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Photochemical Hydrogen Abstraction and Electron Transfer Reactions of Tetrachlorobenzoquinone with Pyrimidine Nucleobases[†]

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Pentachlorophenol, a widespread environmental pollutant that is possibly carcinogenic to humans, is metabolically oxidized to tetrachloroquinone (TCBQ) which can result in DNA damage. We have investigated the photochemical reaction dynamics of TCBQ with two pyrimidine type nucleobases (thymine and uracil) upon UVA (355 nm) excitation using the technique of nanosecond time-resolved laser flash photolysis. It has been found that 355 nm excitation populates TCBQ molecules to their triplet state $^3\text{TCBQ}^*$, which are highly reactive towards thymine or uracil and undergo two parallel reactions, the hydrogen abstraction and electron transfer, leading to the observed photoproducts of $\text{TCBQH}\cdot$ and $\text{TCBQ}\cdot^-$ in transient absorption spectra. The concomitantly produced nucleobase radicals and radical cations are expected to induce a series of oxidative or strand cleavage damage to DNA afterwards. By characterizing the photochemical hydrogen abstraction and electron transfer reactions, our results provide potentially important molecular reaction mechanisms for understanding the carcinogenic effects of pentachlorophenol and its metabolites TCBQ.

Key words: Tetrachlorobenzoquinone, Thymine, Uracil, Triplet state, Hydrogen abstraction, Electron transfer, Laser flash photolysis

I. INTRODUCTION

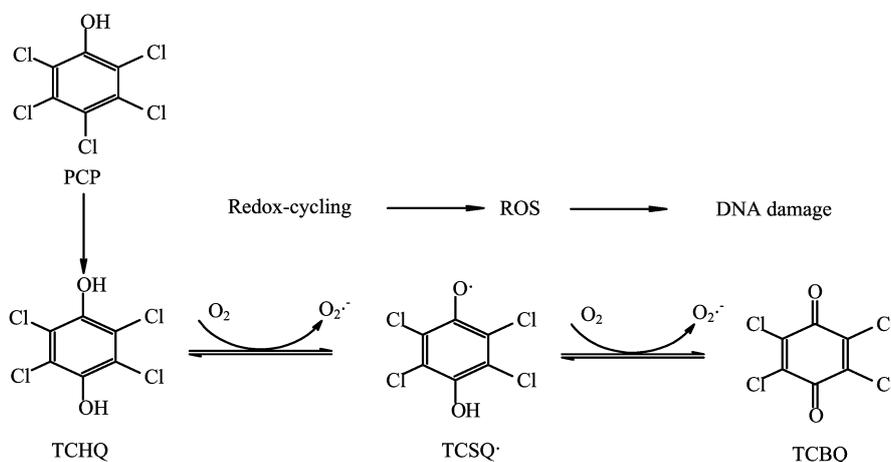
Polyhalogenated quinones represent a class of toxicological intermediates that can create a variety of hazardous effects *in vivo*, including acute hepatotoxicity, nephrotoxicity, and carcinogenesis [1–5]. Tetrachlorobenzoquinone (TCBQ) is one of the major genotoxic and carcinogenic quinoid metabolites of the widely used preservative pentachlorophenol (PCP) [3, 4]. Because of its efficiency, broad spectrum, and low cost, PCP has been used as an algacide, bactericide, fungicide, herbicide, insecticide, and molluscicide. In China and other developing countries, PCP has also been used to kill snails to prevent snail fever. Its worldwide usage and relative stability make PCP a ubiquitous environmental pollutant [1, 2]. In fact, PCP has been detected in body fluids, such as human urine, blood, breast milk, and adipose tissue. While the precise mechanism of PCP's genotoxicity remains to be elucidated, it has been suggested that its quinone and semiquinone metabolites play an important role (Scheme 1 [1]). The carcinogenesis of PCP is thought to involve enzyme-mediated oxidative biotransformations to produce reac-

tive oxygen species (ROS) and the corresponding hydroquinone and semiquinone [1, 3]. Hydroquinone metabolites can be oxidized further to generate quinones in a process mediated by mammalian peroxidases such as lactoperoxidases and myeloperoxidase (MRP). A final quinone metabolite of pentachlorophenol is TCBQ, which induces DNA damage in Chinese hamster ovary cells and single-strand breaks from the para-isomer but not the corresponding ortho-isomer [1, 6]. Recently, several modes of action have been investigated for DNA damage induced by TCBQ. Due to the reactive electrophilic nature of TCBQ, DNA adducts were identified in calf thymus DNA reacting with TCBQ. Structure of a deoxyguanosine TCBQ-dG adduct was characterized carefully, and was found to be a dichlorobenzoquinone nucleoside [6, 7]. The reaction was dependent on the type of nucleobases, as TCBQ reacted more readily with dG than that with other bases. In the presence of Cu(II) and NADPH, low concentrations of TCBQ can induce increased production of 8-hydroxydeoxyguanosine (8-HO-dG) and abasic sites.

Although TCBQ has been demonstrated to be an important and carcinogenic quinoid metabolite of PCP and there is some knowledge of the thermal reactions of TCBQ towards DNA as discussed above, it remains unknown regarding the photochemical reaction process of TCBQ with DNA. Some works have been done on the interaction of unsubstituted quinone with DNA bases [8–13] which may provide valuable hints

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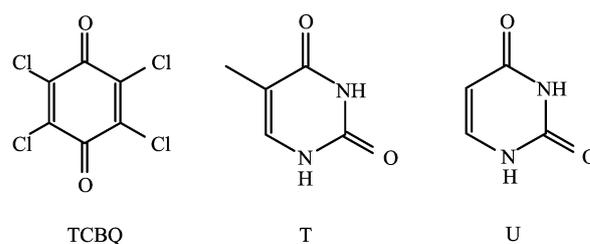
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Scheme 1. Formation of the PCP carcinogenic quinone metabolites [1].

for the current reaction system. Quinones of small size, such as *p*-benzoquinone, 1,4-naphthoquinone, and 9,10-anthraquinone are good electron transfer (ET) agents due to the presence of the electron accepting quinone group, whose photochemical properties in solution have been studied by means of laser flash photolysis and pulse radiolysis. Breslin *et al.* have shown that photo excited $^3\text{AQ}^*$ (anthraquinone) is responsible for DNA damage through electron transfer from DNA base [12] and Bergeron *et al.* have observed UV-induced cross-links in anthraquinone-DNA duplexes [9]. DNA is well known to be sensitive to UV radiations, which cause the formation of pyrimidine dimers by direct photoexcitation, or indirect photosensitization by the interaction of DNA in the ground state with another excited molecule. Quinones embedded in DNA have been extensively used as a photosensitizer to study a variety of photoinduced damages, as alkaline labile breaks, interstrand cross-links, damages at all four DNA bases and relaxation of supercoiling circular DNA, which demonstrate the reactivity of base radicals imbedded in DNA duplexes. Very recently, Basu *et al.* carried out a series of studies on the interaction of menadione and anthraquinone with the DNA bases by laser flash photolysis technique [14–17], showing that most nucleobases reacted with quinones by electron transfer along with hydrogen abstraction. The difference in the quinone structures was also indicated to have some influence on their reactivity towards DNA bases.

In this work, we are motivated to examine the severe detrimental effects of TCBQ by looking into its photochemical reactions with nucleobases upon UV irradiation at 355 nm. Due to the heavy atom effect, TCBQ molecules are expected to undergo primarily intersystem crossing after excited to its singlet state, and thus produce large yields of triplet $^3\text{TCBQ}^*$, which are extremely reactive towards nucleobases. By following the reactions of these highly reactive triplet $^3\text{TCBQ}^*$ with two typical pyrimidine bases, *i.e.* thymine and uracil,



Scheme 2 Molecular structures of the chemical reagents.

using the technique of nanosecond time-resolved laser flash photolysis, we have found that, the electron transfer product $\text{TCBQ}^{\cdot-}$ and hydrogen abstraction product TCBQH^{\cdot} are formed rapidly within 1 μs . By characterizing the photochemical hydrogen abstraction and electron transfer reactions, our results provide potentially important molecular reaction mechanisms for understanding the carcinogenic effects of the widespread environmental pollutants PCP and its metabolites TCBQ.

II. EXPERIMENTS

Tetrachlorobenzoquinone (TCBQ), thymine (T), and uracil (U) were purchased from Sigma-Aldrich and used as received. To dissolve both the hydrophobic TCBQ and the hydrophilic nucleobases, a mixture of HPLC-grade acetonitrile (ACN) and Millipore Milli-Q water (4:1) was used as solvents. Chemical structures of the compounds used in the current work are shown in Scheme 2.

Nanosecond time-resolved transient absorption spectra were measured using a laser flash photolysis setup LP920 spectrometer (Edinburgh Instruments), combined with an Nd:YAG laser (Surelite II, Continuum). The sample was excited by a 355 nm laser pulse (1 Hz,

5 mJ, FWHM \approx 7 ns). The analyzing light was from a 450 W pulsed xenon lamp (Osram XBO 450/OFR). The laser and analyzing light beams crossed at right angles and passed through a quartz cell with 1 cm path length. A symmetrical Czerny-Turner monochromator (TMS300, Bentham) equipped with a photomultiplier (R928, Hamamatsu) for detecting the spectral range from 185 nm to 870 nm was used to analyze transient absorption spectra. The signals from the photomultiplier were collected and recorded as a function of time on an oscilloscope (Tektronix TDS3012C, 100 MHz, 1.25 Gs/s sampling rate), and the data were transferred to a computer via TekVISA software. Each data point was obtained with 10 times average to improve the signal-to-noise ratio. The transient absorption spectra were obtained from a series of oscilloscope traces measured with the same solution in a point-by-point manner with respect to the wavelength and then the data were analyzed by the online software L900 of the LP920 spectrophotometer. Prior to laser irradiation, all solutions were bubbled with the high-purity N₂ gas for at least 20 min to avoid the triplet state quenching by oxygen. All experiments were carried out at room temperature.

III. RESULTS AND DISCUSSION

Excitation of TCBQ alone in ACN/H₂O (4:1) solution by 355 nm laser leads to efficient and fast population to its triplet state ³TCBQ* instantaneously following the laser pulse. As shown in Fig.1(a), there are two strong triplet-triplet (T-T) absorption bands of ³TCBQ* spanning a broad spectral range from 300 nm to 600 nm. The first absorption band of ³TCBQ* has a maximum at about 510 nm and a shoulder at 480 nm. The second absorption band of ³TCBQ* at 370 nm is attributed to a π - π^* transition [18]. Due to the quenching process by the ground state TCBQ molecules or solvent, the triplet state ³TCBQ* decays with a time constant of 377 ± 5.5 ns in degassed solutions, as shown from the single exponential fit (Fig.1(b)) to the decay kinetics of the transient absorption bands of ³TCBQ*. Upon oxygenation, all of the transient features in Fig.1(a) disappear, which confirms assignment of these transient bands to the lowest triplet state ³TCBQ*.

For the solution of TCBQ mixed with excess of pyrimidine bases, obvious photoproduct formation, in addition to the T-T absorption of ³TCBQ*, is observed in the transient optical absorption spectra upon 355 nm excitation, as shown in Fig.2. Nucleobases do not absorb 355 nm and no noticeable transient species appear in the spectra upon 355 nm excitation of the nucleobases. Only TCBQ is excited by 355 nm in the mixed system. Thus, the observed photoproducts arise from the excited TCBQ with the ground state nucleobases.

Figure 2(a) displays the transient absorption spectra obtained on irradiating 0.4 mmol/L TCBQ in the presence of thymine dissolved in ACN/H₂O (4:1) after laser

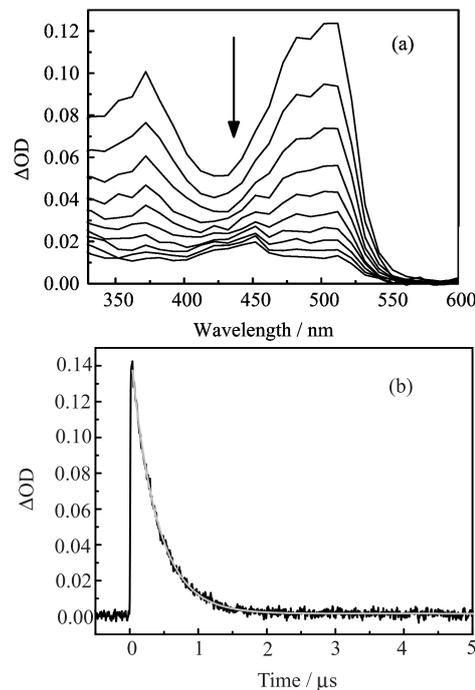


FIG. 1 (a) Transient absorption spectra of TCBQ (0.4 mmol/L in ACN/H₂O (4:1)) at typical delay times with arrow showing 300, 600, 900, 1200, 1500, 1800, 2100, 2400, 2700, 3000 ns respectively, after pulsed excitation at 355 nm. (b) Temporal profiles of the T-T absorption band of ³TCBQ* at 510 nm (with noise). The smooth curve is the single exponential fitting results for the decaying process.

flash at 355 nm. Compared to the pure TCBQ system in Fig.1(a), it is first noticeable that the quenching of the T-T absorption bands of ³TCBQ* at 510 and 370 nm is markedly accelerated when 1 mmol/L thymine was added to TCBQ solution, indicating the occurrence of a photochemical reaction between ³TCBQ* and thymine. Secondly, a new absorption band with two peaks centered at 420 and 450 nm grows in and reaches its maximum intensity within 1 μ s, simultaneously when the two T-T absorption bands of ³TCBQ* at 510 and 370 nm are rapidly quenched to their lowest intensity at 1 μ s. This reflects the gradual forming process of the photoproducts from a bimolecular reaction of ³TCBQ* with thymine, and the photoproducts absorb at 420 and 450 nm. Similar results are observed for the photochemical reaction system of TCBQ with uracil, as shown in Fig.2(b).

According to the previous related photolysis study results on TCBQ [19, 20], the photoproducts absorbing at 420 and 450 nm can be assigned to TCBQH \cdot and TCBQ \cdot^- , respectively. The TCBQH \cdot product comes from the hydrogen abstraction reaction between ³TCBQ* and thymine or uracil, while TCBQ \cdot^- is the electron transfer product. The reaction processes can be summarized with Scheme 3.

Actually, the photochemical electron transfer and hy-

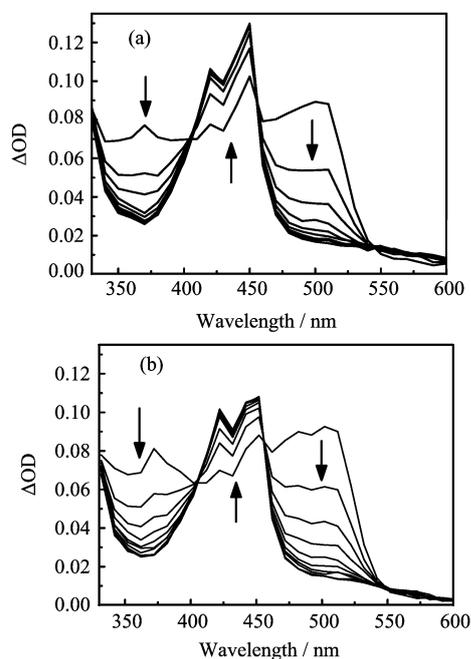
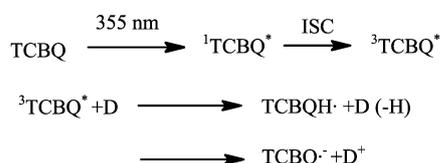


FIG. 2 Transient absorption spectra of TCBQ (0.4 mmol/L) with nucleobases (1 mmol/L) in ACN/H₂O (4:1) at typical delay times with arrows showing 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 ns respectively, after a pulsed excitation at 355 nm. (a) TCBQ+T and (b) TCBQ+U.



Scheme 3 Photochemical reaction processes of TCBQ with pyrimidine bases T or U. D represents the nucleobases T or U.

drogen abstraction products have also been observed in the reaction of anthraquinone and menadione with DNA bases by Basu *et al.* [14–17], indicating that quinones are subject to these types of photochemical reactions readily once populated to their reactive triplet state by photo excitation. Thus, ${}^3\text{TCBQ}^*$ are also expected to be susceptible to electron transfer and hydrogen abstraction reactions with nucleobases, as shown clearly in Fig.2. The two products TCBQH \cdot and TCBQ \cdot^- are generated simultaneously within 1 μs and also decay with identical temporal profiles, as displayed with their characteristic kinetic traces in Fig.3. The similar time evolution implies that both TCBQ \cdot^- and TCBQH \cdot are generated from the initial reaction of ${}^3\text{TCBQ}^*$ with T or U. In other words, the hydrogen abstraction and electron transfer reactions are parallel, but not sequential, to each other.

It has been reported that some solvents, such as CH₃CN, could also be involved in the photochemical

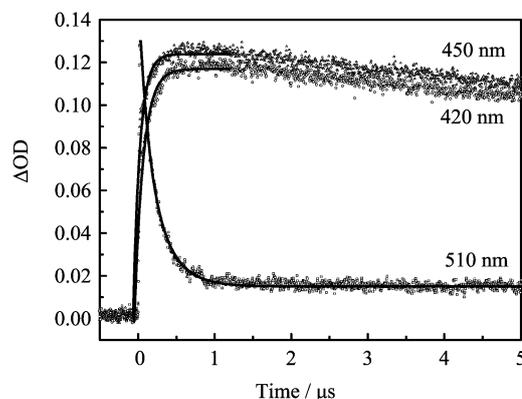


FIG. 3 Temporal profiles for the characteristic absorption bands (420, 450, and 510 nm) involved in the photochemical reaction of TCBQ with T. The dotted curves with noise are experimental data and the smooth solid curves are the exponential fitting results for the forming process of photoproducts and the quenching process of ${}^3\text{TCBQ}^*$.

reaction processes [19], as illustrated in Scheme 3. If re-examining Fig.1, the spectra taken for the pure TCBQ system in CH₃CN/H₂O solution, it can be found that after the T-T absorption bands of ${}^3\text{TCBQ}^*$ at 510 and 370 nm relax to their lowest intensity, a quite weak absorption band exists at 420 and 450 nm, which could be ascribed to the hydrogen abstraction and electron transfer reaction products of ${}^3\text{TCBQ}^*$ with acetonitrile. But compared to the prominent formation of TCBQH \cdot and TCBQ \cdot^- from the ${}^3\text{TCBQ}^*$ reacting with thymine or uracil, as demonstrated with the strong bands of 420 and 450 nm observed in Fig.2, the photoproducts from the ${}^3\text{TCBQ}^*$ reacting with acetonitrile are negligible.

The temporal profiles for the characteristic absorption bands involved in the photochemical reaction are plotted in Fig.3, taking the system of TCBQ with thymine as an example. The kinetic results for TCBQ with uracil are similar. The accelerated quenching rate of ${}^3\text{TCBQ}^*$ at 510 nm in the presence of T indicated the occurrence of a photochemical reaction between ${}^3\text{TCBQ}^*$ and T. For the two photoproducts, TCBQH \cdot and TCBQ \cdot^- which absorb respectively at 420 and 450 nm, the growing process reflects their gradual formation from the photochemical reaction of ${}^3\text{TCBQ}^*$ with thymine, which can be described by a single exponential function without considering the influence of the decaying process, for the simplicity of the kinetics model. The decaying process is complicated by several effects including diffusion and radical extinction by unknown secondary reactions. With the simplified model, the single exponential fitting to the growing process of TCBQH \cdot and TCBQ \cdot^- can yield their formation rate, which is 86.9 and 108.4 ns respectively. For the triplet absorption band of ${}^3\text{TCBQ}^*$ at 510 nm, its decay is comprised of two components, one is the quenching by itself (k_1), the ground state TCBQ or the solvent, and the other is the quenching due to the

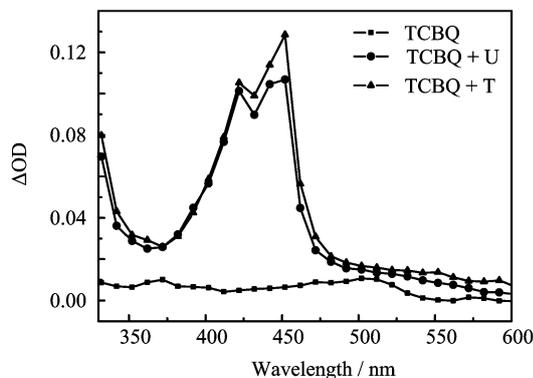


FIG. 4 Comparison of the transient absorption spectra at their maximum photoproduct intensity for TCBQ (0.4 mmol/L) with T and U, respectively.

photochemical reaction with thymine (k_2). k_1 can be obtained by the single exponential fitting to the decay curves of $^3\text{TCBQ}^*$ for the pure TCBQ system in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ solution, which is 377.6 ns. Since k_2 is the photochemical reaction rate of $^3\text{TCBQ}^*$ with thymine, it is equal to the formation rate of photoproducts $\text{TCBQH}\cdot$ and $\text{TCBQ}\cdot^-$, which is 97.6 ns if averaging the formation rates for these two products (86.9 and 108.4 ns). With k_1 and k_2 known, reasonable fitting results are obtained for the decay curves of $^3\text{TCBQ}^*$ with a double exponential decay function ($a_1\exp(-t/k_1) + a_2\exp(-t/k_2) + c$). The contribution of the photochemical reaction of $^3\text{TCBQ}^*$ with thymine to the whole quenching process of $^3\text{TCBQ}^*$ can be obtained from the fitting, which is $a_2/(a_1 + a_2) = 63\%$. This kinetics data, together with the spectral observation of the prominent formation of the photoproducts, indicate that the triplet $^3\text{TCBQ}^*$ species are highly reactive towards the pyrimidine bases, either thymine or uracil, leading to the hydrogen abstraction product $\text{TCBQH}\cdot$ and the electron transfer product $\text{TCBQ}\cdot^-$.

Figure 4 shows the maximum transient absorption spectra of TCBQ with 1 mmol/L of thymine or uracil. Both thymine and uracil are found to undergo electron transfer and hydrogen abstraction to $^3\text{TCBQ}^*$. But a comparison of the intensity of the peaks of thymine and uracil reveals interesting features. As shown in Fig.4, spectrum of the $^3\text{TCBQ}^* + \text{T}$ reaction has higher intensity than spectrum of $^3\text{TCBQ}^* + \text{U}$. If looking their molecular structure shown in Scheme 2, thymine differs from uracil only by a methyl moiety. Presence of this methyl moiety in thymine can be very significant with respect to electron transfer and hydrogen abstraction chemistry. Methyl group has electron donating ability, so it has the tendency to push electrons towards the pyrimidine ring, which will result in an increasing in the electron density over the six-membered ring and therefore can facilitate the electron transfer process. The absence of a methyl substituent deprives uracil of such advantage. Therefore, electron transfer from thymine will

be more favorable than that from uracil. In addition, redox potential (E_{ox}) value of thymine is lower than that of uracil, which also facilitates the electron transfer reaction of $^3\text{TCBQ}^*$ with thymine. In terms of hydrogen abstraction, this methyl group has also the capability of acting as a better hydrogen donor in thymine. The methyl group possesses three additional hydrogen atoms, which can be transferred during hydrogen abstraction. So the hydrogen abstraction reaction is also expected to be favorable for thymine too. For both the two photochemical reaction processes involved, thymine serves as a better donor of electrons and hydrogen atoms than uracil and thus explains the stronger amplitude of photoproducts formed from the thymine reaction system than those from the uracil system.

Overall, our experiments demonstrate that the UVA (355 nm) excitation populates TCBQ molecules to their triplet state $^3\text{TCBQ}^*$ which are highly reactive towards the pyrimidine bases (T or U), leading to the hydrogen abstraction product $\text{TCBQH}\cdot$ and the electron transfer product $\text{TCBQ}\cdot^-$. Concomitantly, nucleobase radical or radical cations are formed, as shown in Scheme 3, which will lead to a series of DNA damage afterwards. Especially, the positive charge may migrate within DNA toward the base having lowest ionization potential, the order being guanine (G) < adenine (A) < cytosine (C) \approx thymine (T), until it is trapped by an irreversible reaction to form 8-oxo-7,8-dihydroguanine (8-oxoG), and other oxidation products [22, 23]. In addition, the nucleobase radicals formed from the triplet state TCBQ hydrogen abstraction may lead to strand cleavage of DNA [24, 25]. Therefore, the photochemical electron transfer and hydrogen abstraction reactions of TCBQ with nucleobases can induce severe detrimental biological consequences.

IV. CONCLUSION

Laser flash photolysis has been used to investigate the photochemical reactions of TCBQ, a quinone metabolite of pentachlorophenol, with two pyrimidine bases, *i.e.* T and U. In transient absorption spectra it is observed that prominent photoproducts of $\text{TCBQH}\cdot$ and $\text{TCBQ}\cdot^-$ are formed concomitantly when the T-T absorption bands of $^3\text{TCBQ}^*$ are rapidly quenched to their lowest intensity, indicating the occurrence of a bimolecular reaction of $^3\text{TCBQ}^*$ with thymine or uracil. Kinetics analysis reveals that photochemical reaction of $^3\text{TCBQ}^*$ with bases accounts for 63% of the total quenching process of $^3\text{TCBQ}^*$. Basically, the UVA (355 nm) excitation populates TCBQ molecules to their triplet state $^3\text{TCBQ}^*$, which are highly reactive towards the pyrimidine bases (T or U), leading to the hydrogen abstraction product $\text{TCBQH}\cdot$ and the electron transfer product $\text{TCBQ}\cdot^-$. Thymine serves as a better donor of electrons and hydrogen atoms than uracil and thus results in stronger amplitude of photoproducts.

ucts formed. These results are of fundamental importance for understanding the carcinogenic effects of the widespread environmental pollutants pentachlorophenol and its metabolites TCBQ from the perspective of photochemical molecular reaction mechanisms.

V. ACKNOWLEDGMENTS

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