, both types of data are referred to as PLC50 in the derivation above. Computational examples for PLC50 can be found in Supplemental Data, section SI3. The experimental PLC50s obtained directly from the reported aqueous concentrations at 50% mortality are shown in Figure 3A and C, distinguished by different colors for different organisms and chemicals, respectively. The experimental PLC50s corresponding to the reported PLT50 data are shown in Figure 3B (in which different colors indicate different organisms) and Figure 3D (color-coded for different chemicals). The plots contain all of the experimental data in the database. The data that produced residuals greater than 1 order of magnitude (shown with the plus symbols in the plots) were considered outliers and were not included in the nonlinear regression calculations. However, they are included in Figure 3. Thirteen data points were eliminated from the regression, decreasing the number of data points to 320 associated with 20 PAHs and 15 species. Note that no chemicals or species were eliminated by removal of the outliers. The lines show the model fit using Equation 30. The dashed lines represent 1 order of magnitude uncertainties.

The experimental PLC50 and PLT50 data are also compared with the PTLM prediction in the Supplemental Data (Figure S2–S5 in section SI4). Each panel presents the similar comparison grouped by chemical (Supplemental Data, Figure S2), by organism (Supplemental Data, Figure S3), by the reference reporting the experimental data (Supplemental Data, Figure S4), and by the light source used in the experiment (Supplemental Data, Figure S5). These comparisons can be used to analyze for biases in the model predictions; for example, the model prediction for a specific organism is always biased high or low. No consistent biases are evident. Also, all predicted PLC50s were below their aqueous solubilities.

The objective for the phototoxic model is to predict the phototoxic concentration for each species and each PAH for the conditions of the experiment. Because the NLC50 is a predicted concentration using the NTLM (Equation 6), it is also part of the model framework. Therefore the PTLM equation to predict PLC50 is

\[
\text{PLC50} = \frac{\text{NLC50}}{1 + \left(\frac{\text{PLC50}}{R^*} \right)} \quad (31)
\]

### Table 2. Nonlinear regression estimated parameters and standard errors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>0.426</td>
<td>0.037</td>
</tr>
<tr>
<td>$R^*$</td>
<td>0.511</td>
<td>0.097</td>
</tr>
</tbody>
</table>

PTLM = phototoxic target lipid model.
Figure 3. Predicted ratio of phototoxic median lethal concentrations (PLC50s) to narcotic median lethal concentrations (NLC50s) versus the number of photons absorbed by the PAH ($P_{abs}$). (A, C) PLC50s are obtained directly from the reported aqueous concentrations at 50% mortality. (B, D) Experimental PLC50s corresponding to the reported phototoxic median lethal time data (PLT50). Organisms are color-coded in panels (A) and (B) and described in the left legend. Chemicals are differentiated by colors (described in the right legend) in panels (C) and (D). The solid lines show the phototoxic target lipid model (PTLM) fit (Equation 30). The dashed lines represent 1 order of magnitude uncertainties. The outliers are indicated by plus symbols. Note that panels (A) and (C) contain the same set of phototoxic LC50 data; (A) is color coded for organisms, (C) is color coded for chemicals. Similarly, panels (B) and (D) are the same set of phototoxic LT50 data; (B) is color coded for organism, and (D) is color coded for chemicals. PAH = polycyclic aromatic hydrocarbon.

Figure 4 compares PLC50s predicted using Equation 31 with the observed PLC50s. The observed PLC50s for LC50-based data are shown in Figure 4A and C, while observed PLC50s associated with the LT50 endpoints are shown in Figure 4B and D. In Figure 4A and B, different colors indicate different organisms, whereas different chemicals are color-coded in Figure 4C and D. Data with residuals greater than 1 order of magnitude are shown with plus symbols. The diagonal line indicates perfect agreement of the experimental data with the predicted values, and the dashed lines represent 1 order of magnitude uncertainties in the prediction. The root mean square error of the prediction, RMSE, is calculated using Equation 32 where $n$ indicates the number of data points.

$$\text{RMSE} = \sqrt{\frac{1}{n} \left( \sum_{i=1}^{n} \left[ \log(\text{PLC50}_{\text{predicted}}) - \log(\text{PLC50}_{\text{observed}}) \right]^2 \right)}$$

(32)

The RMSE of the LC50-based data is 0.473 ($n = 120$), whereas RMSE associated with the LT50-based data is 0.382 ($n = 213$). The RMSE of prediction for the entire data (including both LC50-based and LT50-based data) is 0.416 ($n = 333$). Note that all data, including the outliers, are included in the RMSE calculations. The NTLM yielded a RMSE of 0.416 ($n = 148$) for NLC50 prediction of PAHs [25]. Interestingly, the PTLM yielded RMSEs comparable to that of NTLM, indicating that they both offer the same level of quality in prediction. For both models, less than approximately 5% of data exceed factor of 10 error.

Statistical analysis

The relatively low standard errors of the fitted parameters, $a = 0.426 \pm 0.037$ and $R^* = 0.511 \pm 0.097$, indicates that both parameters are estimated with high confidence. Two assumptions have been made to simplify the model to the point that only these parameters are necessary. They are that the quantum yield for moles of RTS produced to moles of photon absorbed, $F_{RTS}$, is constant for all PAHs, and that the ratio of critical body burden for phototoxicity to narcosis toxicity, $R^*$, is constant for all species. Unfortunately, it is not possible to validate these assumptions directly, since $F_{RTS}$ and $R^*$ occur as a ratio in the model

$$R^* = \frac{R^* \Phi_{RTS}}{F_{RTS}}$$

(33)

An analysis of the residuals $= \log(\text{predicted PLC50}) - \log(\text{observed PLC50})$ versus various features of the data is used to determine whether there are any trends that suggest these 2 critical assumptions are being violated. Figure 5 is a boxplot of log of residuals for individual PAHs sorted by increasing log ($K_{OW}$). The width of each box is proportional to the square root of the number of data points for each PAH. For the PAHs with the most data, anthracene and pyrene appear to be biased high (predicted > observed), whereas fluoranthene is biased slightly low. However, the magnitude of the biases is relatively small: median residuals are a few tenths of a log unit, and it is not possible to know if this is also attributable to a species-specific effect that is correlated to the PAHs involved.
A similar boxplot of residuals for individual organisms sorted by increasing CTLBB (decreasing organism sensitivity) is provided in Figure 6. Again, there are variations but no species with a very large bias, and the boxplot exhibits no specific trend for the median of the residuals versus the species sensitivities.

In an attempt to provide an analysis of the magnitude of the improvement that could be derived from chemical-specific or species-specific model parameters, the following analysis has been performed. The medians for each PAH (PAH-specific medians) were subtracted from the corresponding residuals. The new predicted PLC50s based on the PAH-specific medians were
compared with the observed PLC50s, resulting in a RMSE of 0.347, which improved the predictions by 17%. Similarly, with organism-specific medians subtracted from the associated residuals, the RMSE of the prediction became equal to 0.374, which decreased the error only 10%. This suggests that errors in the model prediction are not attributable entirely to the 2 simplifications resulting in a constant $R_0^*$ but are also the result of other random or uncontrolled features in the experiments.

Although it is well-known that quantum yields and species sensitivity vary with chemicals and organisms, respectively, it is remarkable and surprising that reasonable RMSEs of predictions result even though these variations are not explicitly considered and $R_0^*$ is assumed to be constant. It appears that these variations are not large enough to degrade the predictions of the model. This is fortunate, because keeping $R_0^*$ constant keeps the model parameterization simple enough so that the PTLM is applicable to a large number of PAHs and organisms without additional chemical- and species-specific parameters that are difficult to estimate.

Additional analyses of the residuals can be found in Supplemental Data, section SI5. The residuals are plotted against the factors related to photo-enhanced toxicity, including the intensity of irradiance ($P_{abs}/T_{exp}$) and the photons absorbed ($P_{abs}$) in Supplemental Data, Figures S6 and S7, respectively. The plots show no trends with respect to light intensity and the photons absorbed, indicating the lack of bias toward these parameters.

With regard to $T_{exp}$, the quantities range from 0.1 h to more than 100 h with 269 h and 485 h for 2 data points. The residuals against the $T_{exp}$ are presented in Supplemental Data, Figure S8, for continuous irradiation (PLT50s), as well as periodic irradiation associated with 48-h and 96-h PLC50s. For the periodic exposure, the light exposure time is up to approximately 80 h. The complete discussion is provided in Supplemental Data, section SI5. Briefly, the analysis suggests no failure of the model in the available exposure time range and no considerable bias of log of residuals toward $T_{exp}$. Therefore, the PTLM parameters ($a$ and $R_0^*$) do not strongly depend on the light intensity, total photons absorbed per mole PAH, and the light exposure duration.

Perhaps the most interesting and puzzling result of this modeling analysis is the fact that the toxicity decreases not as the unity power of the photon dose, but rather at a markedly lower rate with an exponent $a = 0.426 \pm 0.037$. The fact that the model prediction is not biased by the length of light exposure or the magnitude of the light intensity suggests that another factor is operating that produces this less than proportional relationship between toxicity and photon dose. This is an area that would profit from further investigation.

**CONCLUSIONS**

The PTLM model is capable of predicting the acute phototoxicity of PAHs measured as either LC50 or LT50. It accounts for the differences in the chemical and physical properties of PAHs, test species sensitivities, and light sources spectra, intensity and duration. The validation is performed using 333 data, including 120 LC50 and 213 LT50 toxicity endpoints associated with 20 PAHs, 15 organisms, and 10 light sources. The model has 2 fitting parameters and does not rely on relative measures of toxicity (e.g., relative photodynamic activities), determined from the experiments. The factors accounting for the chemical properties in the modeling framework ($K_{OW}$ and the molar absorption coefficient) can be estimated from the compound structure. The organism-specific factor in the PTLM is the target lipid model, CTLBB, which is available in the target lipid model database for the CTLBB and has been determined for 47 aquatic [25] and 5 algal species [26].

An important addition to the PTLM would be to determine the applicability of the acute to chronic ratio so that a reasonable...
prediction for chronic phototoxicity could be made. Unfortunately, that is not possible currently because there are no experimental data in the literature specifically for chronic phototoxicity of PAHs.

The PTLM can predict the phototoxicity of PAHs in laboratory settings. However, to evaluate the extent of phototoxicity in a field setting, additional factors such as UV attenuation and organisms’ movement pattern influence the species exposure to the light and chemical and need to be considered. It would be interesting and useful to make such an evaluation where the requisite data are available. Additionally, an application to a field setting requires that mixtures of PAHs be considered. An approach is available [58] and will be the subject of a future publication.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3601.

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Data availability—The data are available online in the Supplemental Data.

REFERENCES


