Phototransformation of the "emerging" BFR
1,3,5-Tris-(2,3-dibromopropyl)-1,3,5-triazine-2,4,6-trione

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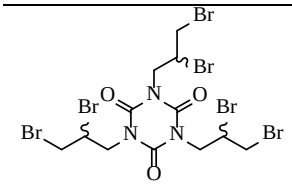
1 Introduction

The occurrence and fate of brominated flame retardants (BFRs) in the environment are topics of increasing concern. In recent years, numerous studies about their global transport, UV degradation, bioaccumulation and toxicity were performed to assess their environmental fate. As a result, most of the first generation BFRs are banned or restricted, and replaced by new ones. However, based on similar properties these new compounds may also pose a serious risk by causing adverse effects to human health and the environment. According to the European Food Safety Authority (EFSA) the class of the "emerging" BFRs are defined as compounds that have been identified in any environmental compartments, but the potential for degradation or bioaccumulation of these emerging BFRs is partially unknown [1].
A representative of this class of compounds is the heterocyclic TDBP-TAZTO (Table 1) which was first detected in mollusks from Chinese bohai sea [2] and in environmental matrices near a manufacturing plant in southern china [3]. Furthermore, Wang *et al*. describe that the growth of the alga *Nannochloropsis* sp. is inhibited by TDBP-TAZTO in a concentration dependent manner [4] but the photo-chemical behavior as well as the formation of possible photo-transformation products (PTPs) are still unknown. In order to clarify this complex issue photo-degradation experiments were by determining the rate constants and degradation half-life times of TDBP-TAZTO in different solvent compositions.

Table 1: Chemical structure and main physico-chemical properties of TDBP-TAZTO [1].

Structure	CAS no.	MW	$LogK_{OW}$	K_{OC}
	52434-90-9	728.69 g mol^{-1}	4.45	6260

In this study, the photodegradation of TDBP-TAZTO was performed for the first time to identify its photolysis products and to get a first understanding about the main degradation pathway in environmental matrices.

2 Materials and methods

Chemicals

Acetonitrile (HPLC-grade), methanol (HPLC-grade) and formic acid (98-100 %) were purchased from Merck (Darmstadt, Germany). TDBP-TAZTO (>97 %), 2-propanol (99 %), hydrogen peroxide solution (30 %) and sodium azide (99 %) were obtained from Sigma Aldrich (Munich, Germany). Ultrapure water was produced by a Seralpur PRO 90 CN system (Ransbach-Baumbach, Germany).

Irradiation experiments

A water-cooled laboratory UV-reactor equipped with a 150 W medium pressure mercury lamp (TQ 150, Heraeus Noblelight, Hanau, Germany) and an external magnetic stirring device were used for all irradiation experiments (λ = 200–280 nm). For the irradiation studies, 200 mL of a 50 µM solution of TDBP-TAZTO in a 55 : 45 volume mixture of acetonitrile and water were placed in the reactor vessel and constantly stirred (600 min^{-1}). To investigate the role of OH radicals H_2O_2 (1/10 mM) and the scavenger 2-propanol (5/50 mM) respectively, were added before irradiation. Furthermore, to determine the effect of $^1O_2$ the scavenger NaN_3 (1/10 mM) was added. The water cooling system of the UV-reactor was set to 10 °C. After equilibration, the UV-lamp was activated and samples (1 mL) were collected with a glass syringe connected to a flexible stainless steel capillary at 0, 5, 10, 20, 30, 40, 50 and 60 min. All UV experiments were performed in triplicate.

2.3 Analytical methods

The primary detection of photo-degradation products was performed by high performance liquid chromatography (HPLC) analyses using an Agilent 1200 series HPLC system hyphenated to a 6130 single quadrupole mass spectrometer equipped with an electrospray interface (Agilent Technologies GmbH, Waldbronn, Germany). The chromatographic separation was achieved using a Gemini® C_{18} analytical column (100 x 2 mm, 3 µm particles; Phenomenex®, Aschaffenburg, Germany), preceded by a Gemini® C_{18} guard column (4 × 2 mm, 3 µm particles). The mobile phase consisted of 0.1 % formic acid in water (A) and a 80 : 20 volume mixture of methanol : acetonitrile (B). The gradient elution program was applied as follows: starting conditions 40 % eluent B, 0–240 min, 100 % B; 240–240.1 min, 40 % B; 240.1–250 min, 40 % B. The solvent gradient was adopted for a total run time of 250 min, while 10 min were used for column regeneration. The flow rate was at 250 µL min^{-1}. All sample and standard solutions were injected in triplicate with an injection volume of 10 µL.

HPLC-MS/MS analyses were performed on an 1200 series HPLC system from Agilent Technologies (Waldbronn, Germany) hyphenated to an API4000 QTrap® MS/MS system (Sciex, Darmstadt, Germany). The chromatographic separation was performed as described above. The instrument was operated in multiple reaction monitoring (MRM) mode with negative electrospray ionization and recording two transitions simultaneously in unit resolution (quantifier (*m/z*) 727.7 → 81.0, qualifier 727.7 → 79.0). The following ion source parameters were used: ion spray voltage, –4500 V; temperature, 475 °C; ion source gas 1, 45 a.u.; ion source gas 2, 72 a.u.; curtain gas, 10 a.u.; collision gas, 12 a.u.. The optimized MRM compound specific parameters were: declustering potential, –40 V; entrance potential, –10 V; collision energy, –62 V; collision cell exit potential, –5 V; dwell time, 50 ms.

High resolution MS analyses were performed on a Finnigan LTQ FT ultra high performance ion trap based fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Thermo electron corporation, Bremen, Germany) equipped with a 7 Tesla superconducting magnet and working in negative electrospray ionization conditions with a spray voltage of 2.4 kV. Mass resolving power was 100,000 (FWHM) at *m/z* 400. FT-ICR mass spectra were acquired within a range of 350 to 1,000 *m/z*.

3 Results and discussion

Determination of the degradation kinetic

The results of the kinetic studies with and without different additions are shown in Table 2. The scavenger 2-propanol (2-propanol + ·OH → propan-2-one) and H_2O_2 were independently added to investigate the role of OH radicals during the UV irradiation of TDBP-TAZTO. Further investigations with NaN_3 were performed to clarify the potential of indirect photolysis with singlet oxygen ($^1O_2$). In order to estimate the degradation kinetics of TDBP-TAZTO, the first order relation was used to calculate the rate constants (k) and the half-life tims ($t_{1/2}$). A significant inhibition of the rate constant k and a subsequently increasing half-life $t_{1/2}$ could be determined by the addition of H_2O_2 and 2-propanol

respectively. Based on these parameters the degradation behavior during the UV irradiation with different concentrations of H_2O_2/2-propanol were almost identical and were both slower compared to TDBP-TAZTO itself. It might be reasonably assumed that other competing reactions preferentially proceed and the PTPs can be formed only after a certain time. One possible reason for the observed issue is that H_2O_2 itself can absorb UV light, which can reduce the light absorption of TDBP-TAZTO. In addition, the reduction of the rate constant with 2-propanol shows that OH radicals play a significant role in the photo-chemical process of TDBP-TAZTO.

During the addition of NaN_3 under UV irradiation it has no inhibition but a promoting effect on the photodegradation in both concentration levels. Therefore, two explanations may be possible: 1) no $^1O_2$ was generated in the reaction solution under UV-(C) and/or 2) NaN_3 itself formed by-products with TDBP-TAZTO. Comprehensively, the results indicate that $^1O_2$ has a low contribution to the indirect phototransformation and the direct photolysis is the main photo-degradation pathway for TDBP-TAZTO.

Table 2: Degradation kinetic parameters with different additions to TDBP-TAZTO (n = 3).

conditions	k (min^{-1})	$t_{1/2}$ (min)	R^2
without	0.0409 ± 0.0054	17.0 ± 2.2	0.9915
10 mM H_2O_2	0.0038 ± 0.0003	184.7 ± 15.4	0.9829
100 mM H_2O_2	0.0038 ± 0.0019	181.5 ± 87.8	0.9896
5 mM 2-propanol	0.0191 ± 0.0017	36.3 ± 3.2	0.9863
50 mM 2-propanol	0.0197 ± 0.0001	35.1 ± 0.2	0.9951
1 mM NaN_3	0.0534 ± 0.0001	13.0 ± 0.02	0.9954
10 mM NaN_3	0.0818 ± 0.0063	8.5 ± 0.7	0.9758

k – rate constant (k = (ln(C/Co)/t)); $t_{1/2}$ – half-life ($t_{1/2}$ = ln2/k); R^2 – coefficient of determination

Identification of photo-transformation products

The chromatogram of a TDBP-TAZTO solution irradiated for 120 min is shown in Figure 1. The appearance of new peaks with different mass to ratio and isotopic pattern compared to TDBP-TAZTO was an indicator for the formation of PTPs. For TDBP-TAZTO, overall six new peaks with 12 main degradation products could be observed by HPLC-MS. The chromatographic behavior (Figure 1) demonstrated that all PTPs formed have probably a higher polarity than TDBP-TAZTO (R_t = 85.565). Based on the corresponding mass spectra for the respective peaks shown in Figure 2 it is not possible to assign one *m/z* relation to each peak until now.

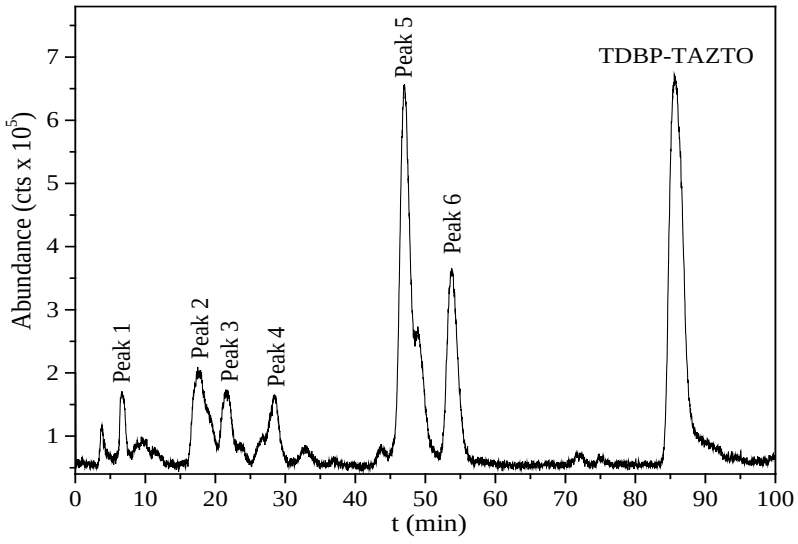


Figure 1: HPLC-ESI$^-$-MS chromatogram of TDBP-TAZTO (c = 50 µM) and its PTPs after 120 min UV-(C) irradiation (0.1 % HCOOH in H_2O : ACN).

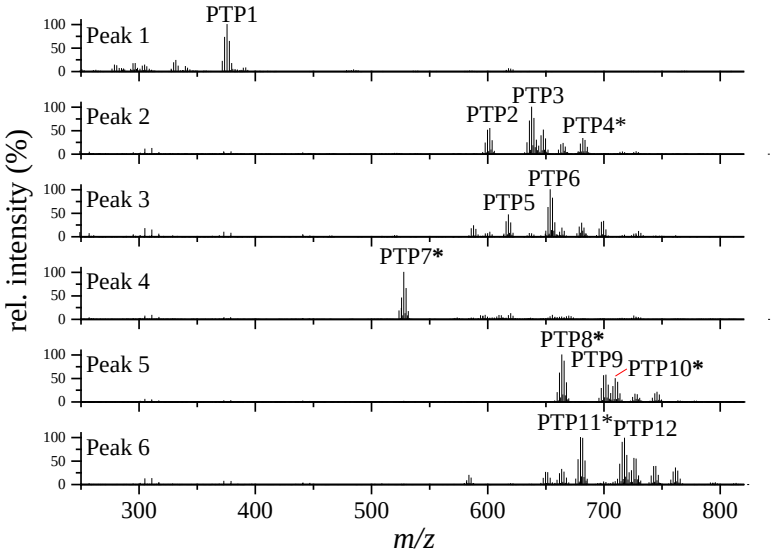


Figure 2: MS spectra (ESI^-) of TDBP-TAZTO (c = 50 µM) and its PTPs after 120 min UV-(C) irradiation (0.1 % HCOOH in H_2O : ACN); marked PTPs are described in Table 3.

To identify these PTPs and to postulate a photo-chemical degradation mechanism high resolution mass spectrometry measurements were performed. As a result, the UV-induced debromination and the hydroxylation at the alky chain could be determined as the main degradation pathways (Table 3). The position of the elimination of the bromine atoms and the hydroxylation is still unclear due to the high symmetry. For this purpose, the PTPs will be isolated by preparative HPLC and to elucidate their structure by using NMR spectroscopy and x-ray crystallography.

Table 3: Accurate and exact mass of PTPs after 120 min UV irradiation.

Experimental mass $[M-H]^-$	Theoretical Mass $[M-H]^-$	Δm/z	δm/m (ppm)	Molecular formula	PTPs
647.7845	647.7833	0.0012	1.9	$C_{12}H_{18}Br_5N_3O_8$	4
527.7423	527.7420	0.0003	0.6	$C_9H_{11}Br_4N_3O_3$	7
663.7794	663.7792	0.0002	0.3	$C_{13}H_{19}Br_4N_3O_8$	8
709.7002	709.6999	0.0003	0.4	$C_{13}H_{18}Br_5N_3O_6$	10
681.7052	681.7050	0.0002	0.3	$C_{12}H_{18}Br_5N_3O_5$	11

Δm/z – absolute mass accuracy; δm/m – relative mass accuracy = $(\Delta m/z)/(m/z)_{exact} * 10^6$

4 References

1. EFSA, 2012.
2. Zhu, N., et al., Environmental Science & Technology, 2012. **46**(13): p. 7174-7181.
3. Ruan, T., et al., Environmental Science & Technology, 2009. **43**(9): p. 3080-3086.
4. Wang, L., et al., Marine Pollution Bulletin.