

## Article

# Re-Examining Cyanuric Acid: An Overlooked Non-Halogenated Cyclic Disinfection Byproduct in Swimming Pool Water

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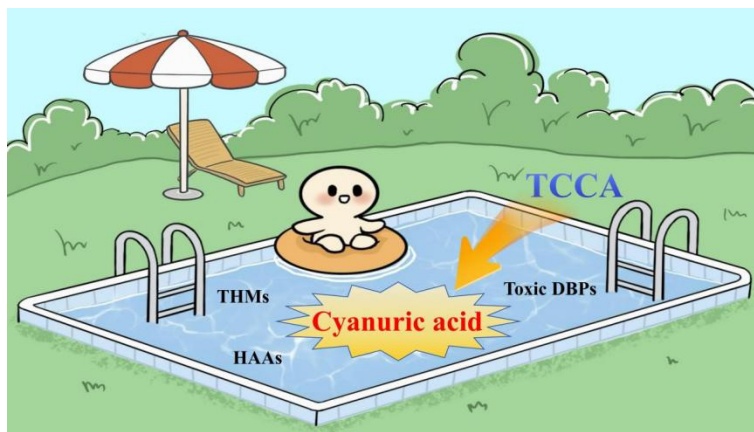
cyanuric acid;  
disinfection by-products;  
swimming pool water;  
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## Highlights

- Cyanuric acid is a dominant non-halogenated cyclic DBP in swimming pool water
- Reported average cyanuric acid levels of 26,600 µg/L, 3–5 orders of magnitude higher than other organic DBPs

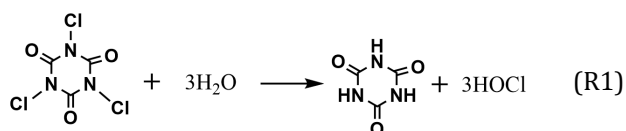
**Abstract:** Cyanuric acid, as a by-product of chloroisocyanuric acids, is frequently detected in swimming pool water. However, its cytotoxicity, persistence, mobility, and bioaccumulation potential remain poorly understood. This study evaluates the cytotoxicity of cyanuric acid and investigates its occurrence and toxicological impact in water samples. Additionally, data on its biodegradation half-life, octanol-water partition coefficient ( $K_{ow}$ ), and bioconcentration factor (BCF) were collected. The average concentrations of cyanuric acid in 20 swimming pools were 26,600 µg/L, which were 3–5 orders of magnitude higher than other known organic disinfection byproducts (DBPs). The median lethal concentration ( $LC_{50}$ ) of cyanuric acid for Chinese hamster ovary (CHO) cells was 13.7 mM. Given its widespread occurrence and high concentration, cyanuric acid is likely a significant contributor to swimming pool cytotoxicity. The calculated cytotoxicity of cyanuric acid (15,054) exceeded that of all reported cyclic DBPs in swimming pools, including halonitrobenzoic acids (0.04), halonitrophenols (0.1), and halobenzoquinones (4.4). Furthermore, its calculated cytotoxicity was also much higher than most aliphatic DBPs. Overall, cyanuric acid contributed approximately 21.4% of pool water cytotoxicity, identifying it as an overlooked cyclic DBP. In terms of environmental persistence, cyanuric acid had a biodegradation half-life of 6.17 days, which is approximately comparable to the median level of aliphatic DBPs. The  $\log K_{ow}$  and BCF of cyanuric acid was  $-0.72$  and  $0.501$  L/kg, suggesting a high mobility and minor bioaccumulation potential. Moreover, due to its extremely high concentration in swimming pool water (up to 79,000 µg/L), these values do not eliminate concerns about its environmental risks. Given that chloroisocyanuric acids have been identified as ideal oxidants in advanced oxidation processes (AOPs) for micropollutant degradation in water in recent years, this study may reveal the potential risk of cyanuric acid in swimming pool water and UV/chloroisocyanuric acid-treated water.

- Quantified cyanuric acid contribution (21.4%) to total cytotoxicity of known DBPs in swimming pool water
- Collected data on biodegradation half-life, Log Kow, and BCF to reveal environmental persistence and mobility characteristics



## 1. Introduction

In recent years, swimming has gained increasing popularity as public awareness of health and fitness continues to rise [1,2]. Compared to indoor swimming pool water, chlorine disinfectant in outdoor pools can undergo degradation upon exposure to ultraviolet (UV), thus necessitating the use of trichloroisocyanuric acid (TCCA) or other chloroisocyanuric acids to maintain adequate chlorine residuals [3]. In theory, TCCA dissociates to produce hypochlorous acid and cyanuric acid, but due to thermodynamic equilibrium, TCCA dissociates only at low chlorine conditions, as illustrated in Reaction (1) [4]. As chlorination proceeds, cyanuric acid accumulates and all TCCA eventually becomes cyanuric acid when chlorine is depleted. Consequently, cyanuric acid has become a characteristic and environmentally relevant compound in swimming pool systems, attracting increasing scientific attention because of its potential implications for pool water quality and safety.



As for toxicity, cyanuric acid was reported to have an oral median lethal dose (LD<sub>50</sub>) of 3400 mg/kg and is not considered teratogenic or carcinogenic [5–7]. Unfortunately, ingesting cyanuric acid can still cause other health problems. A case-control study showed that high-dose cyanuric acid intake can increase the risk of urinary tract stone development (Odds ratio: 1.11, 95% CI: 1.02–1.21) [8]. There is also evidence showing that cyanuric acid irritates skin, eyes and respiratory system and damages gastrointestinal tract and liver [9–11]. Recent toxicological studies have demonstrated that the combined exposure to cyanuric acid and melamine induces marked synergistic toxicity, activating the renin-angiotensin-aldosterone system (RAAS) and enhancing the generation of reactive oxygen species (ROS) within cells, thereby triggering apoptosis. Such pathological

changes can ultimately result in severe renal injury and fibrosis [12]. Therefore, further research is needed to thoroughly evaluate the toxicity and potential health risks arising from exposure to cyanuric acid.

Disinfection by-products (DBPs) constitute a broad class of chemical pollutants that pose significant risks to swimming pool water quality and the health of individuals who use or work in these facilities [13]. These compounds are ubiquitously present in pool environments, occurring not only in the water but also in the air above the pools, and can enter the human body via dermal contact, ingestion, and inhalation [14,15]. Epidemiological evidence links DBP exposure to an increased incidence of rectal and bladder cancers, while experimental studies indicate that many DBPs are cytotoxic, genotoxic, neurotoxic, hepatotoxic, and nephrotoxic [16–22]. Therefore, identifying the DBPs with the higher toxic contributions and developing corresponding control strategies are critical for safeguarding the health of swimmers and pool staff.

As a swimming pool DBP, cyanuric acid has been widely detected worldwide at exceptionally high concentrations [23]. For instance, in swimming pools, cyanuric acid typically occurs at tens of mg/L, which is 1000 to 100,000 times higher than other known organic DBPs [24]. More importantly, in recent years, chloroisocyanuric acids have been identified as ideal oxidants in advanced oxidation processes (AOPs) for micropollutant degradation in water, owing to their high persistence and molar absorption coefficients [25–29]. Cyanuric acid is an important byproduct of the UV/chloroisocyanuric acid system [26]. Since information regarding their cytotoxicity, persistence, mobility, and bioaccumulation is unavailable, we lack a good understanding of its potential threat to human health and the ecosystem.

Current health risk assessments of DBPs in swimming pool water have overlooked cyanuric acid. Therefore, addressing this data gap and conducting a comprehensive re-evaluation are urgently needed. Moreover, given the frequent detection and relatively

high concentrations of cyanuric acid, further investigation into its persistence, mobility, and bioaccumulation is highly relevant to ecological and environmental protection. In this study, cyanuric acid concentrations were surveyed in 20 indoor and outdoor swimming pools, its cytotoxicity was evaluated, and its calculated cytotoxicity, biodegradation half-life, octanol-water partitioning coefficient ( $K_{ow}$ ), and bioconcentration factor (BCF) were compared with those of other organic DBPs. We hope this work will provide a clearer understanding of the potential risks posed by cyanuric acid in swimming pool water and in UV/chloroisocyanuric acid-treated water, as well as a scientific basis for developing effective management and control strategies.

## 2. Materials and Methods

### 2.1. Materials

The standard of cyanuric acid (>98% purity) was obtained from Aladdin (Shanghai, China). Methyl tert-butyl ether, sulfuric acid, and anhydrous sodium sulfate were purchased from Macklin (Shanghai, China) and Aladdin (Shanghai, China) at reagent-grade purity. All laboratory water was ultrapure Milli-Q water (>18 M $\Omega$ -cm), produced using Elix Essential 5 and Milli-Q filtration systems.

### 2.2. Water Sample Collection and Instrumental Analysis

From August 2015 to August 2016, water samples were randomly collected from 20 swimming pools in Kunshan City that were disinfected with TCCA. To ensure comparability, sampling was conducted during normal pool operation hours (9:00–17:00) and under stable weather conditions without recent rainfall events. For each pool, three water samples (3 L each) were obtained. Sampling points were strategically planned according to the dimensions of each pool, with five points assigned per unit area. Water samples were collected in 500 mL sterilized plastic bottles and immediately transported to the laboratory at 4 °C, where they were analyzed within three days to maintain sample integrity and data accuracy. All analyses were performed without quenching.

To determine the cyanuric acid concentration in the water samples, the study adhered to the CJT 244-2016 standard of China, using the PTH090 turbidimeter (Palintest, UK) and Pool Water Quality test kit (Palintest, UK) through a turbidimetric method. The procedure involved several steps: initially, a filtered water sample served as the control group to calibrate the turbidimeter, setting the cyanuric acid concentration indicator to 0. Subsequently, a cyanuric acid detection reagent (Melamine, CAS:108-78-1) was added to the water sample, resulting in a precipitation reaction with cyanuric acid. The turbidity of the water sample, which directly correlated with the cyanuric acid concentration, was then

measured using the water quality analyzer [30]. Each water sample was tested three times to ensure consistency and reliability. The average cyanuric acid concentration derived from the samples collected from the pool was considered representative of the cyanuric acid concentration in the pool water. The reporting limit of cyanuric acid was 5000  $\mu$ g/L. The methods and detail results for turbidity, pH, urea concentration and free residual chlorine concentration of water were listed in the Text S1, S2, and Table S1.

### 2.3. Data Source of Other Known Organic Disinfection By-Products in Global Swimming Pool

To evaluate the concentration of cyanuric acid in swimming pools, its levels were compared with those of other commonly reported organic DBPs. To ensure representative data, concentrations of these DBPs in pool water were collected from studies worldwide. Relevant literature published before April 2023 was retrieved from Web of Science, ACS Publications, ScienceDirect, and Wiley Online Library. Searches were performed using combinations of the following keywords: swimming pools, disinfection by-products (or DBPs), cytotoxicity, and DBP control. The detailed data of known organic DBPs are listed in Table 1.

### 2.4. Cell Cytotoxicity Assay

CHO cells were used as the experimental model following established protocols [31,32]. The cytotoxicity evaluation was conducted using CCK-8 kits and 96-well plates. The selected dose range was determined based on preliminary experiments and literature relevant to the detected concentrations, ensuring it covered levels sufficient to evaluate potential cytotoxic effects while maintaining cellular viability for analysis. The experimental design included blank controls, solvent controls, negative and positive controls, and experimental groups.

Blank controls contained 200  $\mu$ L of F12 medium with 5% fetal bovine serum (FBS) to measure baseline absorbance. Solvent controls consisted of 200  $\mu$ L F12 + FBS containing up to 0.5% DMSO, matching the DMSO concentration of the highest-dose experimental group [33]. Negative controls included approximately 3000 CHO cells (100  $\mu$ L) with 100  $\mu$ L F12 + FBS. Positive controls contained approximately 3000 CHO cells (100  $\mu$ L), F12 + FBS, and a mixture of known DBPs (trichloroacetonitrile (TCAN), dibromoacetonitrile (DBAN), trichloroacetaldehyde (TCAL), and 2-iodophenol (2IP)) to a final volume of 200  $\mu$ L. Experimental wells included approximately 3000 CHO cells (100  $\mu$ L), F12 + FBS, and cyanuric acid to reach 200  $\mu$ L.

The 96-well plates were incubated at 37°C with 5% CO<sub>2</sub> for 72 h, then treated with 10% CCK-8 reagent and incubated further. Absorbance at 450 nm was measured and corrected against the blank. All assays were performed in at least three independent experiments to

ensure reproducibility. The percentages of live cells were calculated according to Equation (1).

$$\text{Cell viability (\%)} = \frac{A_E - A_D}{A_0 - A_D} \times 100\% \quad (1)$$

where  $A_E$ ,  $A_D$ , and  $A_0$  represent the absorbance of the experimental, solvent, and negative control groups, respectively. Cell viability data were analyzed using GraphPad Prism 8, and concentration-response curves were generated to determine median lethal concentration ( $LC_{50}$ ).

### 2.5. Calculations of Toxicity Index

The individual cytotoxic potencies of cyanuric acid in water samples were indicated using the “TIC-Tox” method [34]. Cyanuric acid and DBP cytotoxicity in swimming pool water was expressed according to Equation (2). This approach has been shown to provide reliable estimates of the cytotoxicity of DBP mixtures in disinfected waters, closely matching bioassay-based measurements [35].

$$\text{Calculated cytotoxicity} = \sum_{i=1}^n ([C] \times LC_{50}^{-1}) \times 10^6 \quad (2)$$

where  $[C]$  is the molar concentration of cyanuric acid and DBPs.  $LC_{50}^{-1}$  is the cytotoxicity index (CTI). Comparison of the calculated cytotoxicity revealed the contributions of cyanuric acid and each DBP class to the overall toxicity in swimming pool water.

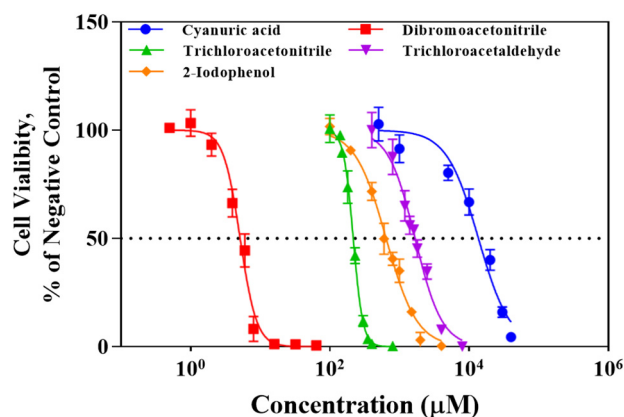
## 3. Result and Discussion

### 3.1. Cytotoxicity of Cyanuric Acid

Absorbance data from the 96-well plates were normalized using Equation (1) to calculate cell viability. The concentration-response curves for the experimental group are presented in Figure 1. The  $LC_{50}$  of cyanuric acid for CHO cells was found to be 13.7 mM. Compared to the known DBPs, the cytotoxicity of cyanuric was similar with chlorate, nitrosamines and nitramines, and BDCM, but was 10–50,000 times lower than almost all of other known organic and inorganic DBPs, e.g., HKs, HAMs, HALs, HANs, and aromatic DBPs [33,36–41]. This mean that in the view of cytotoxicity, cyanuric is unlike an important DBPs. Considering cyanuric acid was reported to have an oral median lethal dose ( $LD_{50}$ ) of 3400 mg/kg and is not considered teratogenic or carcinogenic [5–7], it is likely that the acute toxicity of cyanuric acid is not significant.

Besides that, the  $LC_{50}$  values of four DBPs (DBAN, TCAN, TCAL, and ZIP) were experimentally determined and are shown in Figure 1. These values, 5.1, 197, 1679,

and 643  $\mu\text{M}$  respectively, closely match previously reported results of 4.2, 170, 1400, and 840  $\mu\text{M}$ , confirming the reliability of the measurements and supporting their use in subsequent analyses of DBP toxicity in swimming pool water [42].



**Figure 1.** The concentration-response curves of cyanuric acid and positive controls in CHO cell. Each dose comprises six replicates.

### 3.2. Concentrations of Cyanuric Acid

The concentrations of cyanuric acid in 20 swimming pools were measured. As shown in Table 1, its concentration ranged from 7000 to 79,000  $\mu\text{g/L}$ , with an average of  $26,600 \pm 19,600 \mu\text{g/L}$ . In China, the regulatory concentration limit of cyanuric acid in swimming pool water is 50 mg/L (GB37488-2019) while in USA it is 100 mg/L (USA ANSI/APSP-11, 2009), so the levels of cyanuric acid in surveyed samples are often below their regulatory limits. In comparison, the concentrations of aliphatic and aromatic DBPs in swimming pool waters in literature usually follow the following order: haloacetic acids (HAAs) (750  $\mu\text{g/L}$ ) > haloamides (HAMs) (290  $\mu\text{g/L}$ ) > haloaldehydes (HALs) (260  $\mu\text{g/L}$ ) > trihalomethanes (THMs) (122  $\mu\text{g/L}$ ) >> haloketones (HKs) (36.8  $\mu\text{g/L}$ ) > haloacetonitriles (HANs) (24.9  $\mu\text{g/L}$ ) > halonitromethanes (HNMs) (6.1  $\mu\text{g/L}$ ) > iodo-haloacetic acids (I-HAAs) (0.8  $\mu\text{g/L}$ ) > iodo-trihalomethanes (I-THMs) (0.7  $\mu\text{g/L}$ ) > halobenzoquinones (HBQs) (0.1  $\mu\text{g/L}$ ) > halophenols (HPs) (0.03  $\mu\text{g/L}$ )  $\approx$  halohydroxybenzaldehydes (HBALs) (0.03  $\mu\text{g/L}$ ) > halohydroxybenzoic acids (HBAs) (0.02  $\mu\text{g/L}$ ) > haloanilines (HAs) (0.01  $\mu\text{g/L}$ ) > halonitrophenols (HNPs) (0.004  $\mu\text{g/L}$ ) (Figure 2a) [2,41,43–67]. The concentration of cyanuric acid in swimming pool water is clearly much higher than that of other DBPs, ranging from approximately 35.5 to 6.7 million times greater.



**Table 1.** Concentrations of DBPs and cyanuric acid in swimming pools, and calculated cytotoxicity <sup>a</sup>.

	DBPs	Average (µg/L)	Calculated Cytotoxicity
THMs	TBM	7.5	7.5
	DBCM	3.8	3.4
	TCM	91.2	79.4
	BDCM	19.9	10.6
HAAs	BAA	4.3	3224
	CAA	32.8	429
	DBAA	9.2	71.6
	DCAA	377	401
	BCAA	82.4	611
	TCAA	210	536
	DBCAA	5.7	112
HANs	BDCAA	28.8	202
	CAN	1.9	n.a.
	BAN	1.7	n.a.
	DCAN	13.4	2127
	BCAN	3.5	2680
	DBAN	1.2	2118
	TCAN	0.4	n.a.
	BDCAN	0.2	n.a.
HNMs	DBCAN	1.6	n.a.
	TBAN	1.0	n.a.
	DCNM	0.4	n.a.
	BCNM	0.2	28.3
	DBNM	0.3	n.a.
	TCNM	2.1	24.1
	BDCNM	0.6	218
HAMs	DBCNM	0.9	269
	TBNM	1.6	n.a.
	CAM	70.7	n.a.
	DCAM	35.2	143
	BCAM	7.0	2374
	DBAM	7.3	n.a.
	TCAM	162.5	488
HKs	BDCAM	3.6	n.a.
	DBCAM	2.2	n.a.
	TBAM	1.4	n.a.
	CP	6.2	n.a.
	11DCP	4.6	n.a.
	13DCP	1.9	n.a.
	111TCP	22.4	n.a.
HALs	113TCP	0.9	n.a.
	1133TeCP	0.8	n.a.
	CAL	0.3	n.a.
	BAL	5.6	n.a.
	DCAL	1.0	n.a.
	BCAL	0.3	n.a.
	DBAL	1.3	n.a.
	TCAL	231	973
I-THMs	BDCAL	12.0	3066
	DBCAL	3.1	2547
	TBAL	0.3	n.a.
	BCIM	0.1	0.2
I-HAAs	DBIM	0.1	0.2
	BDIM	0.5	1.0
	IAA	0.2	365
HBQs	CIAA	0.3	4.5
	DIAA	0.3	2.9
	2,6-DCBQ	0.03	4.4
HPs	2,3,5-TCBQ	0.03	n.a.
	2,6-CIBQ	0.03	n.a.
	4-MCP	0.01	n.a.
HBAs	2,4-DCP	0.02	n.a.
	3,5-DCHBA	0.01	0.4
	5-MCHBA	0.01	n.a.

Table 1. Cont.

	DBPs	Average (µg/L)	Calculated Cytotoxicity
HBALs	3-MCHBAL	0.01	n.a.
	3-MBHBAL	0.01	n.a.
	3,5-DCHBAL	0.01	0.1
HAs	2-MBA	0.01	n.a.
	2,4-DCA	0.003	n.a.
	2,4-DBA	0.003	n.a.
HNPs	2,6-DCNP	0.002	108
	2-MCNP	0.002	n.a.
	2,4-CBNP	0.002	n.a.
	Cyanuric acid	26600	15054

Noting: n.a. = not available. <sup>a</sup> All data except cyanuric acid were from references [36–41,43–67].

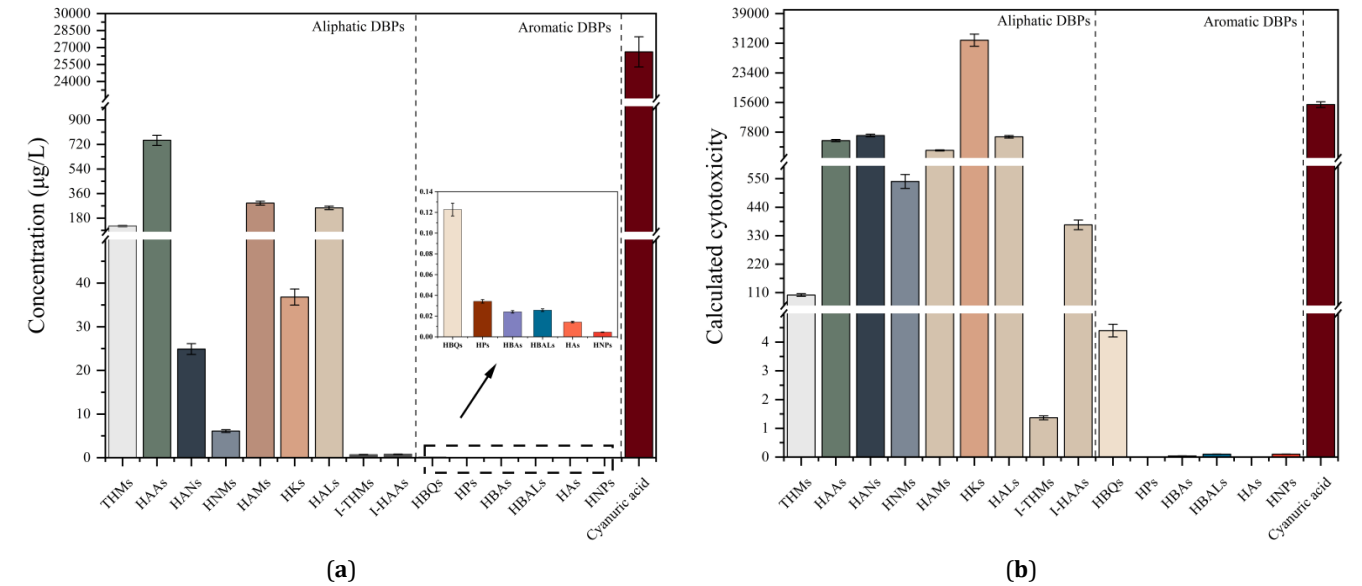


Figure 2. Concentrations and calculated cytotoxicity of disinfection by-products and cyanuric acid in swimming pools. (a) Concentration; (b) Calculated cytotoxicity.

3.3. A Comparison of Calculated Cytotoxicity between Cyanuric Acid and Other Known Organic DBPs in Swimming Pool

As shown in Figure 2b, the calculated cytotoxicity of cyanuric acid, ranged from 2262 to 44,677, with an average of 15,054. Based upon a holistic evaluation of average concentrations and specific toxicities, the cytotoxic contributions of analyzed compounds from highest to lowest were ranked as follows: HKs (32,014) > cyanuric acid (15,054) >> HANs (6924) > HALs (6587) > HAAs (5585) > HAMS (3006) >> HNMs (539) > I-HAAs (372) > THMs (101) >> HBQs (4.4) > I-THMs (1.4) > HBALs (0.1) ≈ HNPs (0.1) > HBAs (0.04).

Previous studies have shown that HKs is a major source of cytotoxicity in drinking water [31], and based on this study they seem to also represent the largest contributor of cytotoxicity to pool water. The findings of this study highlight the need to pay close attention to HKs as a key class of DBPs in swimming pools. Notably, although cyanuric acid itself has the lowest specific cytotoxicity, its extremely high concentration (94.7%) in swimming pool water eventually led to significant cytotoxic contribution (21.4% of the total DBP calculated

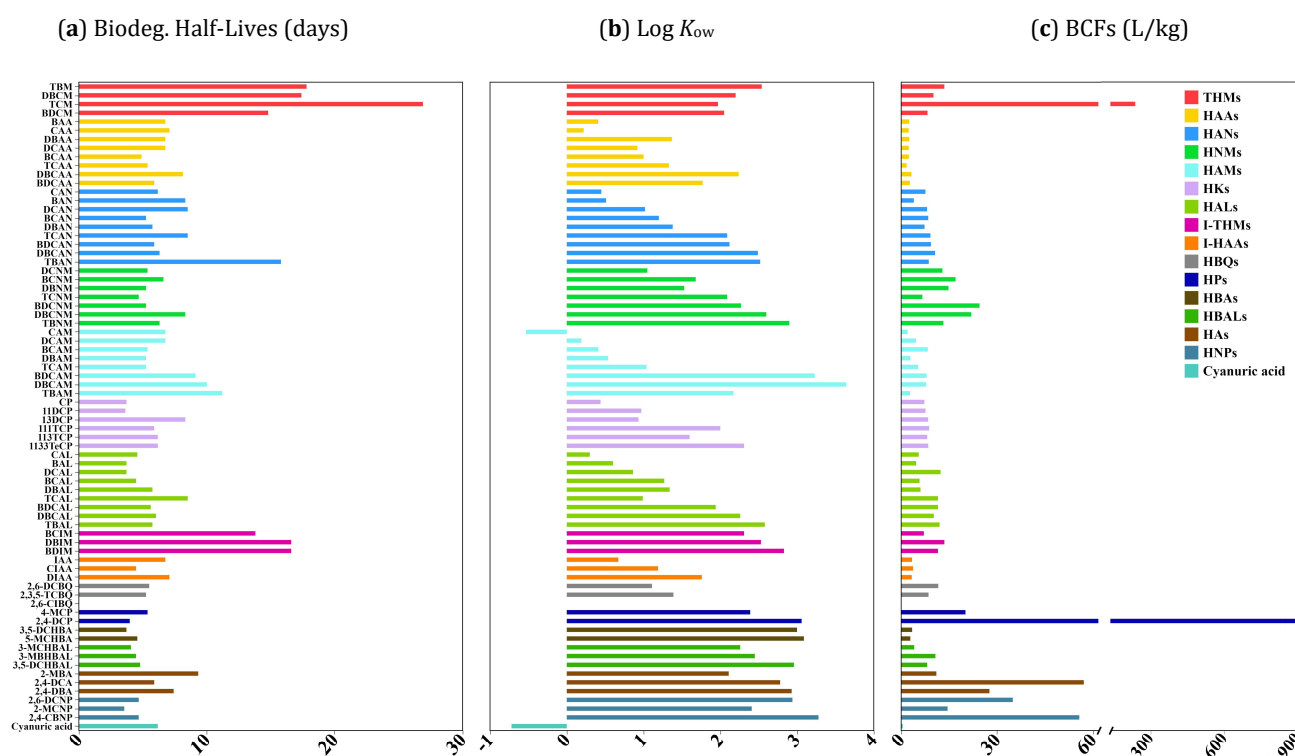
cytotoxicity in this study). In fact, the toxicity of cyanuric acid was often overlooked before. Its existence may lead to potential health risks. However, the calculated cytotoxicity in this study was only referred to CHO cells. The situations of other cell lines still require further research.

3.4. Biodegradability of Cyanuric Acid and Known DBPs

Biodegradation half-lives are an important indicator of the environmental persistence of chemicals [4,68]. A further comparison of the biodegradation half-lives of cyanuric acid with other aliphatic and aromatic DBPs is crucial for understanding their environmental occurrence and their long-term impacts on ecosystems and human health. Data were retrieved from the U.S. EPA's CompTox Chemicals Dashboard (<https://comptox.epa.gov/dashboard/>, accessed on 1 September 2024). As shown in Figure 3a, the biodegradation half-life of cyanuric acid is 6.17 days, which is comparable to that of CAN (6.17 days). In comparison, the biodegradation half-lives of aliphatic DBPs range from 3.63 to 26.9 days, with an average of 7.77 days and a median of 6.17 days. The biodegradation half-lives of aromatic DBPs range from 3.55 to 9.33 days, with

an average of 5.12 days and a median of 4.68 days. Overall, the biodegradation half-life of cyanuric acid is comparable to the median of aliphatic DBPs and slightly longer than that of aromatic DBPs. Because cyanuric acid often reaches much higher concentrations than other DBPs in

swimming pools, its moderate persistence allows it to remain present even after partial biodegradation, potentially contributing significantly to the overall DBP load despite its low cytotoxicity.



**Figure 3.** The bioconcentration, persistence and mobility of cyanuric acid and other known DBPs. (a) Biodegradation Half-Lives; (b) Octanol-Water Partitioning Coefficients ( $K_{ow}$ ); (c) Bioconcentration Factor.

It should also be noted that the biodegradation behavior of cyanuric acid may vary substantially depending on the physicochemical conditions of the aquatic environment [69–71]. In chlorinated pool systems, continuous chlorination and elevated oxidative potential may suppress microbial activity, thereby reducing the effective biodegradation rate compared with that observed under standard aerobic laboratory conditions [72,73]. Conversely, once released into natural waters with lower chlorine residuals and more diverse microbial communities, the degradation of cyanuric acid is likely to proceed more rapidly. Understanding these system-dependent differences is also essential for accurately characterizing its persistence and for distinguishing between its behavior in managed pool environments and in natural aquatic systems.

### 3.5. $K_{ow}$ and Bioconcentration of Cyanuric Acid and Known DBPs

The  $K_{ow}$  reflects the balance of a chemical's affinity between polar and non-polar regions, making it a crucial parameter for evaluating the mobility and bioaccumulation potential of chemicals [74]. A positive

$\log K_{ow}$  indicates higher hydrophobicity, lower mobility, and greater potential for bioaccumulation. The greater the value, the stronger the hydrophobicity, and vice versa [75]. As shown in Figure 3b, the  $\log K_{ow}$  of cyanuric acid is  $-0.72$ . In contrast, aliphatic DBPs exhibit  $\log K_{ow}$  values ranging from  $-0.530$  to  $3.64$ , with a mean of  $1.54$  and a median of  $1.39$ . Aromatic DBPs, on the other hand, have  $\log K_{ow}$  values between  $3.55$  and  $9.33$ , with a mean of  $5.12$  and a median of  $4.68$ . Overall, cyanuric acid exhibits a relatively low  $\log K_{ow}$  compared to most DBPs, with the notable exception of chlorate ( $\log K_{ow} = -4.63$ ). [76,77]. Furthermore, the generally higher  $\log K_{ow}$  values of aromatic DBPs compared to aliphatic DBPs suggest that chemicals with aromatic ring structures may have a greater bioaccumulation potential.

BCF is another critical property for assessing chemical bioaccumulation. A higher BCF indicates greater long-term accumulation risk and higher ecological risk, and vice versa [78–80]. Chemicals with a BCF  $< 100$  L/kg are classified as having low bioaccumulation potential. As shown in Figure 3c, the BCF of cyanuric acid is  $0.501$  L/kg. In comparison, aliphatic DBPs exhibit BCF values ranging from  $1.7$  to  $236$  L/kg, with a mean of  $11.9$  L/kg and a median of  $8.09$  L/kg, while aromatic DBPs have BCF

values ranging from 2.86 to 898 L/kg, with a mean of 88.3 L/kg and a median of 14.5 L/kg. Overall, cyanuric acid exhibits a relatively low Log  $K_{ow}$  compared to most DBPs, leading to conclusions similar to those based on Log  $K_{ow}$ .

Although the low Log  $K_{ow}$  and BCF values suggest limited bioaccumulation potential under typical environmental conditions, interpreting these properties within the context of swimming pool systems highlights additional considerations. Cyanuric acid is maintained at high concentrations to stabilize chlorine, and pool water is continuously recirculated, leading to repeated and prolonged human and aquatic exposure [70]. Furthermore, Sinclair et al. reported that more than a quarter of individuals have a 24-h cyanuric acid metabolism rate of less than 80% [77], indicating incomplete human clearance in some cases. These factors suggest that even cyanuric acid with low intrinsic bioaccumulation potential can accumulate transiently in localized environments or human tissues during repeated exposures.

In contrast, when pool water containing cyanuric acid is discharged into the environment, its ecological impact is expected to be limited. The compound undergoes moderate biodegradation under aerobic conditions and is substantially diluted in receiving waters. As a result, environmental concentrations typically decrease to levels several orders of magnitude lower than those found in pools, making chronic accumulation in aquatic organisms unlikely. The potential risk associated with cyanuric acid is thus primarily confined to the swimming pool environment, where continuous recirculation and high residual levels may lead to repeated human exposure, whereas post-discharge dilution and biodegradation considerably mitigate its persistence and bioaccumulation potential in natural waters.

#### 4. Conclusions

This study evaluated the occurrence and significance of cyanuric acid in swimming pool water. The LC<sub>50</sub> of cyanuric acid for CHO cells was determined to be 13.7 mM. In 20 swimming pools, the average concentration of cyanuric acid was 26,600 µg/L, which were 3–5 orders of magnitude higher than other known organic DBPs. Cyanuric acid accounted for 21.4% of the total cytotoxicity estimated from known DBPs, suggesting it is an overlooked DBPs before. Furthermore, compared with other known organic DBPs, although the bioconcentration and persistence of cyanuric acid were lower than most of known DBPs, concerns about its environmental risks cannot be eliminated owing to its extremely high concentration in swimming pool water (up to 79,000 µg/L). Considering the concentration and cytotoxic contribution of cyanuric acid in swimming pool water exceed significantly those of most DBPs, further investigation is warranted to better understand its fate, transport, and control techniques.

#### Supplementary Materials

The additional data and information can be downloaded at: <https://media.sciltp.com/articles/others/2511130926593722/GES-25090038-SI-FC-done.pdf>. Table S1. Turbidity, pH, urea concentration and free residual chlorine concentration in 20 swimming pools. Text S1. Method of turbidity test. Text S2. Method of pH, urea concentration, and free residual chlorine concentration test.

#### Author Contributions

Z.D and T.Q: conceptualization, methodology, software; Z.D: data curation, writing—original draft preparation; J.C.: visualization, investigation; J.L. and B.C.: supervision; writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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#### Institutional Review Board Statement

Not applicable for studies not involving humans or animals.

#### Data Availability Statement

The data will be availability based on request.

#### Conflicts of Interest

The authors declare no conflict of interest.

#### Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used Chatgpt to enhance the language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

#### List of Abbreviations

Abbreviation	Full Name
2-MBA	2-bromoaniline
2,4-DCP	2,4-dichlorophenol
2,4-DCA	2,4-dichloroaniline
2,4-DBA	2,4-dibromoaniline
2,6-CIBQ	2-chloro-6-iodo-1,4-benzoquinone
2,6-DCBQ	2,6-dichloro-1,4-benzoquinone benzoquinone
2,6-DCNP	2,6-dichloro-4-nitro-phenol
2,3,5-TCBQ	2,3,5-trichloro-1,4-benzoquinone
3-MCHBAL	3-chlorobenzaldehyde



3-MBHBAL	3-bromobenzaldehyde
3,5-DCHBA	3,5-dichlorobenzoic acid
3,5-DCHBAL	3,5-dichlorobenzaldehyde
4-MCP	4-Chlorophenol
5-MCHBA	5-Chlorosalicylic acid
BAA	Bromoacetic acid
BAL	Bromoacetaldehyde
BAM	Bromoacetamide
BAN	Bromoacetonitrile
BCAL	Bromochloroacetaldehyde
BCAM	Bromochloroacetamide
BCAN	Bromochloroacetonitrile
BCF	Bioconcentration Factor
BCNM	Bromochloronitromethane
BDCAA	Bromodichloroacetic acid
BDCAL	Bromodichloroacetaldehyde
BDCAM	Bromodichloroacetamide
BDCM	Bromodichloromethane
BDCNM	Bromodichloronitromethane
CAA	Chloroacetic acid
CAL	Chloroacetaldehyde
CAM	Chloroacetamide
CAN	Chloroacetonitrile
CP	Chloroacetone
DBAA	Dibromoacetic acid
DBAL	Dibromoacetaldehyde
DBAM	Dibromoacetamide
DBAN	Dibromoacetonitrile
DBCAA	Dibromochloroacetic acid
DBCAL	Dibromochloroacetaldehyde
DBCAM	Dibromochloroacetamide
DBCNM	Dibromochloronitromethane
Abbreviation	Full name
DBNM	Dibromonitromethane
DCAA	Dichloroacetic acid
DCAL	Dichloroacetaldehyde
DCAM	Dichloroacetamide
DCAN	Dichloroacetonitrile
DCNM	Dichloronitromethane
1,1-DCP	1,1-dichloroacetone
1,3-DCP	1,3-dichloroacetone
HA	Haloaniline
HAA	Haloacetic acid
HAL	Haloaldehyde
HAM	Haloamide
HAN	Haloacetonitrile
HBA	Halohydroxybenzoic acid
HBAL	Halohydroxybenzaldehyde
HBQ	Halobenzoquinone
HK	Haloketone
HNM	Halonitromethane
HNP	Halonitrophenol
HP	Halophenol
TBM	Tribromomethane
TCAA	Trichloroacetic acid
TCAL	Trichloroacetaldehyde
TCAM	Trichloroacetamide
TCAN	Trichloroacetonitrile
TCCA	Trichloroisocyanuric acid
TCM	Trichloromethane
TCNM	Trichloronitromethane
THM	Trihalomethane
I-THM	Iodo-trihalomethane
I-HAAs	Iodo-haloacetic acid
IAA	Iodoacetic acid
CIAA	Chloroiodoacetic acid
BIAA	Bromoiodoacetic acid
DIAA	Diiodoacetic acid
1,1,1-TCP	1,1,1-trichloroacetone

1,1,3-TCP	1,1,3-trichloroacetone
1,1,3,3-TeCP	1,1,3,3-Tetrachloroacetone

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