# Disinfection By-Products Foramtion Potential of Twenty Amino Acids

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***Abstract*—DBPs formation potential, including THMs, HAAs, HKs, chloropicrin, and chloral hydrate, of 20 amino acids was evaluated. Chlorination experiments were carried out at pH=8.0±0.1 by using phosphates buffer and performed at $Cl_2$:N=8:1(molar ratio) at 20±1℃ for a reaction duration of 24h. Chlorination experiments shown the DBPs formation potential of 20 amino acids differed considerably and without any obvious relationship to TOC contents. THMs, HAAs, HANs, HKs, chloropicrin, and chloral hydrate formation potential were in the range of 0.001~0.467 mol/mol, 0~0.669 mol/mol, 0.005~1.170 mmol/mol, 0.031~9.270 mmol/mol, 0~1.380 mmol/mol, and 0.059~39.080 mmol/L, respectively. THMs and HAAs were two largest classes of DBPs formed which accounts for 61~99% of total DBPs detected in the experiments except for Glu(43%) and Ile(32%). However, unlike NOM and resorcinol, THMs and HAAs formation were not related which indicated that the THMs and HAAs formation paths were different as compared with NOM. Certain amount of N-DBPs were found, but the formation potential are relatively small when compared with THMs and HAAs.**

***Keywords- disinfection by-products; DBPs formation potential; organic nitrogenous compounds***

## I. INTRODUCTION

Disinfection by-products (DBPs) are formed during natural organic matters (NOM) reacting with chlorine or other disinfectants. As chloroform has been first detected in chlorinated water in 1974, DBPs formation and control are always the focus of researches in water and wastewater treatment. In the last three decades, more and more DBPs are found in chlorinated water, and these DBPs are usually grouped into six distinct categories and include trihalomethanes (THMs), haloacetic acids (HAAs), haloactonitriles (HANs), haloketones (HKs), chlorophenols (CPs) and others. Epidemiological studies and/or toxicological studies using laboratory animals have verified that most of the DBPs are carcinogenicity, teratogenicity, and mutagenicity. USEPA, EU, WHO, China and many countries have drawn up strict criterion on DBPs to ensure the safety of potable water.

Numerous researchers have documented that NOM is the principal precursor of organic DBPs formation [1~3]. Due to the complexity and uncertainty of NOM, DBPs formation potential (DBPFP), including THMs formation potential (THMFP), HAAs formation potential (HAAFP), HANs formation potential (HANFP), etc. are often used as surrogates to assess NOM. DBPFP is a test measuring method and refers to the quantity of DBPs formed with a high dosage of free chlorine and a long reaction time. Other factors affecting formation of DBPs include chlorine dose, contact time, bromide ion concentration, pH, temperature, and organic nitrogen concentration.

Besides NOM, dissolved organic nitrogen(DON) can also reacts with chlorine to yield various DBPs. DCAN (dichloroactonitriles) was found to be formed during chloramination of glutamic acid, cytosine, cysteine, and tryptophan[4]. W.H. Chu et al studied the chlorination of tyrosine (Tyr) in water, and found that increased chlorine contact time and/or Cl2/Tyr ratio increased the formation of most C-DBPs, with the exception of 4-chlorophenol, dichloroacetonitrile, and dichloroacetamide. Chloroform and dichloroacetic acid increased with increasing pH, dichloroacetonitrile first increased and then decreased, and other DBPs had maximum yields at pH 7 or 8[5]. Chloroform formation in chlorination of aniline (Ala) was studied, and the results indicated that chloroform reached a maximum value of 0.143% at the molar ratio of Cl/N=1.0 over a Cl/N range of 0.2~5.0 (pH = 7.0, reaction time = 5 d, and initial Ala = 0.1mM) [6]. Chlorination of algal cells and EOM of Microcystis aeruginosa, and Chlorella vulgaris which were demonstrated to inriched with protein-like and soluble microbial by-product-like matters by Fluorescence excitation-emission matrix was examined. The evaluation showed that algal cells and EOM exhibited a high potential for DBP formation, and algae under bloom seasons contributing significantly to the DBP precursor pool when compared to natural organic matter was indicated in the study [7]. Bond. T. et al reviewed the DBPs precursors of DBPs in water treatment, and pointed that total amino acid, free amino acid are the precursors of DBPs (especially HANs, CNCl, and NDMA)[8]. However, the DBPFP of different DON are not so clear.

The objective of this study is to evaluate formation potential of regulated DBPs (including THMs, HAAs, HAAs, HKs, chloropicrin, and chlorohydrate) during chlorination of a variety of 20 amino acids which is usually detected in water and wastewater to improve better understanding of DBPs formation during water and wastewater chlorination.

## II. MATERIALS AND METHODS

### A. Materials

Chlorine-demand free water is used in all of the

experiments, including the preparation of chemical solutions and chlorination reaction experiments. The chlorine-demand free water is prepared by chlorinating ultra-pure water produced by Milli-Q Academic System to ensure the chlorine residual at 1.0±0.2mg/L as $Cl_2$ after a contact period of 24h, then the water was boiled to evaporate the chlorine residual and cooled to room temperature. All chemicals used in the experiment are reagent grade without further purification.

20 amino acids, including alanine(Ala), arginine(Arg), asparagines (Asn), aspartic acid(Asp), cysteine (Cys), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine(Leu), lycine(Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine(Ser), threonine (Thr), tryptophan(trp), tyrosine(Tyr), and valine (Val), are used as organic nitrogenous compounds in the experiments.

Amino acids stock solutions are prepared by dissolving the amino acids powder in chlorine-demand free water at concentration of 1.00g $L^{-1}$(calculated as α-$NH_2$-N). For insoluble amino acids, aliquots of HCl or NaOH are added and stirred by a magnetic stirrer until all powder is dissolved. The amino acids stock solutions are preserved at 4℃ in dark and prepared weekly.

Chlorine stock solution is prepared by dilute the commercial hypochlorite sodium to 2.00g $L^{-1}$(calculated as $Cl_2$) and also preserved at 4℃ in dark and prepared weekly. The chlorine stock solution is standardized before use.

### *B. Experimental Procedures*

Chlorination of amino acids is performed in a 4L amber glass bottle which is gently stirred on a magnetic stirrer. The chlorination procedure is adopted the method recommended by Summers R.S. et al and listed as below:

- Fill the bottle by chlorine-demand free water to three quarters full, dose certain amount of amino acids stock solution to make the amino acids concentration to 5.0mg $L^{-1}$(0.35mmol $L^{-1}$)when the bottle is full filled;
- Add 5.0mL pH=8.0 borate buffer to water, and adjust to pH=8.0 with $H_2SO_4$/NaOH if necessary;
- Dose chlorine stock solution to ensure the chlorine dosage at 200mg $L^{-1}$(2.82 mmol $L^{-1}$, $Cl_2$:N molar ratio at 8:1) when the bottle is full filled;
- Vigorously stirred the solution by magnetic stirrer by 30s;
- Fill to top with chlorine-demand free water and cap headspace-free, total mixed;
- Incubate in dark at 20.0℃ for 24h;

After incubation, sample was grabbed and quenched using 400mg crystalline ammonia chloride and extracted immediately for disinfection by-products analysis (quenched sample must be extracted immediately to reduce the DBPs formation by any possible chloramines, but the extract can be preserved for less than 3days at 4℃ in dark before further processing.

### *C. Analytical Methods*

THMs, HKs, HANs, Chloropicrin, and Chloral Hydrate Analysis: THMs, including trichloromethane ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), chlorodibromomethane ($CHClBr_2$), and tribromomethane ($CHBr_3$), HANs, including bromodichloroacetonitrile (BDCAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), and trilchloroacetonitrile(TCAN), haloketones (HKs), including dichloropropanone and trichloropropanone, chloropicrin, and chloral hydrate are measured by EPA Methods 551.2. The quenched water sample is extracted by 3mL MTBE, and 2μL of the extract is then injected into a GC (Agilent 6890) equipped with a fused silica capillary column (HP-5) and μECD for separation and analysis. HAAs Analyssis: HAAs are measured using the liquid-liquid extraction and derivatization method by EPA Methods 552.2. A 40mL volume of quenched sample is adjusted to pH <0.5 and extracted with 4 mL of (MTBE) to partition the HAAs to MTBE phase. HAAs are then converted to methyl esters by the addition of acidic methanol followed by slight heating for derivatization. The acidic extract is neutralized by a backextraction with a saturated solution of sodium bicarbonate and the target analytes are identified and measured by capillary column gas chromatography using an electron capture detector (GC/ECD).

## III. RESULTS AND DISCUSSION

### *A. THMs Formation Potential*

THMs is one of the most imprtant categories of DBPs found in chlorinated water and wastewater. The THMs formation potential (THMFP) of 20 amino acids are shown in Figure.1. In 20 amino acids studied, Trp and Tyr have the highest THMFP which was 0.46mol/mol (amino acids). The formation mechanism of THMs by Trp and Tyr may be analoguos to that of resorcinol which was proposed and demonstrated by many researches [10-12]. THMs reaction usually occurs with the resorcinol type moiety becasue of the fast chlorination of the carbon atom that are acivated by ortho-hydroxide (OH). For Trp, the aromatic structure is activated by o-amino and o-alkyl group, these strong electron donating groups at o-postion make the electron cloud relatively concentrated, and make the benzene ring are easily been attacked by chlorine. The chlorination of electron-rich carbon opens the benzene ring, and the following cleavage and hydrolysis causes THMs formation. The hydroxide (OH) and alkyl group in Tyr are at the p-postion which caused the electron cloud is scattered all over the benzene ring, thus cause less THMs formation. Besides Trp and Tyr, Phe also has an benzene ring in its structure, but there is only one alkyl group which makes relatively small electron cloud density. This samll electron cloud desity makes the chlorine attack more difficult and results in less THMs formaiton.

THMs formation by Ala, Asn, Asn, and His are about 0.2mol/mol which are less than Trp and Tyr, but is still far more than other amino acids. This THMs formation may be caused by the carbon atom activated by $–NH_2$ in the side-chain. The $Cl_2$:N used in this experiment is 8:1 which is larger than that used by Chu et al(1:1)[6], thus causes more THMs formation.

THMs formation in chlorination process are usually assumed to be related with NOM (calculated as TOC). However, THMFP of amino acids had no obvious relationship with TOC (not measured but calculated by the concenrtation of amino acids) in the reaction system. TOC of Phe and Tyr was similar at 38.6mg/L, but the THMFP of Tyr is 50-times of Phe. This demonstrated that THMFP of amino acids was controlled by the reactivity of amino acids structure rather than carbon content.

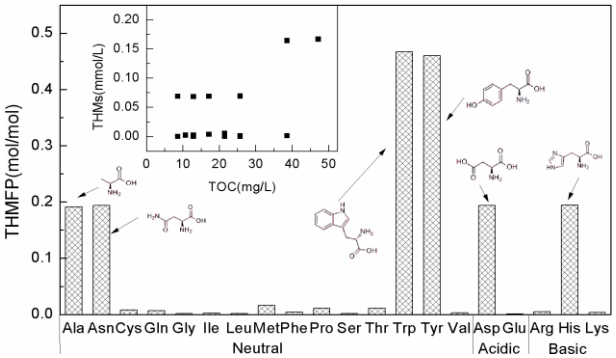


Figure 1. THMs formation potential of 20 amino acids

## B. *HAAs Formation Potential*

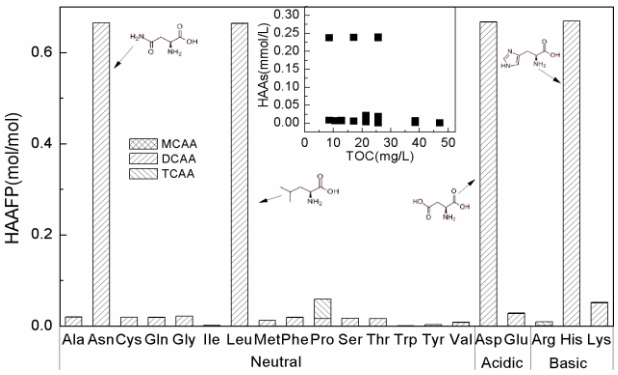


Figure 2. HAAs formation potential of 20 amino acids

HAAs formation potential of 20 amino acids are illustrated in Fig.2. HAAs formaiton potential of Asn, Leu, Asp, and His are at about 0.7mol/mol which is far more than other amino acids. Trp and Tyr, with large THMs formation potential, also have relatively larger HAAs formation potenial. As compared with THMs largest formation in chlorination of amino acids with activated benzene ring, the largest HAAs formation occur in the chlorination of amino acids without aromatic structure. Moreover, the HAAs formation is usually found to be half of THMs on a weight basis [13]. But is the chlorination of amino acids, the HAAs and THMs formation is not related. As shown in Fig. 7, the THMs formation potential of Trp and Tyr is relatively high while the HAAs formation potential of them is negligible, and the HAAs formation potential of Leu is relatively high while the THMs formation potential is negligible. But the possible reaction scheme needs more detailed study to confirm. This differences may suggested that the HAAs formation may be occur at different carbon site other than researches proposed that THMs and HAAs formation are caused by chlorination of same carbon atom and the following different cleavage position controls the DBPs species[14].

For the HAAs species, no MCAA are not measured in this study. This is concided with with that in water and wastewater chlorination in practice, and may be caused by the larger $Cl_2$:N used. Similar to MCAA, TCAA were also found in all the chlorination process of amino acids but Pro, this results indicated that increasing chlorine dosage may cause even larger HAAs formation. As similar to THMs formation, the HAAs formation is not related to TOC, either.

## C. *HANs Formation Potential.*

Researches have demonstrated that the DON greatly affect the HANs formation [15,16]. But the experiment results showed that HANs formation potential of all amino acids are very small as compared with THMs and HAAs formation potential as illustrated in Fig. 3. The HANs formation potential of 20 amino acids is less than 1.2mmol/mol, which is only ~1‰ of THMs and HAAs formation potential. This results suggested that the HANs formation in water and wastewater chlorination may not mainly caused by the direct chlorination of DON. Yang, et al demonstrated that nitrogen atom in DCAN formed by chloramination of Trp, Glu, and NOM from Suwannee River was mainly originated from monochloramine [4]. But they also found the nitrogen atom in DCAN formed by chloramination of Cys was originated from Cys itself. This difference in nitrogen origin indicated that DCAN were formed through different paths, and this may explain the different HANs formation potential of 20 amino acids.

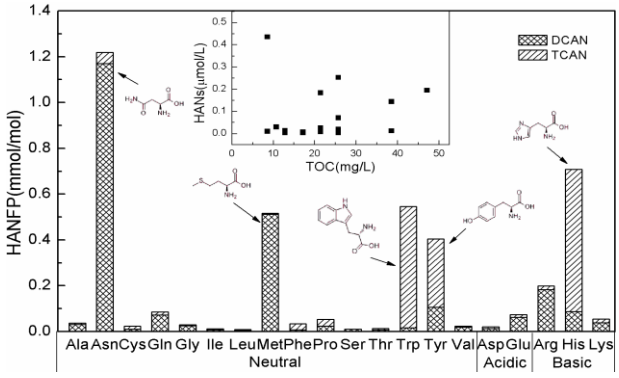


Figure 3. HANs formation potential of 20 amino acids

Unlike the uniform species of THMs and HAAs formation during amino acids chlorination, the HANs species are different in chlorination of different amino acid. For Asn, Met, Arg, and many other amino acids, the DCAN is the dominant species of HANs. But for Trp, Tyr, and His, the TCAN is the dominant species, and the TCAN formation are also considerable in chlorination of Phe and Pro despite of less HANs formation. Trp, Tyr, His, Phe, and Pro are the only 5 amino acids which has ring structure among the 20 amino acids, which indicated that the different formation paths of DCAN and TCAN. TCAN may mainly formed by chlorination of carbon atom in ring structure,

while DCAN are formed by chlorination of atoms in chain structure.

### *D. HKs Formation Potential.*

Haloketones(HKs) include many substances. But in practice, HKs usually refers to 1,1-dichloroacetone and 1,1,1-trichloroacetone. For the 3-carbons-chain in di- and tri-chloroacetone, HKs are generally formed in chlorination of amino acids with linear carbon chain as shown in Fig. 4. Due to the benzene ring rapture, the HKs formation of Phe is also considerable. Therefore, for greatly activated benzene ring in Trp and Tyr, the ring rapture may be more thoroughly and leads to more THMs formation with smaller molecule but HKs formation. And it must be noted that, Gly has only two carbon atoms in its structure, but considerable HKs are measured in its chlorination. This HKs formation by chlorination of Gly indicates that the HKs may form by carboxyl re-combination. Dichloropropanone is the main species detected in the experiments, and indicates that larger HKs formation can be found when increasing the chlorine dosage.

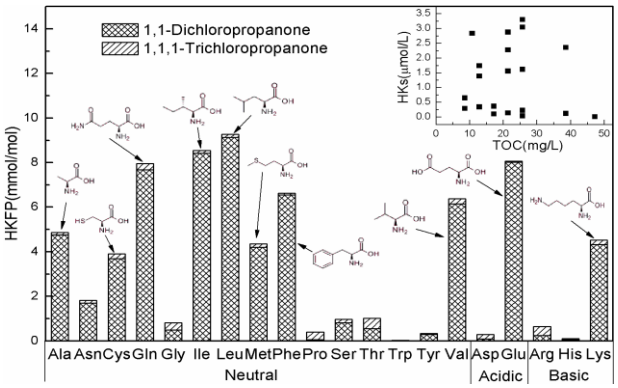


Figure 4. HKs formation potential of 20 amino acids

### *E. Chloropicrin Formation Potential.*

Despites little formation amounts as compared with THMs and HAAs, chloropicrin is detected in many studies all over the world. A survey on 7 authorities across 5 states of Australia shows that the chloropicrin formation at about <0.01~1ug/L[17], and the survey of Canadian drinking water also shows the chloropicrin formation at 0.2~1.1μg/L[18]. The chloropicrin formation potential of 20 amino acids is less than 1.4mmol/mol which similar to HANs as illustrated in Fig.5. Ser has the greatest chloropicrin formation potential which is about 1.4mmol/mol while Leu, Thr, and Val has the second chloropicrin formation potential which is around 0.5mmol/mol. Chloropicrin formation when chlorination of other amino acids are relatively small which is less than 0.1mmol/mol. Researches also demonstrates that chloropricrin formation in chlorination of amino acids is insignificant[19]. However, other researchers found that the chloropicrin formation of Lys and Gly in ozonation-chlorination process are as higher as 1.1% and 8.7%[20]. The result indicates that although the chloropicrin formation in direct chlorination of amino acids is insignificant, but the intermediates of amino acids in certain treatment process must not be ignored.

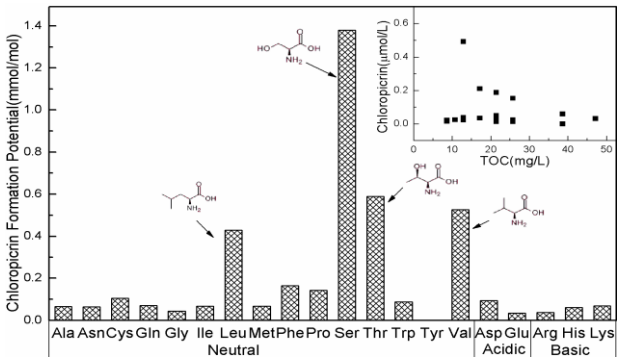


Figure 5. Chloropicrin formation potential of 20 amino acids

### *F. Chloral Hydrate Formation Potential.*

As shown in Fig. 6, chloral hydrate formation potential of 20 amino acids are at 0~40mmol/mol, which is higher than HANs and chloropicrin but much less than THMs and HAAs. This result is coincidental to chloral hydrate formation in water and waste water chlorination [17,18]. As similar to HANs formation, chloral hydrate formation are relatively higher in chlorination of Trp, Tyr, and His that with a activated ring structure. The formation potentials of Asp and Glu that with –COOH in side-chain are also considerable.

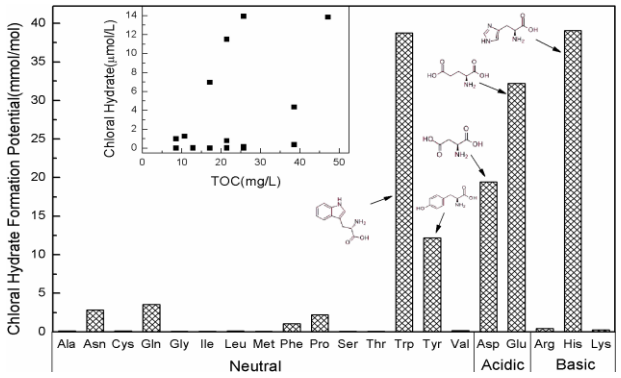


Figure 6. Chloral hydrate formation potential of 20 amino acids

### *G. DBPs Formation Potential and Species Distribution.*

The DBPs formation potential of 20 amino acids differs considerably. DBPs formation potential of His, Asp, and Asn are the highest and at about 0.9mol/mol. DBPs formation potential of Leu is 0.7mol/mol which is second highest. DBPs of Trp and Tyr are similar, which are about 0.5mol/mol. DBPs formation potential of Ala is about 0.2mol/mol. As discussed above, in the structure of these amino acids, the strong electron donating groups in the structure make the electron cloud denser in certain sites and make the sites easily to be attacked by chlorine thus cause DBPs formation. DBPs of other amino acids are relatively small and less than 0.1mol/mol. Besides, DBPs formation potential has no obvious relationship with TOC content. This indicated that the DBPs formation is control by the

reactivity of amino acids with chlorine rather than the TOC contents.

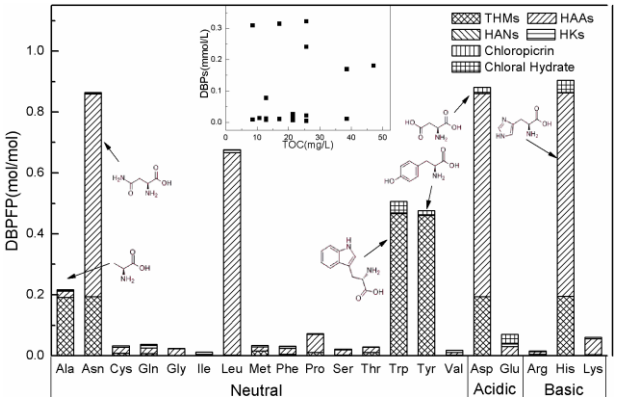


Figure 7. DBPs formation potential of 20 amino acids

As shown Fig.7, THMs and HAAs are the dominant species of DBPs, the sum of THMs and HAAs accounts for more than 95% of DBPs measured in this experiments for Asn, Leu, Asp, Ala, Tyr, Pro, Gly, and His (including amino acids that with both larger and less DBPs formation potential), and accounts for more than 85% for other 7 amino acids. This indicates that the usually regulated THMs and HAAs formation are the main DBPs in amino acids chlorination. Chloral hydrate formation was the second largest category of DBPs measured in this experiment, but it is much less than THMs and HAAs. The other DBPs, including HANs, HKs, and chloropicrin, are negligible as compared with THMs and HAAs.

## IV. CONCLUSIONS

DBPs formation potential of 20 amino acids differs considerably and is controlled by the reactivity of amino acids with chlorine other than TOC contents. Amino acids that have electron-rich sites activated by strong electron donating groups, such as Asn, Leu, Trp, Tyr, Asp, and His, exhibits the greatest DBPs formation potential which is more than 0.5mol/mol. For these amino acids, THMs and HAAs formation potential accounts for more than 95% of DBPs formation potential. But unlike NOM or resorcinol that usually used as surrogate for NOM, the THMs and HAAs formation potential is not related and indicated that different formation paths. Certain amounts of N-DBPs, such as HANs, and chloropicrin are found in the chlorination of 20 amino acids, but the formation potential are not related with nitrogen contents.

## ACKNOWLEDGMENTS

This study was supported by the National Science Foundation of China (NSFC) Award No. 50908074, and the Fundamental Research Funds for the Central Universities Award No. 2009B17314.

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