MECHANISTIC QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP MODEL FOR THE PHOTOINDUCED TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS: I. PHYSICAL MODEL BASED ON CHEMICAL KINETICS IN A TWO-COMPARTMENT SYSTEM

SERGEY N. KRYLOV, XIAO-DONG HUANG, LORELEI F. ZEILER, D. GEORGE DIXON and BRUCE M. GREENBERG*

Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

(Received 5 November 1996; Accepted 28 March 1997)

Abstract—A quantitative structure–activity relationship model for the photoinduced toxicity of 16 polycyclic aromatic hydrocarbons (PAHs) to duckweed (Lemna gibba) in simulated solar radiation (SSR) was developed. Lemna gibba was chosen for this study because toxicity could be considered in two compartments: water column and leaf tissue. Modeling of photoinduced toxicity was described by photochemical reactions between PAHs and a hypothetical group of endogenous biomolecules (G) required for normal growth, with damage to G by PAHs and/or photomodified PAHs in SSR resulting in impaired growth. The reaction scheme includes photomodification of PAHs, uptake of PAHs into leaves, triplet-state formation of intact PAHs, photosensitization reactions that damage G, and reactions between photomodified PAHs and G. The assumptions used were: the PAH photomodification rate is slower than uptake of chemicals into leaves, the PAH concentration in aqueous solution is nearly constant during a toxicity test, the flow rate of actinic radiation is lower within leaves than in the aqueous phase, and the toxicity of intact PAHs in the dark is negligible. A series of differential equations describing the reaction kinetics of intact and photomodified PAHs with G was derived. The resulting equation for PAH toxicity was a function of treatment period, initial PAH concentration, relative absorbance of SSR by each PAH, quantum yield for formation of triplet-state PAH, and rate of PAH photomodification. Data for growth in the presence of intact and photomodified PAHs were used to empirically solve for a photosensitization constant (PSC) and a photomodification constant (PMC) for each of the 16 PAHs tested. For 9 PAHs the PMC dominates and for 7 PAHs the PSC dominates.

Keywords—Lemna gibba Growth inhibition Photochemistry Polycyclic aromatic hydrocarbons Mathematical model

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are environmentally ubiquitous contaminants [1,2] whose biological activity is greatly enhanced by biotic and abiotic activation. Although a number of possible routes of activation exist [3–7], one of the most important in the environment is photoactivation by sunlight. Polycyclic aromatic hydrocarbons are, by virtue of their highly conjugated π-orbital systems, prime examples of photoactive contaminants [5,8–12]. Polycyclic aromatic hydrocarbons strongly absorb in the ultraviolet-B (UV-B) (290–320 nm) and UV-A (320–400 nm) spectral regions [7,13,14], both of which are present in sunlight [15]. The toxicities of PAHs have been shown to be greatly enhanced [5,7,11,14,16] when the UV components of sunlight are present, as either natural sunlight or a light source mimicking sunlight.

Quantitative structure–activity relationships (QSARs), which correlate the physicochemical properties of molecules to observable biological responses, are useful for understanding the mechanisms of action of groups of related chemicals and for predicting the environmental risks associated with those chemicals [17]. Quantitative structure–activity relationships can predict the potential hazards of untested compounds, establish which physical traits of chemicals contribute to biological impacts, describe the routes of chemical interaction with an organism, and aid in elucidating chemical and biological mechanisms of toxicity. In developing a QSAR model, it is essential to consider the attributes of the environmental compartment in which the contaminant of interest resides, as this will dictate which physicochemical properties are likely to be most influential in toxicity. Because solar radiation is ubiquitous in the environment and can enhance the toxicity of PAHs [4,7,9,18], solar radiation is a factor that should be used in QSAR modeling of PAH toxicity.

To generate a QSAR model describing the photoinduced toxicity of PAHs, both the mechanisms of the primary photochemical reactions involving light-sensitive molecules [18,19] and the potential negative impacts of those reactions on living organisms [9] must be considered. Light can alter the toxicity of PAHs by essentially two routes: photosensitization reactions initiated by the PAHs and photomodification of the PAHs (Fig. 1). Photosensitization reactions initiated by PAHs often proceed via the formation of singlet-state oxygen (1O2) [9,19]. This process begins with the sensitizing PAH absorbing a photon, which elevates the PAH to an excited singlet state. The excited singlet-state PAH can undergo intersystem crossing to the excited triplet state, where it can react with ground triplet-state oxygen (3O2) to form 1O2. Due to its low energy barrier to reaction, 1O2 is capable of oxygenating or oxidizing many different biomolecules, altering their chemical structure, and consequently inhibiting or inactivating them [18,19]. Photomodification of PAHs, usually via oxidation, results in the formation of new compounds that will have different bioactivities than the parent compounds [7,20,21]. These two physicochemical processes, photosensi-
icity. To minimize the number of parameters to be considered, merging both photosensitization and photomodification into a test plant. Physiologically and photomodification, can be used in toxicity modeling [10].

Most PAHs possess triplet-state lifetimes that are sufficiently long for diffusion-limited reactions with $^{3}\text{O}_2$, rendering them effective photosensitizers [5,8,11,22,23]. Indeed, previous QSAR models for the photoinduced toxicity of PAHs were based primarily on photosensitization reactions [5,8,11]. In these models correlations between PAH bioactivity and the triplet-state energy levels were observed and used to explain toxicity. However, some of the chemicals did not fit the models. It should be noted that at the time these models were developed, the substantial role of photomodification in enhancing the toxicity of PAHs had not been established. Photomodification (defined here as photooxidation and photolysis) can significantly alter PAH toxicity [7,10,20,21]. Photomodification products of PAHs are more toxic than the intact PAHs to plants, bacteria, and invertebrates [7,20,21, unpublished observations]. Because the rates of PAH photomodification in sunlight are relatively rapid, the photoproducts must be considered as major factors in the net environmental loads of PAHs [7,24]. To wit, it is the PAHs with the slowest photomodification rates that are generally observed at the highest concentrations in the environment [1,7,25–27], implying that PAHs with faster rates of photomodification have been structurally altered and do not exist in high concentration in intact form.

Because photomodified PAHs are highly toxic, it follows that a complete model for the photoreactions of PAHs and their resultant photoinduced toxicity must incorporate determinants for both photosensitization and photomodification occurring both inside and outside of the target organism. In a preliminary study based on only six PAHs, we demonstrated the merits of this approach for modeling the toxicity of PAHs to the aquatic higher plant *Lemna gibba* L. [10]. However, merging both photosensitization and photomodification into a QSAR model requires incorporation of a large number of parameters that may have varying degrees of importance in toxicity. To minimize the number of parameters to be considered, *L. gibba* was chosen as a test plant. Physiologically and photosynthetically, *L. gibba* is fairly typical of other C-3 higher plants. Because *L. gibba* is an aquatic plant and takes up waterborne chemicals through the underside of the leaf [28], only two compartments need to be considered: plant tissue and water column. This eliminates the need to factor a vascular system into the model. In addition, toxicity of intact PAHs to *L. gibba* in the absence of light is very low [6,14], so modeling could be performed without large interferences of other physiological factors. In this study, a detailed chemical kinetic model was developed to describe the key photochemical reactions of PAHs within leaf tissue and the surrounding aqueous medium. The model showed that photosensitization and photomodification additively contribute to toxicity. The results of two series of experiments on the photoinduced toxicity of 16 PAHs to *L. gibba* were used to solve for two complex constants in the model. The first series, which determined the toxicity of the intact PAHs, was used to address that component of toxicity due to photosensitization reactions (photosensitization constant [PSC] for each PAH). The second series, which determined the toxicity of the PAHs after photomodification, was used to address that component of toxicity due to the photomodified PAHs (photomodification constant [PMC] for each PAH). Overall, the model explains toxicity on the basis of both photosensitization reactions initiated by the intact chemicals and direct toxicity of the photomodification products. This QSAR model was tested for predictive and practical value with an empirical application in the companion paper [29].

**MATERIALS AND METHODS**

**Plant growth and toxicity assessment**

Prior to chemical treatment, *L. gibba* L. G-3 was cultured axenically on half-strength Hutner’s medium under 60 $\mu$mol/m$^2$/s of continuous photosynthetically active radiation (400–700 nm) (PAR) generated with cool-white fluorescent lamps [7]. The irradiation source for plant growth during chemical treatment was simulated solar radiation (SSR). This light source and its spectral output are described in detail elsewhere [7,15,20]. The light was filtered through the polystyrene petri dish top (Phoenix Biomedical, Baxter-Canlab, Mississauga, ON, Canada) covering the plants. The polystyrene absorbed all of the UV-C and 50% of the UV-B. The spectral output of the SSR source after filtration through polystyrene had a PAR: UV-A : UV-B ratio of 100:10:1 based on photon fluence rates, which approximates the relative levels of these three spectral regions in sunlight [9,15,20]. For all experiments the fluence rate was 100 $\mu$mol/m$^2$/s as measured by a spectroradiometer (Oriel, Stratford, CT, USA) and is the integrated fluence rate from 290 to 700 nm. This fluence rate is low compared to natural sunlight (~2,000 $\mu$mol/m$^2$/s), so this study will tend to underestimate the contribution of light to toxicity in the natural environment.

Toxicity of intact and photomodified PAHs was assessed as plant growth as determined by monitoring leaf production (leaf count) at 2-d intervals over an 8-d period [7,28]. In the companion paper leaf count is shown to be as accurate a measure of toxicity as tissue fresh weight and chlorosis [29]. The number of leaves at $t = 0$ was 10 and after 8 d the controls had $140 \pm 10$ leaves. Toxicity was determined as diminished leaf production, represented by the metric $Y = \ln(N/N_0)$, where $N_0$ is the number of leaves in the sample treated with a given PAH at each time point and $N$ is the number of leaves in the control at the same time point. The value $Y$ was calculated for each 2-d interval in the 8-d test. Note, when there was a toxic...
Theoretical QSAR model for photoinduced toxicity of PAHs

Environ. Toxicol. Chem. 16, 1997 2285

Fig. 2. Structures of the 16 polycyclic aromatic hydrocarbons used for modeling and their three-letter abbreviations.

Table 1. Physicochemical parameters of polycyclic aromatic hydrocarbons (PAHs) used in modeling. [C]₀ is the initial concentration of PAH. $t_{1/2}$ is the half-life of the PAH in simulated solar radiation (SSR). $k_m$ is the pseudo-first-order rate constant for photomodification. $R$ is the fraction of the original amount of intact PAH remaining after 48 h. $J$ is the absorbance of SSR by the PAH. $\varphi$ is the triplet-state quantum yield.

<table>
<thead>
<tr>
<th>PAH</th>
<th>[C]₀ (µM)</th>
<th>$t_{1/2}$ (h)</th>
<th>$k_m$ (h⁻¹)</th>
<th>$R$</th>
<th>J</th>
<th>$\varphi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT</td>
<td>10.60</td>
<td>2</td>
<td>0.347</td>
<td>0.060</td>
<td>36.9</td>
<td>0.60</td>
</tr>
<tr>
<td>BAA</td>
<td>8.33</td>
<td>5</td>
<td>0.139</td>
<td>0.150</td>
<td>58.7</td>
<td>0.80</td>
</tr>
<tr>
<td>BBA</td>
<td>8.33</td>
<td>27</td>
<td>0.026</td>
<td>0.575</td>
<td>37.0</td>
<td>0.65</td>
</tr>
<tr>
<td>BBF</td>
<td>8.70</td>
<td>70</td>
<td>0.010</td>
<td>0.794</td>
<td>46.5</td>
<td>0.50</td>
</tr>
<tr>
<td>BGP</td>
<td>6.90</td>
<td>100</td>
<td>0.007</td>
<td>0.849</td>
<td>126.2</td>
<td>0.60</td>
</tr>
<tr>
<td>BAP</td>
<td>7.56</td>
<td>52</td>
<td>0.013</td>
<td>0.739</td>
<td>200.3</td>
<td>0.40</td>
</tr>
<tr>
<td>BEP</td>
<td>7.56</td>
<td>75</td>
<td>0.009</td>
<td>0.808</td>
<td>78.8</td>
<td>0.70</td>
</tr>
<tr>
<td>CHR</td>
<td>8.33</td>
<td>56</td>
<td>0.012</td>
<td>0.754</td>
<td>34.8</td>
<td>0.67</td>
</tr>
<tr>
<td>COR</td>
<td>6.29</td>
<td>100</td>
<td>0.007</td>
<td>0.851</td>
<td>102.0</td>
<td>0.80</td>
</tr>
<tr>
<td>DAA</td>
<td>6.95</td>
<td>16</td>
<td>0.043</td>
<td>0.421</td>
<td>144.2</td>
<td>0.50</td>
</tr>
<tr>
<td>DAP</td>
<td>6.41</td>
<td>40</td>
<td>0.017</td>
<td>0.679</td>
<td>309.5</td>
<td>0.50</td>
</tr>
<tr>
<td>FLA</td>
<td>9.35</td>
<td>40</td>
<td>0.017</td>
<td>0.679</td>
<td>84.4</td>
<td>0.60</td>
</tr>
<tr>
<td>FLE</td>
<td>11.49</td>
<td>19</td>
<td>0.036</td>
<td>0.472</td>
<td>3.0</td>
<td>0.31</td>
</tr>
<tr>
<td>PHE</td>
<td>10.60</td>
<td>14</td>
<td>0.050</td>
<td>0.382</td>
<td>8.9</td>
<td>0.80</td>
</tr>
<tr>
<td>PYR</td>
<td>9.43</td>
<td>46</td>
<td>0.015</td>
<td>0.711</td>
<td>72.7</td>
<td>0.27</td>
</tr>
<tr>
<td>TRI</td>
<td>8.40</td>
<td>65</td>
<td>0.011</td>
<td>0.782</td>
<td>8.9</td>
<td>0.95</td>
</tr>
</tbody>
</table>

PAHs were purchased from Sigma (St. Louis, MO, USA), Aldrich (Milwaukee, WI, USA), or Accustandard (New Haven, CT, USA). Purity was confirmed by high-performance liquid chromatography (HPLC) and the chemicals were used as purchased. The PAHs were dissolved in dimethylsulfoxide (DMSO) to 2 g/L and delivered to the plant growth medium by dilution to a final concentration of 2 mg/L. The plant growth medium was not stirred during the toxicity test, making movement of the chemicals through the medium diffusion controlled. Because PAHs have low solubility in water [30], delivery with DMSO was necessary to achieve a concentration of 2 mg/L. Polycyclic aromatic hydrocarbons at 2 mg/L have been shown to be in the log-linear phase of the toxicity dose–response curves for L. gibba [7,14]. High PAH concentrations were used because the modeling process required the presence of a large excess of chemical to avoid changes in concentration during a toxicity test. A DMSO concentration of 0.1% (v/v) does not affect L. gibba growth [7,28]; control plants were grown in the presence of 0.1% DMSO. Other delivery solvents have been tested in previous studies [7,28], where similar levels of PAH toxicity have been found. Furthermore, DMSO does not dramatically alter the rate or extent of PAH uptake [31]. The accuracy of PAH delivery to the medium was confirmed by UV absorbance spectroscopy and HPLC analysis as previously described [21]. Once the medium containing plants is placed in SSR, however, photomodification and uptake of the chemicals become concurrent processes, making it impractical to monitor PAH concentrations during a toxicity experiment [7].

Photomodification of PAHs and application of photomodified PAHs

To measure the rate of PAH photomodification, growth medium containing 2 mg/L PAH was incubated in SSR (100 µmol/m²/s). At various time points the aqueous phase was
extracted with chloroform and analyzed by UV/visible spectroscopy. The amount of the intact PAH remaining was determined by following loss of an absorbance band specific to the parent PAH [7]. The results from the spectroscopic analyses were confirmed by HPLC [21].

To generate photomodified PAHs for use in the second series of toxicity tests, the intact PAHs were delivered to growth medium to a final concentration of 8 mg/L using DMSO, and photomodified in UV-B (6 μmol/m²/s) until less than 10% of the parent PAH remained. The incubation time ranged from 1 to 7 d depending on the PAH. The extent of photomodification was assayed as above. Ultraviolet-B radiation was used instead of SSR to provide a higher fluence rate of actinic photons, and accelerate the photomodification process. Complex mixtures of photoproducts are formed from each PAH, in some cases more than 30 products [21,25,27,32]. Use of complex photomodification mixtures was crucial because similar mixtures will form when the intact PAHs are applied to the plants in the presence of light. The kinetic model presented here is based on chemical changes to intact PAHs in the presence of SSR.

To apply the photomodified PAHs to the plants, the growth medium containing the photomodified PAHs was diluted by a factor of four with fresh medium to give a 2 mg/L solution, based on the amount of intact PAH prior to photomodification. For the control plants, a 0.1% solution of DMSO in growth medium, which had been preincubated in UV-B for the same period as the corresponding PAH-containing medium, was diluted four fold with fresh growth medium and incubated with the plants. The control plants grew at the same rate in UV-treated DMSO-medium and fresh medium free of DMSO [7].

Computer modeling and statistics

An IBM-compatible personal computer (486 processor) was used for computer modeling and calculations. The program for calculating the integral of overlap between the absorption spectra of the PAHs and the irradiation spectrum of SSR was written in BASIC. Linear regressions through the diminished leaf production data (Y) were used to solve for two complex constants in the model presented below. The linear regressions of the growth suppression data for the intact and photomodified PAHs as a function of time were performed using the MGLH module of SYSTAT (SYSTAT, Version 5.05 for Windows, Evanston, IL, USA). The quality of fit of the regressions (r²) and the significance of the regressions (p) were determined with SYSTAT.

RESULTS AND DISCUSSION

Primary toxicity data

The rates of PAH photomodification as well as the data for growth of the plants in the presence of intact and photomodified PAHs were required to numerically solve for the PSC and PMC that are described in the model below. The half-lives and exponential decay constants for the photomodification of the PAHs in SSR are presented in Table 1. It has been previously demonstrated that in SSR, photomodification of PAHs is essentially the only modifying reaction impacting on PAHs in aqueous solution, and that the reaction proceeds with exponential decay kinetics [7,14,21]. For all 16 PAHs, the half-lives for photomodification are ≥100 h, fast enough for the photomodification products to have an impact in the 2-d static renewal period of the toxicity test. It should be noted that the half-lives should be shorter (and toxicity potentially greater) in natural environments where the fluence rate of sunlight (2,000 μmol/m²/s) is far greater than that of the SSR source (100 μmol/m²/s) used here.

Toxicity tests with L. gibba were performed with the 16 PAHs. Diminished leaf production, Y, was used to quantitate toxicity, and in the companion paper [29], Y was found to be as an effective measure as plant fresh weight or chlorosis. In intact form, the least toxic PAHs were COR and BGP with a Y of −0.180 and −0.190, respectively, on day 8. Anthracene was the most toxic (almost no plant growth) with a Y of −2.51 on day 8 (Table 2). To determine the contribution of the photomodified PAHs to photoinduced toxicity, the 16 PAHs were tested after extensive photomodification (Table 3). Impacts of the photomodified PAHs ranged from no new leaf production for ANT and BAA (Y = −2.52) to a Y of −0.41 for DAA, the least toxic photomodified PAH.

Table 2. Diminished leaf production, Y, caused by the intact polycyclic aromatic hydrocarbons (PAHs) in simulated solar radiation (SSR) as a function of time. The number of leaves in the control (Nc) and treated (Nt) samples were counted on the day indicated. Y was calculated as ln(Nt/Nc). The data are averages of nine repeats ± the standard deviation. The number of leaves at t = 0 was 10 and the number of leaves in the control at t = 192 h (8 d) was 140.

<table>
<thead>
<tr>
<th>PAH</th>
<th>48</th>
<th>96</th>
<th>144</th>
<th>192</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT</td>
<td>−0.62 ± 0.03</td>
<td>−1.36 ± 0.03</td>
<td>−1.95 ± 0.04</td>
<td>−2.51 ± 0.04</td>
</tr>
<tr>
<td>BAA</td>
<td>−0.09 ± 0.05</td>
<td>−0.21 ± 0.06</td>
<td>−0.42 ± 0.07</td>
<td>−0.44 ± 0.09</td>
</tr>
<tr>
<td>BBA</td>
<td>0.01 ± 0.02</td>
<td>−0.06 ± 0.06</td>
<td>0.11 ± 0.08</td>
<td>−0.22 ± 0.09</td>
</tr>
<tr>
<td>BBF</td>
<td>−0.06 ± 0.03</td>
<td>−0.28 ± 0.05</td>
<td>−0.48 ± 0.07</td>
<td>−0.65 ± 0.09</td>
</tr>
<tr>
<td>BGP</td>
<td>−0.03 ± 0.02</td>
<td>−0.15 ± 0.03</td>
<td>−0.14 ± 0.04</td>
<td>−0.19 ± 0.07</td>
</tr>
<tr>
<td>BAP</td>
<td>−0.04 ± 0.02</td>
<td>−0.16 ± 0.05</td>
<td>−0.34 ± 0.05</td>
<td>−0.41 ± 0.04</td>
</tr>
<tr>
<td>BFP</td>
<td>−0.03 ± 0.02</td>
<td>−0.17 ± 0.04</td>
<td>−0.37 ± 0.06</td>
<td>−0.51 ± 0.10</td>
</tr>
<tr>
<td>CHR</td>
<td>−0.04 ± 0.03</td>
<td>−0.11 ± 0.02</td>
<td>−0.23 ± 0.03</td>
<td>−0.32 ± 0.04</td>
</tr>
<tr>
<td>COR</td>
<td>−0.04 ± 0.03</td>
<td>−0.06 ± 0.05</td>
<td>−0.09 ± 0.09</td>
<td>−0.18 ± 0.12</td>
</tr>
<tr>
<td>DAA</td>
<td>−0.06 ± 0.03</td>
<td>−0.08 ± 0.03</td>
<td>−0.15 ± 0.05</td>
<td>−0.23 ± 0.05</td>
</tr>
<tr>
<td>DAP</td>
<td>−0.04 ± 0.03</td>
<td>−0.16 ± 0.04</td>
<td>−0.34 ± 0.05</td>
<td>−0.41 ± 0.10</td>
</tr>
<tr>
<td>FLA</td>
<td>−0.16 ± 0.05</td>
<td>−0.47 ± 0.09</td>
<td>−0.81 ± 0.13</td>
<td>−1.02 ± 0.14</td>
</tr>
<tr>
<td>FLE</td>
<td>−0.04 ± 0.02</td>
<td>−0.19 ± 0.04</td>
<td>−0.30 ± 0.04</td>
<td>−0.41 ± 0.06</td>
</tr>
<tr>
<td>PHE</td>
<td>−0.07 ± 0.07</td>
<td>−0.20 ± 0.07</td>
<td>−0.36 ± 0.08</td>
<td>−0.42 ± 0.08</td>
</tr>
<tr>
<td>PYR</td>
<td>−0.13 ± 0.04</td>
<td>−0.35 ± 0.07</td>
<td>−0.55 ± 0.11</td>
<td>−0.67 ± 0.12</td>
</tr>
<tr>
<td>TRI</td>
<td>−0.03 ± 0.08</td>
<td>−0.10 ± 0.02</td>
<td>−0.28 ± 0.07</td>
<td>−0.36 ± 0.08</td>
</tr>
</tbody>
</table>

* Names of the PAHs are given in Figure 2.
the excited triplet-state (3 \text{CA}) via intersystem crossing from the ground triplet-state oxygen (3 \text{O}_2).

Strikingly, relative to the intact chemicals, the toxicity of 13 of the 16 compounds increased dramatically following photomodification. The exceptions were ANT, BBA, and PYR, which only showed slight increases in toxicity following photomodification. The exceptions were ANT, BBA, and PYR, respectively; Table 1), it is suspected that the mixtures of photomodification products have toxic strengths similar to the parent PAHs.

**General scheme for modeling the photoinduced toxicity of PAHs**

To model the toxicity of PAHs we considered the dynamics of PAHs in the presence of leaf tissue and SSR. *Lemma gibba* rapidly takes up contaminants from the aqueous phase through the underside of the leaves, and not via the rizoid (or root) [28,31]. Thus, one can consider the interactions of the PAHs with the leaf tissue as a two-compartment system (Fig. 3): the aqueous medium represents one compartment, and the inside of the leaf the other. As well, two chemical species occur, the intact PAH, designated “C,” and the mixture of photomodification products of the PAH, designated “P.” For purposes of modeling, all the photoproducts of a particular PAH are regarded as one entity. Both intact and photomodified PAHs occur in the aqueous phase and in the leaf tissue, with all chemical species reaching a steady-state in the two phases. Processes and chemical species occurring in the aqueous phase are denoted with the subscript “A” and those occurring in the leaves are denoted with the subscript “L.”

In the aqueous media, two types of reactions occur that can result in photomodification of PAHs; type II and type I photosensitization reactions. During a type II photosensitization, after absorption of a photon, ground-state PAH (C_A) goes to the excited triplet-state (3 \text{CA}) via intersystem crossing from the excited singlet-state (Fig. 1). The experimental system is open to the air and the plants are oxygenic, so the main quencher of 3 \text{CA} in all cases will be ground triplet-state oxygen (3 \text{O}_2).

### Table 3. Diminished leaf production, Y, caused by the photomodified polycyclic aromatic hydrocarbons (PAHs) in simulated solar radiation (SSR) as a function of time. Experiments were performed and data are presented as described in Table 2

<table>
<thead>
<tr>
<th>PAH</th>
<th>Time (h)</th>
<th>48</th>
<th>96</th>
<th>144</th>
<th>192</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT</td>
<td>0.73 ± 0.00</td>
<td>1.42 ± 0.00</td>
<td>1.90 ± 0.00</td>
<td>2.53 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>BAA</td>
<td>0.73 ± 0.00</td>
<td>1.42 ± 0.00</td>
<td>1.90 ± 0.00</td>
<td>2.53 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>BBA</td>
<td>0.70 ± 0.10</td>
<td>1.39 ± 0.20</td>
<td>1.87 ± 0.27</td>
<td>2.50 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>BBF</td>
<td>0.18 ± 0.06</td>
<td>0.49 ± 0.10</td>
<td>0.83 ± 0.16</td>
<td>1.15 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>BGP</td>
<td>0.14 ± 0.03</td>
<td>0.34 ± 0.09</td>
<td>0.39 ± 0.09</td>
<td>0.51 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>BAP</td>
<td>0.71 ± 0.10</td>
<td>1.40 ± 0.21</td>
<td>1.88 ± 0.28</td>
<td>2.51 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>BFP</td>
<td>0.26 ± 0.10</td>
<td>0.79 ± 0.19</td>
<td>0.97 ± 0.32</td>
<td>1.45 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>CHR</td>
<td>0.50 ± 0.06</td>
<td>0.63 ± 0.16</td>
<td>0.99 ± 0.30</td>
<td>1.29 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>COR</td>
<td>0.25 ± 0.04</td>
<td>0.39 ± 0.09</td>
<td>0.59 ± 0.07</td>
<td>0.88 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>DAA</td>
<td>0.06 ± 0.02</td>
<td>0.30 ± 0.12</td>
<td>0.29 ± 0.05</td>
<td>0.41 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>DAP</td>
<td>0.16 ± 0.07</td>
<td>0.51 ± 0.24</td>
<td>0.86 ± 0.41</td>
<td>1.09 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>FLA</td>
<td>0.70 ± 0.14</td>
<td>1.10 ± 0.36</td>
<td>1.31 ± 0.43</td>
<td>1.73 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>FLE</td>
<td>0.16 ± 0.03</td>
<td>0.47 ± 0.14</td>
<td>0.39 ± 0.06</td>
<td>0.54 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>PHE</td>
<td>0.17 ± 0.04</td>
<td>0.37 ± 0.12</td>
<td>0.36 ± 0.10</td>
<td>0.52 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>PYR</td>
<td>0.16 ± 0.04</td>
<td>0.31 ± 0.06</td>
<td>0.51 ± 0.10</td>
<td>0.75 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>TRI</td>
<td>0.21 ± 0.05</td>
<td>0.48 ± 0.12</td>
<td>0.70 ± 0.20</td>
<td>0.95 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

*Names of the PAHs are given in Figure 2.*
Fig. 3. Reaction scheme for the phototoxicity of polycyclic aromatic hydrocarbons (PAHs) in leaf tissue. C denotes the intact PAH and P the mixture of photomodification products. The subscripts A and L denote the aqueous media and leaf tissue, respectively. G, G_{ox}, and G^• denote the intact, oxidized, and radical forms, respectively, of hypothetical biomolecules required for normal growth of plants. Q_1 and Q_2 are endogenous quenchers of excited states of indicated molecules. Half arrows denote reversible reactions and full arrows denote irreversible reactions. O_2 is ground triplet-state oxygen, O^•_2 is excited singlet-state oxygen, and hv is a photon of energy.

Simplified scheme for modeling photoinduced toxicity of PAHs

The general scheme for photoinduced toxicity described above (Fig. 3) is too complex for detailed mathematical analysis. To simplify the scheme (Fig. 4), a few assumptions can be made. As stated above, the main quencher for C_A in the aqueous solution is molecular oxygen. A major route for the interaction between C_A and O_2 is the generation of singlet-state oxygen, O^•_2 [19,35], which can react with the PAH that react with O_2 generating O^•_2. If O^•_2 is formed in the leaf in close proximity to G, G can be oxidized by the O^•_2. This type II photosensitization pathway can lead directly to inactivation of G [19]. Alternatively, the energy in C_A and O^•_2 can be dissipated by two other routes: quenchers (Q_1 and Q_2) and phosphorescence (Fig. 3). The quenchers are endogenous plant molecules that deactivate C_A or O^•_2 by energy transfer, which transforms them back to C_L or O_2, respectively. Carotenoids and ascorbate are examples of compounds that protect plants by scavenging active oxygen species [19,34]. Phosphorescence results from a spontaneous return of C_A to C_L with the release of a photon.

Type I photosensitization is another route that intact PAHs can use to oxidize G. In this instance, a free radical species, G^•, is generated from direct reaction of C_A with G (Fig. 3). These free radical species (or ion radicals) are readily oxidized by ground state molecular oxygen (O_2) or other biomolecules, rendering G inactive [19]. It should be noted that in both type II and type I photosensitization reactions, C_A is returned unaltered to the ground state.

The C_A can react directly with O_2 resulting in PAH oxidation products (P_L) (Fig. 3). The P_L molecules formed intracellularly, or formed extracellularly and taken up by the plant, can react directly with G. This direct attack would be a process that does not require light [7,14]. Ground state C_L molecules can also react directly with G (i.e., without photon activation). Furthermore, G can be damaged by type I and II photosensitization reactions initiated by excited state P_L molecules (Fig. 3) [7].

The reactions shown in Figure 3 describe the potential mechanisms of action of PAHs in plant cells in the presence of actinic radiation. However, defining the dynamics of a contaminant in a cell in this way is general in nature and can be applied to any mechanism of toxicity. In other cases, reactions describing the dynamics of the chemical in the absence of light but with other modifying factors might be relevant.

Fig. 4. Simplified reaction scheme for the phototoxicity of polycyclic aromatic hydrocarbons (PAHs) in leaf tissue. The reactions are described in the detail in the text. C, P, G, G_{ox}, G^•, O_2, Q_1, Q_2, and Q_3 are as described in Figure 3. k_2, k_{-2}, k_0, k_{ox}, k_m, and k_{-m} represent rate constants for the depicted reactions. f is the quantum yield for excited triplet-state formation. J is the integral of the overlap between the absorbance spectrum of a PAH and irradiation spectrum of the simulated solar radiation source. Half arrows denote reversible reactions and full arrows denote irreversible reactions.
formed it to create $P_A$ by a type II photosensitization reaction. The $P_A$ can also be formed by type I (radical-mediated) photosensitization reactions. However, the steps of both photomodification processes can be combined into an overall rate for the photomodification of PAHs in solution described by an exponential decay constant, $k_m$ (Table 1). Furthermore, as discussed above, there is not sufficient time for diffusion of $^1O_2$, $^3C_A$, and $P_X$ from the growth medium into the leaves. Therefore, the only reaction in aqueous solution that must be considered is the light-dependent modification of PAHs (Fig. 4).

Intact PAHs and their photoproducts diffuse from the aqueous solution into the leaves until a steady state is reached. The rate of uptake of PAHs and their photoproducts into the plants has been demonstrated to be much faster than the rate of PAH photomodification [31]. Further, a steady state is established for the PAHs in the two compartments within 24 h for both intact and photomodified PAHs [31]. During the PAH exposures of $L. gibba$ reported here, the chemicals were applied at 2 mg/L, well in excess of their solubility limits [30]. As such, there was a large reservoir of the PAH at the solid–aqueous interface to replenish the pool of PAHs photomodified and/or taken up by the plants. Therefore, we can assume that the concentration of $C_A$ was approximately constant throughout each toxicity test.

Many chemical processes in the plant tissue can be assumed to have a negligible contribution to toxicity. The leaf tissue strongly attenuates UV radiation and PAR, whereas the growth medium essentially does not absorb SSR. Because the plants only cover a small portion of the surface of the growth medium, the fluence rate of UV radiation will be much lower in the leaves than in the medium. Thus, the formation of $P_X$ from $C_L$ will be slow compared to the formation of $P_A$ from $C_A$. It then follows that the light-mediated conversion of $C_L$ to $P_L$ will be small compared to the uptake of $P_A$ that was formed in the aqueous medium. We also assume that metabolic (enzymatic) oxidation of PAHs, which is known to be slow in plants [36,37], will contribute negligibly to the concentration of oxidized PAHs.

With respect to intact PAHs, damage to G can be caused either by photosensitization reactions triggered by $C_L$ or by direct attack of $C_L$ on G in processes that do not require light. However, direct toxicity of intact PAHs in the absence of light or other forms of activation has been demonstrated to be negligible [7,20]. Therefore, we will not consider the direct reactions of intact PAHs with G in this model. Accordingly, the only route by which $C_L$ molecules can inhibit plant growth is via photosensitization.

One can also assume that the phosphorescence lifetime ($\tau_{ph}$, triplet-state lifetime in the absence of any quenchers) of $^3C_L$ is not an important factor to the photoinduced toxicity of PAHs. Indeed, phosphorescence lifetimes of all PAHs are relatively long ($10^{-6}$ to $10^{-3}$ s) [22], whereas the average interval between collisions of $^3C_L$ with $O_2$ is less than $10^{-6}$ s (see above). As such, phosphorescence in the presence of $O_2$ will be negligible; essentially all $^3C_L$ will be quenched by $O_2$ or other endogenous quenchers, $Q_L$. So $\tau_{ph}$, or the triplet-state lifetime, need not be considered as an essential factor in the photoinduced toxicity of PAHs.

When considering photomodified PAHs, two possible routes of G deactivation exist: photosensitized oxidation initiated by $P_L$ via $^1O_2$ or direct attack of $P_X$ on G. Previous studies have demonstrated that although many photomodified PAHs have enhanced activity in actinic radiation, they also have significant bioactivity in the absence of actinic radiation [7,20]. Currently, experimental data do not exist that would allow separation of the light-dependent and -independent components of photomodified PAH toxicity. Therefore, the toxicity of $P_L$ will be considered as a single group of reactions that represent both light-independent processes and photosensitive processes.

On the basis of the assumptions above, the general scheme in Figure 3 can be simplified (Fig. 4). We will consider only the rate of photooxidation of PAHs in the growth medium; the equilibrium of intact and photooxidized PAHs between the growth medium and leaf tissue, the photosensitized oxidation of G caused by the intact PAHs, the quenching of $^3C_L$, and finally the photochemical and nonphotochemical damage to G initiated by $P_L$.

**Proportionality of leaf production to the concentration of G**

Leaf production as a function of time in the presence of each of the 16 PAHs was used to model toxicity. Because the growth of $L. gibba$ proceeds exponentially when nutrients and the surface of the growth medium are not limiting [28], the growth rate as measured by leaf production is proportional to the number of leaves ($N$) present at any given time. Therefore

$$\frac{dN}{dt} = k'N$$

where $k'$ is the rate constant for growth of the plants. Based on our definition of G, one can assume that the growth rate is also proportional to the concentration of [G]. Therefore

$$\frac{dN}{dt} = k[G]$$

where $k$ is the rate constant for growth based on the concentration of G. By combining the two equations for $dN/dt$ one obtains

$$N = k[G]$$

where $k = k'k'$. Therefore, assuming all toxicity tests start with the same number of leaves, at any given time during a test the number of leaves present will be proportional to [G].

**Reactions of G in the presence of a PAH**

A chemical kinetic model describing G as a function of the chemical reactions in Figure 4 can be derived. This kinetic model can then be solved using empirical data for the number of leaves ($N$) produced in a given length of time in the presence of intact and photomodified PAHs (Tables 2 and 3).

According to the scheme in Figure 4, G is consumed via the following four reactions:

$$P_L + G \rightarrow P_G$$

(4)

$$^1O_2 + G \rightarrow G_{ox}$$

(5)

$$^3C_L + G \rightarrow ^{3}C_L + G^*$$

(6)

$$G^* + O_2 \rightarrow G_{ox}$$

(7)

where $k_{p}, k_{1}, k_{2},$ and $k_{3}$ are the rate constants for their respective reactions. Reaction 4 describes photochemical and nonphotochemical consumption of G by PAH photoproducts. Reaction 5 is a type II photosensitized oxidation of G where the $^1O_2$ was generated by reaction with $^3C_L$. Reactions 6 and
7 describe a two-step type I photooxygenation of G initiated by \(^{1}\)C\(_{L}\). The concentration of \(^{18}\)O\(_{2}\) is sufficiently high in plants to assume that the rate-determining step in this type I photosensitized oxidation is Reaction 6 rather than Reaction 7 [22].

The concentration of G can only increase exponentially with plant growth according to

\[ G \rightarrow 2G \tag{8} \]

where \( k_{g} \) is a growth-dependent unimolecular rate constant for production of G. Using Reactions 4 through 8, a differential equation can be generated describing the rate at which [G] changes

\[ \frac{d[G]}{dt} = k_{g}[G] - (k_{d}[P_{A}] + k_{p}[^{13}\text{C}_{L}] + k_{o}[^{18}\text{O}_{2}])[G] \tag{9} \]

To solve this equation for [G] one must derive expressions for \([P_{A}],[C_{A}],[^{13}\text{C}_{L}],[^{18}\text{O}_{2}]\).

**Derivation of \([P_{A}],[C_{A}]\)**

To mathematically solve for the concentration of \(P_{A}\), the rate of PAH photomodification in aqueous solution and the rate of uptake of photomodified PAHs into the leaves must be determined. The rate of PAH photomodification is described by a unimolecular reaction with first-order kinetics:

\[ C_{A} \xrightarrow{k_{m}} P_{A} \tag{10} \]

where \( k_{m} \) is the photomodification rate constant based on the \(t_{1/2}\) for each PAH in SSR. The half-lives of the PAHs in SSR were determined empirically (Table 1). Because PAH photomodification proceeds with exponential decay kinetics, this indicates a quasi-unimolecular reaction dependent only on PAH concentration under a constant fluence rate. Under the experimental conditions employed, an excess of the PAH was added to the growth medium. Therefore, the concentration of the PAH and its photoproducts, \([C_{A}]+[P_{A}]\), in the aqueous phase should be approximately constant and much greater than the amount of \(C_{A}\) plus \(P_{A}\) throughout the toxicity test. Given that \([C_{A}]\) is the initial concentration of PAH delivered to the growth medium, and that pseudo-first-order photodegradation of PAHs based on \([C_{A}]\) was observed, expressions describing the amounts of \(C_{A}\) and \(P_{A}\) present at any given time are

\[ [C_{A}] = [C_{A}]_{0}e^{-k_{m}t} \tag{11} \]

\[ [P_{A}] = [C_{A}]_{0}(1 - e^{-k_{m}t}) \tag{12} \]

The rate of PAH photomodification is slower than the rate of uptake of both intact and photomodified PAHs by \textit{L. gibba} [31]. It then follows, based on equations 11 and 12, that the concentrations of \(C_{A}\) and \(P_{A}\) can be described as

\[ [C_{A}] = K_{c}[C_{A}]_{0}e^{-k_{c}t} \tag{13} \]

\[ [P_{A}] = K_{p}[C_{A}]_{0}(1 - e^{-k_{p}t}) \tag{14} \]

where \( K_{c} = k_{c}/k_{c}^{*} \) and \( K_{p} = k_{p}/k_{p}^{*} \). \( K_{c} \) and \( K_{p} \) are the equilibrium constants (or bioconcentration factors) for partitioning of C and P between the aqueous solution and the plants. The assimilation rate constants are \( k_{c} \) and \( k_{p} \), and the depuration rate constants are \( k_{c}^{*} \) and \( k_{p}^{*} \). Note, for the photomodified PAHs, the rate limiting step in uptake is \( k_{c} \) (the rate of photomodification). Indeed, in the empirical model in the companion paper [29] we found that a parameter for the uptake of the photomodified compounds was not required to predict toxicity.

**Derivation of \([^{3}\text{C}_{L}]\)**

Excited triplet-state PAHs, \(^{3}\text{C}_{L}\), are formed by intersystem crossing from the excited singlet-state \((^{1}\text{C}_{L}\)) which, in turn, is generated from a ground-state molecule of \(C_{L}\) by the absorption of a photon (h\(\nu\))

\[ C_{L} + h\nu \xrightarrow{k_{p}(\lambda)}^{3}\text{C}_{L} \rightarrow ^{1}\text{C}_{L} \tag{15} \]

where \(k_{p}(\lambda)\) is the spectral photon fluence rate (i.e., spectral density or output) of the SSR source used in the experiments [7,15,20], \(\epsilon(\lambda)\) is the extinction coefficient of \(C\) as a function of wavelength, and \(\varphi\) is the quantum yield of triplet-state generation. To use \(I(\lambda)\) and \(\epsilon(\lambda)\) more conveniently they were combined into a single value \(J\) (Table 1), where \(J\) is the integral of overlap between the SSR output spectrum and the absorbance spectrum for each PAH:

\[ J = \int_{\lambda_{\text{min}}}^{\lambda_{\text{max}}} I(\lambda)\epsilon(\lambda)d\lambda \tag{16} \]

One can then write an equation for the rate of PAH triplet-state generation:

\[ \left( \frac{d[^{3}\text{C}_{L}]}{dt} \right)_{\text{gen}} = \varphi J[^{1}\text{C}_{L}] \tag{17} \]

Consumption of \(^{3}\text{C}_{L}\) occurs by the following reactions:

\[ ^{3}\text{C}_{L} + G \rightarrow ^{1}\text{C}_{L} + G^{*} \tag{18} \]

\[ ^{3}\text{C}_{L} + ^{18}\text{O}_{2} \rightarrow ^{1}\text{C}_{L} + ^{1}\text{O}_{2} \tag{19} \]

\[ ^{3}\text{C}_{L} + Q_{1} \rightarrow ^{1}\text{C}_{L} + Q_{1} + \text{heat} \]

where \(k_{p}^{(1)}, k_{d}^{(1)}, \text{and} k_{d}^{(1)}\) are the rate constants for their respective reactions. Reactions 6 and 18 lead to damage of G by type I and type II photosensitized oxidations, respectively. In the case of Reaction 18, it is \(^{18}\text{O}_{2}\) that will oxidize G. Reaction 19 represents quenching of \(^{3}\text{C}_{L}\) by endogeneous molecules, denoted as \(Q_{1}\), such as carotenoids, which putatively protect the plants from oxidative damage [19,34,38,39]. Combining Equations 6, 18, and 19, the rate of \(^{3}\text{C}_{L}\) consumption is

\[ \left( \frac{d[^{3}\text{C}_{L}]}{dt} \right)_{\text{consump}} = k_{d}^{(1)}[^{3}\text{C}_{L}][G] + k_{d}^{(1)}[^{3}\text{C}_{L}][^{18}\text{O}_{2}] \]

\[ + k_{d}^{(1)}[^{3}\text{C}_{L}][Q_{1}] \tag{20} \]

The differential equation for the rate of change in \(^{3}\text{C}_{L}\) is

\[ \frac{d[^{3}\text{C}_{L}]}{dt} = \left( \frac{d[^{3}\text{C}_{L}]}{dt} \right)_{\text{gen}} - \left( \frac{d[^{3}\text{C}_{L}]}{dt} \right)_{\text{consump}} \]

\[ = \varphi J[^{1}\text{C}_{L}] - k_{d}^{(1)}[^{3}\text{C}_{L}][G] - k_{d}^{(1)}[^{3}\text{C}_{L}][^{18}\text{O}_{2}] \]

\[ - k_{d}^{(1)}[^{3}\text{C}_{L}][Q_{1}] \tag{21} \]

The rates of \(^{3}\text{C}_{L}\) formation and degradation are rapid (ns to ms) and dependent on the concentrations of \(C_{L}\), \(G\), \(^{18}\text{O}_{2}\), and \(Q_{1}\), as well as on the fluence rate of the SSR source. Because the levels of \(C_{L}\), \(G\), \(^{18}\text{O}_{2}\), and \(Q_{1}\) are constant for relatively long time periods (minutes to hours), it can be assumed that the steady state \([^{3}\text{C}_{L}]\) is also approximately constant on the long time scales of a toxicity test. Thus, \(d[^{3}\text{C}_{L}]/dt\) can be assumed to be very close to zero. Equation 21 can then be set equal to zero and algebraically rearranged to solve for \([^{3}\text{C}_{L}]\) giving
\[ [1^1C_1] = \frac{\varphi[C_1]J}{k_1^{[1]}[G] + k_1^{[1]}[O_2] + k_1^{[1]}[Q_1]} \] (22)

To further simplify this equation, the frequency of reactions between \(1^1C_1\) and \(G\) can be assumed to be small compared with other routes of \(1^1C_1\) consumption. This is because we assume that the concentration of any given \(G\) will be small relative to \([O_2]\) and \([Q_1]\) (quenchers such as carotenoids and ascorbate occur in high concentrations in plants) [38,39]. Therefore, it can be assumed that \(1^1O_2\) will be at an approximate steady-state level on the time scale of the toxicity test. Therefore, \(1^1O_2\) can be written

\[ [1^1O_2] = \frac{\varphi[C_1]J}{k_1^{[1]}[O_2] + k_1^{[1]}[Q_1]} \] (24)

**Derivation of \([1^1O_2]\)**

Within plant cells \(1^1O_2\) is generated by energy transfer from \(1^3C_1\) to \(1^3O_2\)

\[ \cdot 1^3C_1 + \cdot 1^3O_2 \xrightarrow{k_1^{[3]}} \cdot 1^1C_1 + 1^1O_2 \] (18)

where \(k_1^{[3]}\) is the rate constant for this reaction. \(1^1O_2\) can be consumed by reaction with \(G\) molecules or by reaction with endogenous quenchers, \(Q_2\)

\[ \cdot 1^1O_2 + G \xrightarrow{k_2^{[1]}} G_n \] (5)

\[ \cdot 1^1O_2 + Q_2 \xrightarrow{k_2^{[2]}} O_2 + Q_2 + \text{heat} \] (25)

where \(k_2^{[1]}\) and \(k_2^{[2]}\) are the respective rate constants for the reactions. Therefore, using Equations 5, 18, and 25 a differential equation describing the rate of change in the concentration of \(1^1O_2\) can be written

\[ \frac{d[1^1O_2]}{dt} = k_1^{[1]}[1^1C_1][O_2] - k_2^{[1]}[1^1O_2][G] - k_2^{[2]}[1^1O_2][Q_2] \] (26)

The same logic used to conclude that \(d[1^1C_1]/dt \approx 0\) can be used to set \(d[1^1O_2]/dt \approx 0\). Because the lifetime of \(1^1O_2\) compared to other molecules in the differential equation is short, the \([1^1O_2]\) will be at an approximate steady-state level on the time scale of the toxicity test. Therefore, Equation 26 can be rearranged to algebraically solve for \([1^1O_2]\)

\[ [1^1O_2] = \frac{k_1^{[1]}[1^1C_1][O_2]}{k_2^{[1]}[G] + k_2^{[2]}[Q_2]} \] (27)

As in the derivation of \([1^1C_1]\), it can be assumed that the concentration of quenchers of active \(O_2\) in plants is very high [19,34,35,38,39], making \([1^1O_2]\) almost negligible. Therefore, it can be assumed that \(1^1O_2\) will be at a steady-state level on the time scale of the toxicity test. Therefore, \([1^1O_2]\) can be written

\[ [1^1O_2] = \frac{k_1^{[1]}[1^1C_1][O_2]}{k_2^{[1]}[Q_2]} \] (28)

Using Equation 24, it is possible to substitute for \([1^1C_1]\), and rewrite Equation 28 as

\[ [1^1O_2] = \frac{k_1^{[1]}\varphi[C_1][O_2]}{k_2^{[1]}[Q_2](k_1^{[1]}[O_2] + k_1^{[1]}[Q_1])} \] (29)

**Derivation of \([G]\)**

At this point, \([C_1]\) (Eqn. 13), \([P_1]\) (Eqn. 14), \([1^1C_1]\) (Eqn. 24), and \([1^1O_2]\) (Eqn. 29) can be substituted into Equation 9 to express the rate of change in \([G]\) as a function of time
\[ Y = -At \left( \frac{B + A}{k_{at}e} \right) (1 - e^{-ktc}) \] (40)

Substituting from Equations 31 and 32, Equation 40 can be written as

\[
Y = -k_dK_p[C]_o t \left( 1 - e^{-ktc} \right) \left( \frac{1}{k_{m}t} \right) \left( \frac{1}{1 - e^{-ktc}} \right)
\]

This equation can be rearranged to isolate the portions of the toxicity equation representing photomodification and photosensitization

\[
Y = -k_dK_p[C]_o t \left( 1 - e^{-ktc} \right) \left( \frac{1}{k_{m}t} \right) \left( \frac{1}{1 - e^{-ktc}} \right)
\]

To simplify Equation 42, the following notations are introduced to define the three parts of the equation that are each a series of PAH-dependent constants:

\[ D = k_dK_p \] (43)

\[ F = \left( k_{1}^{1} + \frac{k_{1}^{2}k_{2}^{1}}{k_{2}^{2}}[O_2] \right) \left( \frac{1}{k_{1}^{1}[O_2] + k_{1}^{2}[Q_1]} \right) \] (44)

and

\[ R = \frac{1}{k_{m}t} \left( 1 - e^{-ktc} \right) \] (45)

where \( D \) represents the uptake and reactivity of the photomodified PAHs, \( F \) represents uptake and photosensitization activity of the intact PAHs, and \( R \) represents the fraction of the original amount of intact PAH remaining at \( t \). Thus, diminished leaf production can be rewritten as

\[
Y = -[C]_o t \left[ D(1 - R) + F\varphi J R \right]
\]

\[ = -[C]_o t \left( PMC + PSC \right) \] (46)

where \( D(1 - R) \) and \( F\varphi J R \) are the photomodification (PMC) and the photosensitization (PSC) constants, respectively, for each PAH. This results in a final equation for the photoinduced toxicity of PAHs that is a linear function dependent on an algebraic sum of independent factors for the bioactivity of the photomodified PAHs and the photosensitization activity of the intact PAHs. Thus, in theory, the contributions of photosensitization and photomodification to the toxicity of PAHs are distinct and additive.

**Solution of PMC and PSC based on empirical data for toxicity**

Based on Equation 46, \( Y \) is a linear function of several experimental parameters: \([C]_o\), \( t, R, \varphi, J, D, \) and \( F \). The values for \([C]_o\), \( R, \varphi, \) and \( J \) have been determined (Table 1); \( R \) was calculated from \( k_m \). \( J \) was calculated from the integral of the overlap between the absorbance spectrum of each chemical and the spectral output of the SSR source (Eqn. 16), and values for \( \varphi \) were obtained from the literature [5,8,13,22,23,40]. Therefore, only \( D \) and \( F \) are unknowns for each PAH. \( D \) combines two chemical-specific constants for characterizing the uptake and toxicity of the photomodification products produced from a given PAH, \( K_p \) and \( k_d \) (Eqn. 43). \( F \) (Eqn. 44) is a complex combination of constants, distinct for each PAH, describing the photoinduced toxicity of intact compounds via type I and type II photosensitization reactions. With empirical data for leaf production as a function of time for each chemical in intact and photomodified form (Tables 2 and 3), both \( D \) and \( F \) can be solved independently for each PAH. This is analogous to calculating rate constants or equilibrium constants for chemical reactions based on data for production of a product of that reaction. It is crucial to understand that these constants represent a mathematical solution based on empirical data, not a statistical proof that the model is correct. The latter is presented in the companion paper [29].

To calculate \( D \), and consequently the PMC = \( D(1 - R) \), the data for diminished leaf production, \( Y \), by fully photomodified PAHs were used (Table 3). The treatment of plants with photomodified PAHs corresponds to \( R = 0 \), because there are no intact PAHs to be photomodified. Therefore, Equation 46 simplifies to

\[ Y = -[C]_o J D \] (47)

Linear regression as a function of time, \( Y = a + bt \), can be used to solve for \( D \) because the slope, \( b \), is \(-[C]_o D \). As such, \( D \) for each PAH can be determined by the quotient of the slope of the linear regression for \( Y \) and the initial concentration of the PAH:

\[ D = \frac{b}{[C]_o} \] (48)

Table 4 contains \( a, b, \) and \( D \) values for the 16 PAHs examined. It should be noted that the linear regressions resulted in \( a \) (the
vertical intercept) having a value of approximately zero at $t = 0$; the plot of $Y$ against $t$ nearly passed through the origin for every PAH, even though the $t_0$ point was not used in the regressions. Indeed, the errors for $a$, based on the toxicity data, in many cases were larger than $a$. This is consistent with Equation 46 for $Y$, which does not contain a constant for the vertical intercept. Using the values $D$ (Table 4) and $R$ (Table 1) the PMC ($= D(1 - R)$) for each PAH was calculated (Table 4). Note, the $D$s only range over about one order of magnitude, implying the photomodified products from each PAH have similar values for $K_y$ and $k_x$.

Having determined a $D$ for each PAH, it is possible to use the experimental data for diminished leaf production of $L$. gibba, $Y = \ln(N_I/N_s)$, by the intact chemicals (Table 2) to solve for the parameter $F$ and the PSC ($= F\varphi JR$). $Y$ is proportional to the period of treatment, $t$ (Eqn. 46), and can be used for the linear regression, $Y = c + ft$, to determine $F$. Once again the vertical intercept, $c$, approached zero for most of the PAHs and the regression is essentially $Y = ft$. Combining this abridged linear equation and Equation 46 results in the following equation, which can be solved for $F$

$$F = \frac{f}{[C]_0} - D(1 - R)$$

The values $c$, $f$, and $F$ are presented in Table 5. Using known values for $[C]_0$, $R$, $\varphi$, and $J$ (Table 1), as well as $D$ (Table 4) and $f$ (Table 5) the values for $F$ and the PSC ($= F\varphi JR$) was calculated for all 16 PAHs (Table 5).

**Graphic representation of growth suppression**

The experimentally determined PSCs and PMCs can be summed according to Equation 46 and plotted against $Y$. The plot of $Y$ versus $-t[C]_0$(PMC $+$ PSC) yields a straight line with the values for all the PAHs falling on this line at the four time points (Fig. 5A, $r^2 = 0.96$, $p < 0.001$). A plot of $Y/t$ versus $[C]_0$(PMC $+$ PSC) can also be generated by averaging the four $Y/t$ values for each PAH (Fig. 5B, $r^2 = 0.95$, $p < 0.001$). This results in a single data point for each of the 16 PAHs, allowing a clearer depiction of where each chemical falls in the model. Both plots reveal that when the PSC and the PMC are used in modeling photoinduced toxicity of PAHs, an excellent relationship between toxicity and the physicochemical parameters can be generated.

**CONCLUSIONS**

This QSAR model illustrates theoretically how photosensitization and photomodification processes can be brought together to describe the photoinduced toxicity of PAHs. We demonstrated that these two processes in photoinduced toxicity are additive, and when used in a single QSAR, can reconcile photoinduced toxicity data for 16 PAHs into a linear function. This indicates that both processes must be considered when evaluating the phototoxicity of PAHs in the environment. To prove that this model is valid, empirical values must be used for the parameters described here to determine if this model can be used to predict toxicity (see companion paper [29]).

Comparison of the PSC and PMC for the chemicals demonstrates that either constant can occupy a key role: for nine PAHs the PMC is larger, for seven the PSC is higher. Interestingly, the PMC for PHE is larger than the PSC. Recently, PHE has been demonstrated to have little photosensitization activity and the photoinduced toxicity of PHE can be attributed almost entirely to its major photomodification product, phenanthrenquinone [21]. Conversely, FLA has the highest PSC of the chemicals tested and has been used by many investigators as an example of a contaminant that behaves as a photosensitizer [5,11]. Indeed, a general trend exists among those chemicals that have a PSC greater than their PMC to be more toxic relative to each other with increasing values of PSC (i.e., FLA $>$ BBF $>$ ... $>$ BGP).

Anthracene has consistently been found to be the most phototoxic PAH to plants. It is very toxic in photomodified form and is the most rapidly photomodified of the PAHs tested. Interestingly, ANT has the second highest PMC value (Table 4). Moreover, the PSC of ANT is not significantly different from zero. This suggests that intact ANT is not a strong photosensitizer, and perhaps only becomes toxic following photomodification. The PAH with the highest PMC is BAA. However, it has a negative PSC; neither constant should be negative.
due to uptake of the intact PAHs into the plants where the PAHs will have slower rates of photomodification.

The log of the octanol/water partition coefficient ($K_{ow}$) is often used as a predictor of toxicity as it describes potential tissue burdens of a chemical. However, the log $K_{ow}$s for PAHs do not predict their photoinduced toxicity [5]. This QSAR modeling exercise demonstrates that understanding the mechanism of toxicity is just as important as the potential for uptake of a chemical. Past QSAR models for photoinduced toxicity of PAHs have accurately described the photosensitization factor. For instance, in a recent model [11], optimum values for photosensitization were used to describe toxicity. Our study should be viewed as complementing previous QSAR models by incorporating photomodification. By considering both photosensitization and photomodification processes, one should be able to predict the toxicity of PAHs to numerous organisms in environments exposed to light.

A key result of the modeling exercise presented here is that the photosensitization and photomodification processes contribute additively to toxicity. This is logical, because the intact PAHs and the photoproducts will exist in solution together after a toxicity test begins. Alternatively, one may consider that an intact PAH will exert itself via photosensitization until it is photomodified, at which point the photoproducts will have a toxic impact. This implies that the intact and photomodified PAHs do not act synergistically. Indeed, the predictive model in the companion paper [29], shows empirically that photosensitization and photomodification contribute additively to toxicity.

Acknowledgement—We wish to thank Lisha Ren and Brendan McConkey for technical assistance. We acknowledge W. Taylor, N. Bols, P. Mezry, and members of the Greenberg and Dixon labs for fruitful discussions. This research was supported by grants from the Canadian Network of Toxicology Centers and the National Science and Engineering Research Council to B.M. Greenberg and D.G. Dixon.

REFERENCES
3. Yang, S.K., D.W. McCourt, P.P. Roller and H.V. Gelboin. 1976. Enzymatic conversion of benzo(a)pyrene leading predominantly to the diol-epoxide r-7,8-dihydroxy-r-9, 10-oxy-7,8,9,10-tetrahydroxypbenzo(a)pyrene through a single enantiomer of r-7,8-dihydroxy-7, 8-dihydrobenzo(a)pyrene. Proc. Natl. Acad. Sci. USA 73:2594–2598.