とし，全項目の平均点を算出している．

口頭試問とは別に，前述のように，全受講生は顕微鏡観察したオーシスト及びシストを写真撮影し，提出することになっており，提出された写真は研修最終日にスライドプロジェクターで投影し，全受講生の前で講師が講評を行う．受講生は自分が提出した写真に加え，他の受講生の多くの写真の評価を視聴することで，形態観察の仕方とそれに必要な顕微鏡の操作，写真撮影の技法などを復習することができる．一方，講師は個々の受講生が習得すべき知識や技術の習得度を，提出された写真を介して確認することができる．

Ⅶ. 受講生からの研修に対する評価

本研修では，研修修了時に受講生を対象に研修内容に対するアンケート調査を実施しているが，本研修は受講生から全体的に高い評価を得ている．平成23年度の研修では，満足度に関して「とても良かった」，「概ね良かった」，「どちらかというと良かった」，「良くなかった」という項目のうち，「とても良かった」と回答した受講生が約70%，「概ね良かった」と回答した受講生が約30%であり，同様のアンケート調査を実施している他の研修と比較しても高い満足度が得られている．また，個別意見としても「最先端の情報，最高峰の指導の下，基本から研修を受けられたのがよかった.」，「演習で（顕微鏡の）微分干渉像の写真の撮り方を覚えられたことは収穫だった．また，一連の操作手順を再度確認できて，研修に参加した甲斐があった.」，「実践的で即役立つ内容になっており，他県，他施設の状況も理解することができた.」等の肯定的な意見が多く出ており，日常業務に役立つ実践的な研修が行われていることが確認できる．

Ⅷ. 今後の課題

水道水の微生物学的安全性の確保のために，水道事業にもHACCPの概念が導入され，ハザードとしての原水の汚染状況を正確に把握することが大前提となっている．そのため，クリプトスポリジウム等の試験法による検査の需要が一層高まっており，それに応えられる検査技術の普及と定着が求められている．新たに遺伝子検査法が提案されているが，水試料からのクリプトスポリジウム・ジアルジア試験法は，今後も顕微鏡観察による方法が基本であることに変わりはない．この試験法の各段階である「濃縮・精製技術」，「染色技術」，「技術を要する顕微鏡操作」および「顕微鏡を用いた観察手法」を多くの受講生が習得し，彼らがそれぞれの地域においてさらに技術を伝達・普及させていくことが本研修の使命である．こうした検査体制を維持・向上させるために，本研修が果たすべき役割は大きい．

前述したように水試料からのクリプトスポリジウム・ジアルジア試験法は実績主義を重視する試験法であるため，対象とする試料に適した試薬，機器・器材を組み合わせ，微分干渉装置付蛍光顕微鏡で観察して判別・計数しなければならない．こうした試験法の選択の決定や試験の工程中の問題点の解決，あるいは顕微鏡観察による判定などに対して，研修後も支援することができれば，本研修がこれまで以上に重要な役割を果たすことになると思われる．

水道水を介したクリプトスポリジウム症あるいはジアルジア症の発生は，健康危機管理上の重大な問題である．本研修は，水試料からのクリプトスポリジウム・ジアルジア試験法に関する知識と技術の習得を主な目的としながら，受講生が地域においてクリプトスポリジウム・ジアルジア対策に取り組みながら，検査体制の中核として活躍できるようにすることも，その役割として担っている．中核となるためには，受講生は単に試験法に関する知識や操作技術を習得するにとどまらず，地域保健行政に関連した幅広い能力が求められる．したがって，今後は試験担当者に求められるコンピテンシーの理解と向上を目的として，基本的能力及び専門能力に関連した幅広い内容を講義ならびに実習に加えることを検討しなければならない．

水試料からのクリプトスポリジウム・ジアルジアの遺伝子検出法は，試験法として今後本格的に導入され，日常的に実施される検査として普及・定着する可能性がある．本研修では，これまでのところデモンストレーションにより操作を紹介し，また短時間の実習が行われているのみであるが，今後は顕微鏡観察操作と同様に，本格的な実習を行い，受講生の技術向上に努める必要があるだろう．

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Selecting analytical target pesticides in monitoring: Sensitivity analysis and scoring

Koji Tani [a], *Yoshihiko Matsui* [b,*], *Kensuke Iwao* [a], *Motoyuki Kamata* [c], *Taku Matsushita* [b]

[a] *Graduate School of Engineering, Hokkaido University, N13W8, Sapporo 060-8628, Japan*
[b] *Faculty of Engineering, Hokkaido University, N13W8, Sapporo 060-8628, Japan*
[c] *College of Engineering, Kanto Gakuin University, Mutsuurahigashi 1-50-1, Kanazawa-ku, Yokohama 236-8501, Japan*

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ABSTRACT

Measuring river water concentrations of all pesticides applied in a catchment area is a daunting task. This study aims to develop new score tables for selecting analytical target pesticides. Sensitivity analyses were conducted using a diffuse pollution hydrologic model to quantitatively evaluate the influence of pesticide properties (e.g., log K_{OC}, degradability [half-life]) on concentrations of rice-farming pesticides in river water. Using the results of the analyses, score tables were systematically designed for the pesticide properties such that the sum of the scores for a particular pesticide, designated as the contamination index, was proportional to the expected/predicted concentration of that pesticide in river water. The contamination indexes for pesticides applied in three river basins were calculated and compared with the corresponding observed pesticide concentrations. Correlations between contamination indexes and observed concentrations were fairly good. Pesticides were ranked according to the quotients obtained by dividing the pesticide concentrations predicted from the contamination indexes by the corresponding drinking-water quality guideline values, and pesticide candidates to be monitored were successfully selected on the basis of a threshold quotient.

1. Introduction

Pesticides have markedly enhanced agricultural productivity and crop yields (e.g. Bolognesi, 2003). However, pesticide releases from agricultural fields and the resulting contamination of surface water threaten human health, as well as the local ecosystem in many regions, because surface water is a primary source of drinking water (Gilliom et al., 1999; Capri and Karpouzas, 2007; Hildebrandt et al., 2008; Vryzas et al., 2009; Wittmer et al., 2010). Governments regulate pesticide concentrations in drinking water. For example, the European Union Drinking Water Directive specifies a maximum acceptable concentration of 0.1 μg/L for individual pesticides and a maximum total acceptable concentration of 0.5 μg/L for all pesticides and their metabolites, degradation products, and reaction products (Drinking Water Directive, 1998). The analytical target pesticides are not defined by the Directive, but those pesticides which are likely to be present in a given supply need be monitored by the monitoring authority. In order to select the analytical target pesticides, therefore, a risk assessment is required which takes into account the pesticide usage and the local circumstances, but this process is not defined in the Directive. Japanese drinking water quality guideline (JDWQG) specifies that *DI* value should not exceed 1; *DI* is the

* *Corresponding author.* Tel./fax: +81 11 706 7280.
E-mail address: matsui@eng.hokudai.ac.jp (Y. Matsui).
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sum of the DV_i/GV_i quotients for monitored pesticides [MHLWJ (Ministry of Health, Labor and Welfare of Japan), 2003a]:

$$DI = \sum_i \frac{DV_i}{GV_i} \quad (1)$$

where DV_i is the observed concentration of pesticide i and GV_i is the reference concentration of pesticide i and is determined in JDWQG by MHLWJ (2003a) based on the acceptable daily intake of the pesticide. A hundred and two pesticides are listed in Pesticide Group 1 of JDWQG on the basis of the quantities sold and the availability of quantification methods, and the monitoring authority can further select the analytical target pesticides depending on its local circumstances. The selection process is, however, not defined.

Chemical scoring and ranking methods are developed as a screening tool for risk assessment of chemicals (Swanson et al., 1997; Finizio et al., 2001; Gramatica and Guardo, 2002; Juraske et al., 2007). A score table that ranks pesticides has been proposed for the selection of analytical target pesticides for JDWQG (Kamata et al., 2007). In this score table (hereafter referred as the old score table), a pesticide is scored between 1 and 5 for each of the following properties: quantity sold, octanol–water partition coefficient (log K_{OW}), degradability, and acceptable daily intake (ADI), as shown in Table 1S in the supplementary information. Ideally, the sum of the first three scores (Scores A–C in Table 1S, which are related to pesticide runoff from rice paddies), should be well correlated with the concentrations of a particular pesticide in river water, but the old score table has not been validated. Generally, the results of chemical ranking approach have rarely been validated against measured concentrations, while the approach is applied for screening pesticides in surface water (Altenburger et al., 1993; Papa et al., 2004). In addition, the old score table does not include any criteria for selecting which pesticides should be monitored. For example, even if a list of pesticides was sorted and prioritized by means of the old score table, the table does not specify an absolute threshold value above which a pesticide should be selected for monitoring. Consequently, the old score table is hardly accepted as a screening tool by monitoring authorities.

In the current study our aim was to develop and test new score tables for (1) selecting pesticides with the potential to contaminate river water and (2) facilitating the development of a program for monitoring water quality. Rice-farming pesticides were investigated in this study as unlike upland field pesticides, they enter river water at high rates due to the large amount of fresh natural water required during the cropping season (Matsui et al., 2002). Therefore, pesticides applied to rice-paddy fields have a greater potential to contaminate river water, some of which is the source of drinking water.

2. Materials and methods

2.1. *Diffuse pollution hydrologic model*

Although many models and their applications have been reported, few are designed to predict runoff of rice-farming pesticides from rice-paddy fields (Inao and Kitamura, 1999; Miao et al., 2003; Nakano et al., 2004; Inao et al., 2008). Moreover, very few studies have attempted to develop a diffuse pollution hydrologic model applicable to basin-scale catchments (Matsui et al., 2002). In this study, we applied a diffuse pollution hydrologic model, which is capable of predicting the rice-farming pesticide concentrations in river water (Matsui et al., 2006a, b, Matsui et al., 2007), for conducting sensitivity analysis and then developing score tables. Sensitivity analysis capability of the model is also verified using the data of pesticide runoff dependency on K_{OW} (Tani et al., 2010). To apply the model to the river basins, we divided each catchment area into a grid of 1-km^2 cells and then subdivided each grid cell into at least 12 compartments: a rice-paddy compartment et al. (Matsui et al., 2006a). It is assumed that applied pesticides reach the paddy fields (the loss due to the drift of pesticide, the effect of application mode, the effect of spray formulation, and the effect of adjuvants were not accounted). A set of differential mass-balance equations was defined to describe the dynamics of the pesticide and water in each compartment. The equations are solved as a system of ordinary differential equations by Gear's stiff method (backward differentiation formulas) from the IMSL MATH/LIBRARY (Visual Fortran Versions 6.6, Compaq). The Chikugo River (33.18°N, 130.28°E, length: 143 km, basin area: 2860 km^2) and Sagami River basins (35.25°N, 139.22°E, length 109 km, basin area 1680 km^2) were selected as the model river basins.

2.2. *Pesticides*

Pesticide concentrations observed in 2004–2008 in the Mabechi River (40.47°N, 141.42°E, length 142 km, basin area 2050 km^2) and in 2004–2007 in the Chikugo and Sagami Rivers were used for evaluating the score tables. Pesticide concentration data were obtained from the local water supply authorities (see Acknowledgments), who measured pesticide concentrations almost weekly according to the JDWQG standard methods (MHLWJ, 2003b). Pesticide property data were obtained by PCKOCWIN (organic carbon partitioning coefficient estimation program), KOWWIN (octanol-water partitioning coefficient estimation program) and BIOWIN (biodegradation factor estimation program) modules in the EPI (Estimation Program Interface) Suite (Aronson et al., 2006; US Environmental Protection Agency, 2007) Tomlin, 2006; Kamrin, 1997), and Ministry of the Environment of Japan (2010). The quantities of pesticides sold in the basins were estimated from pesticide sale data books (Japan Plant Protection Association, 2005–2008) after allocation of the pesticide sales to the river basins on the basis of land-use data (Matsui et al., 2006a; Kamata et al., 2008).

2.3. *Sensitivity analyses for constructing the new score tables*

2.3.1. *Score for quantity of pesticide sold*

Pesticides applied in fields reach the river after having been diluted. Therefore, the basic potential for the pesticide concentration in river water should be proportional to the quantity of pesticide sold divided by the river flow rate. In the current study, the score for the quantity of pesticide sold, defined as Score X, is given by

$$\text{Score } X = \log\left(\frac{M}{Q}\right) \quad (2)$$

where M is the quantity of pesticide sold (mg/year), Q is the annual river flow rate (km^3/year).

2.3.2. *Score for pesticide degradation and adsorption*

Using the diffuse pollution hydrologic model, sensitivity analyses were previously conducted to evaluate the influence of each pesticide property independently and indicated that adsorption and degradation in soil are the most influential properties and water solubility somewhat affects pesticide runoff (Tani et al., 2010). However, the analyses were not aimed to design score tables. For designing score tables, interdependency of pesticide properties needs to be incorporated in sensitivity analyses. In the current sensitivity analyses, interdependency of soil adsorption coefficient and water solubility are incorporated: highly soluble compounds tend to have low soil adsorption coefficients and vice versa. The following regression equation for the relationship between K_{OC}, the organic-carbon-based soil adsorption coefficient (mL/g), and water solubility (S, mg/L) has been proposed to be applicable for most pesticides (Lyman et al., 1990):

$$0.55\log S = -\log K_{OC} + 3.64 \quad (3)$$

Therefore, we considered two parameters [K_{OC} for adsorption and half-life in soil (HLS) for degradation] in our sensitivity analyses, and water solubility was treated as a subparameter represented by the soil adsorption coefficient according to Eq. (3). Finally, in the sensitivity analyses of this study, input values of HLS, which is an index of soil degradation, were varied between 10^{-2} and 10^3 day, and K_{OC}, an index of organic-carbon-based soil adsorption in soil, was varied between 1 and 10^6 mL/g [the ranges covers all pesticides listed in JDWQG and covers almost all pesticides listed in The e-Pesticide Manual (Tomlin, 2006)]). Because the runoff rates of herbicides and fungicides differ owing to differences in application dates (Tani et al., 2010), sensitivity analyses for herbicides and fungicides were conducted separately. Details of the sensitivity analysis procedure are seen in the paper of Tani et al. (2010).

Fig. 1 shows a graph of the results of the fungicide sensitivity analysis for adsorption and degradation in soil of the Sagami River basin; the vertical axis is the dimensionless simulated pesticide concentration (C/M_V), where C is the average simulated concentration (μg/L) for the 4-month period starting with the date of fungicide application, and M_V is a parameter determined by the quantity of the applied fungicide (mg) divided by the total river flow volume (L) for the 4 months starting from the date of application. Another fungicide sensitivity analysis was conducted for Chikugo River basin and a similar result to Fig. 1 was obtained. Because Score X is designed as such that a score increment of 1 corresponds to a 10-fold increase in pesticide concentration, scores for soil adsorption and soil degradation, defined as Score Y (view A in Table 1), were also designed similarly as Eq. (4) after the C/M_V values for the two rivers were averaged geometrically.

$$\text{Score } Y = \log\left(\frac{C}{M_V}\right) \quad (4)$$

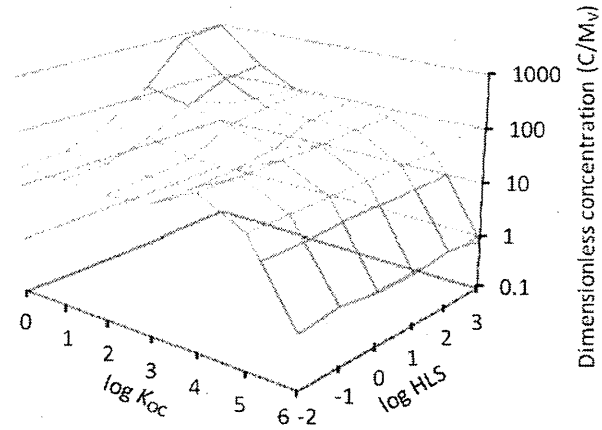

Fig. 1 – Plot of results of sensitivity analysis for adsorption and degradation in soil for fungicides in the Sagami river basin. Dimensionless pesticide concentrations (C/M_V), where C is the average simulated concentration for the 4-month period starting from the date of pesticide application (μg/L), and M_V is a parameter determined by the quantity of the applied fungicide (mg) divided by the total river flow volume (L) for the 4 months starting from the date of application, are plotted against K_{OC} and HLS (half-life with regard to degradation in soil).

Because the concentration is normalized by the quantity of the applied pesticide in Eq. (4), Score Y value is not affected by the quantity of the applied fungicide but it is reflected by HLS and K_{OC} values. A score table for herbicides (view B in Table 1) was determined by means of a process similar to that used for fungicides.

With regard to degradation in water, Tani et al. (2010) reported that pesticide concentrations are influenced and decreased only when the rate constant of degradation in water was large. The sensitivity analyses for degradation in water of the Sagami and Chikugo River basins were conducted in the current study and similar results to Tani et al. (2010) were obtained. Using the results of the sensitivity analyses and Eq. (5), the score tables for values of half-life in water (HLW) were determined in the same way as that of Score Y (Score Z in view C of Table 1).

$$\text{Score } Z = \log\left(\frac{C}{M_V}\right) \quad (5)$$

3. Results and discussion

3.1. *Correlation between contamination index and pesticide concentration*

Pesticide concentrations observed from 2004 through 2008 in the Mabechi River as well as from 2004 through 2007 in the Chikugo and Sagami Rivers were used for the evaluation of the score tables. Contamination index values, defined as the sum of the scores, were calculated for each of the pesticides used in the Mabechi, Chikugo, and Sagami River basins by means of

Table 1 – New score tables for soil adsorption, soil degradation, and water degradation of fungicides and herbicides. K_{OC} is the organic-carbon-based soil adsorption coefficient (mL/g), and HLS is the half-life (day) with respect to degradation in soil. HLW is the half-life (day) with respect to degradation in water.

A. Values of score Y for fungicides

log HLS	log K_{OC}											
	6.0≥ & >5.5	5.5≥ & >5.0	5.0≥ & >4.5	4.5≥ & >4.0	4.0≥ & >3.5	3.5≥ & >3.0	3.0≥ & >2.5	2.5≥ & >2.0	2.0≥ & >1.5	1.5≥ & >1.0	1.0≥ & >0.5	0.5≥ & >0.0
3.0≥ & >2.5	0.4	1.1	1.5	1.6	1.7	1.8	1.8	1.8	1.9	2.1	2.2	2.5
2.5≥ & >2.0	0.4	1.1	1.5	1.6	1.7	1.7	1.8	1.8	1.9	2.0	2.2	2.5
2.0≥ & >1.5	0.4	1.1	1.5	1.6	1.7	1.7	1.7	1.8	1.8	2.0	2.1	2.3
1.5≥ & >1.0	0.5	1.1	1.5	1.6	1.6	1.6	1.6	1.7	1.8	1.9	2.0	2.1
1.0≥ & >0.5	0.4	1.1	1.5	1.6	1.6	1.5	1.4	1.5	1.6	1.7	1.8	1.9
0.5≥ & > 0.0	0.4	1.1	1.5	1.6	1.6	1.4	1.3	1.3	1.4	1.5	1.6	1.7
0.0≥ & > −0.5	0.4	1.1	1.5	1.6	1.5	1.3	1.2	1.2	1.3	1.4	1.4	1.5
−0.5≥ & >−1.0	0.3	1.0	1.5	1.6	1.5	1.3	1.2	1.1	1.2	1.2	1.3	1.3
−1.0≥ & >−1.5	0.3	1.0	1.5	1.6	1.5	1.3	1.1	1.1	1.1	1.1	1.1	1.2
−1.5≥ & >−2.0	0.4	1.1	1.5	1.6	1.5	1.3	1.1	1.1	1.0	1.1	1.1	1.1

B. Values of score Y for herbicides

log HLS	log K_{OC}											
	6.0≥ & >5.5	5.5≥ & >5.0	5.0≥ & >4.5	4.5≥ & >4.0	4.0≥ & >3.5	3.5≥ & >3.0	3.0≥ & >2.5	2.5≥ & >2.0	2.0≥ & >1.5	1.5≥ & >1.0	1.0≥ & >0.5	0.5≥ & >0.0
3.0≥ & >2.5	0.4	1.1	1.5	1.7	1.7	1.8	1.8	1.8	1.9	2.0	2.1	2.3
2.5≥ & >2.0	0.3	1.1	1.5	1.6	1.7	1.7	1.8	1.8	1.9	2.0	2.1	2.2
2.0≥ & >1.5	0.3	1.1	1.5	1.6	1.7	1.7	1.7	1.7	1.8	1.9	2.0	2.1
1.5≥ & >1.0	0.3	1.0	1.5	1.6	1.6	1.6	1.6	1.7	1.7	1.8	1.9	2.0
1.0≥ & >0.5	0.3	1.0	1.5	1.6	1.6	1.5	1.5	1.5	1.6	1.7	1.7	1.8
0.5≥ & >0.0	0.3	1.0	1.5	1.6	1.5	1.4	1.3	1.4	1.5	1.5	1.6	1.7
0.0≥ & >−0.5	0.3	1.0	1.5	1.5	1.5	1.3	1.3	1.3	1.3	1.4	1.5	1.5
−0.5≥ & >−1.0	0.3	1.0	1.5	1.5	1.5	1.3	1.2	1.2	1.3	1.3	1.3	1.4
−1.0≥ & >−1.5	0.3	1.1	1.5	1.5	1.5	1.3	1.2	1.2	1.2	1.2	1.3	1.3
−1.5≥ & >−2.0	0.3	1.0	1.5	1.6	1.5	1.3	1.2	1.2	1.2	1.2	1.2	1.2

C. Values of score Z for fungicides and herbicides

Log HLW of fungicides	Log HLW of herbicide	Score Z
>1.59	>1.38	3.0
1.59≥ & >0.87	1.38≥ & >0.86	2.9
0.87≥ & >0.55	0.86≥ & >0.62	2.8
0.55≥ & >0.32	0.62≥ & >0.43	2.7
0.32≥ & >0.14	0.43≥ & >0.29	2.6
0.14≥ & >−0.02	0.29≥ & >0.16	2.5
−0.02≥ & >−0.15	0.16≥ & >0.05	2.4
−0.15≥ & >−0.27	0.05≥ & >−0.05	2.3
−0.27≥ & >−0.38	−0.05≥ & >−0.14	2.2
−0.38≥ & >−0.48	−0.14≥ & >−0.23	2.1
−0.48≥ & >−0.57	−0.23≥ & >−0.31	2.0
−0.57≥ & >−0.66	−0.31≥ & >−0.38	1.9
−0.66≥ & >−0.74	−0.38≥ & >−0.45	1.8
−0.74≥ & >−0.82	−0.45≥ & >−0.52	1.7
−0.82≥ & >−0.89	−0.52≥ & >−0.59	1.6
−0.89≥ & >−0.97	−0.59≥ & >−0.65	1.5
−0.97≥ & > −1.03	−0.65≥ & >−0.71	1.4
−1.03≥ & >−1.10	−0.71≥ & >−0.77	1.3
−1.10≥ &>−1.16	−0.77≥ & >−0.83	1.2
−1.16≥ & >−1.22	−0.83≥ & >−0.88	1.1
−1.22≥ & >−1.28	−0.88≥ & >−0.94	1.0
−1.28≥ & >−1.34	−0.94≥ & >−0.99	0.9
−1.34≥ & >−1.39	−0.99≥ & >−1.04	0.8
−1.39≥ &>−1.44	−1.04≥ & >−1.09	0.7
−1.44≥ & >−1.49	−1.09≥ & >−1.14	0.6
−1.49≥ & >−1.55	−1.14≥ & >−1.19	0.5
−1.55≥ & >−1.59	−1.19≥ & >−1.24	0.4
−1.59≥ & >−1.64	−1.24≥ & >−1.28	0.3
−1.64≥ & >−1.69	−1.28≥ & >−1.33	0.2
−1.69≥ & >−1.74	−1.33≥ & >−1.38	0.1
−1.74≥	−1.38≥	0.0

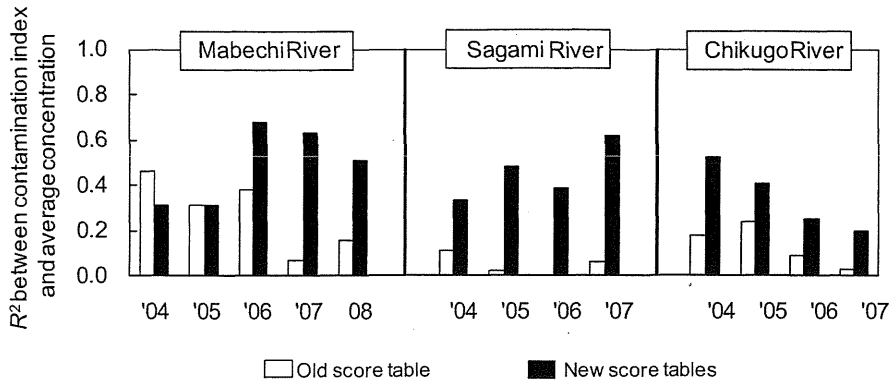

Fig. 2 – Comparison of the coefficients of determination (R^2) for plots of average pesticide concentrations versus contamination index values calculated with the new score tables and the old score table.

the new score tables (Score X was calculated from Eq. (2), and Scores Y and Z were obtained from Table 1) and the old score table (Scores A–C, Table 1S in the supplementary information). Fig. 1S in the supplementary information shows an example of the correlation between the contamination index values and average measured concentrations (sum of the measured concentrations divided by the number of water samples) of pesticides in the Chikugo River in 2004. Higher correlations were obtained with the new score tables than with the old score table. The coefficient of determination R^2 increased from 0.18 to 0.52 when the new score tables were applied (compare views A and B in Fig. 1S). However, the concentrations for any given contamination index value were scattered in a range around the logarithm of 2. The data scattering may have been due to the uncertainty for the application dates and application methods (including drift and adjuvant) for the pesticides; the acquisition of accurate application dates and the accurate evaluation for the effects of application methods were hard (Matsui et al., 2006a) and therefore these factors were not considered in the calculation of the contamination index. It may also be due to the accuracy of Score X values. In evaluating Score X value it is assumed that the quantity of pesticide applied was equal to the amount of the sales in that year. This assumption may, however, not be very accurate because all the purchased pesticides may not necessarily be applied in the agricultural field in a year after the purchase.

Fig. 2 summarizes the R^2 values for the plots of the average concentrations of pesticides against the contamination index values for the three rivers. Overall, the contamination index values calculated with the new score tables were better correlated with pesticide concentrations than the values calculated with the old score table, indicating that the new score tables are effective tools for preliminary ranking or prioritizing pesticides to be monitored.

3.2. *Use of the tables to select pesticides to be monitored*

3.2.1. *Estimating maximum pesticide concentration with the new score tables*

Fig. 3 shows the relationship between the highest observed pesticide concentrations and contamination index values. Since score is defined such that a score increase of 1 corresponds to a 10-fold increase in the pesticide concentration in river water, the relationship between highest observed concentration and contamination index, which is given by a sum of scores, is described, in the ideal situation, by the following equation:

$$\log C_H = \text{Contamination Index} - A \quad (6)$$

where C_H is the highest observed pesticide concentration (μg/L) and A is a constant. The mean A value was 11.6. The A value for the one-sided 95% upper confidence limit was 10.7. Therefore, C_U, the one-sided 95% upper confidence limit of the maximum pesticide concentration (μg/L), is predicted by the following equation:

$$\log C_U = \text{Contamination Index} - 10.7 \quad (7)$$

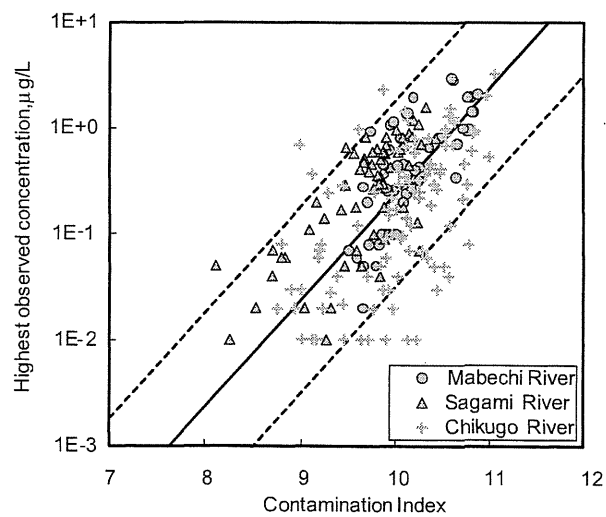

Fig. 3 – Relationship between the highest observed concentrations of pesticides and the contamination index values calculated with the new score tables. The solid line is a plot of the equation log C_H = Contamination index - 11.4. The dashed lines indicate one-sided 95% upper and lower confidence limits.

3.2.2. *Use of possible maximum concentration to select pesticides to be monitored*

JDWQG specifies that *DI* value, defined by Eq. (1), should not exceed 1. However, the calculation of *DI* value still has a potential problem that the value is heavily dependent on the number of monitored pesticides. In the current study, we predicted the maximum possible concentration of pesticide i, PV_i, in river water by using Eq. (7), and we then calculated PV_i/GV_i values. The PV_i/GV_i values were compared with the DV_i/GV_i values for the highest observed concentration.

Fig. 4 shows the DV_i/GV_i and PV_i/GV_i quotients of pesticides applied in the Sagami River basin in 2004. Because the quantification of pesticide concentrations above 1/100 of each GV_i value is recommended in evaluating *DI* value in JDWQG (MHLWJ, 2003b), a PV_i/GV_i quotient of 0.01 can be regarded as a threshold limit in selecting pesticides to be monitored and used for *DI* value calculation. That is, if the PV_i/GV_i value of pesticide i is less than 0.01, nonnecessity for monitoring that pesticide is suggested. By applying this threshold limit, we selected 16 pesticides from the 34 pesticides applied in the Sagami River basin for monitoring in the year 2004, as shown in Fig. 4. Among the selected pesticides, seven compounds DV_i/GV_i values exceeded the 0.01 threshold (iprobenfos, molinate, mefenacet, benthiocarb, esprocarb, simetryn, and bromobutide). However, other seven pesticides had DV_i/GV_i values of <0.01 although their PV_i/GV_i values were ≥0.01. This is understandable because PV_i is the maximum possible pesticide concentration predicted by the one-sided 95% upper confidence limit, and PV_i can be regarded as a conservative estimate. Two pesticides (diquat monohydrate and cafenstrole) were selected as pesticide candidates, but their concentrations were not actually measured.

The numbers of pesticide candidates for monitoring selected by this method are summarized in Table 2: 16–21 pesticide candidates among the 30–34 in the Mabechi River, 16–18 among the 31–34 in the Sagami River, and 22–25 among the 32–33 in the Chikugo River. The efficiency of the selection method was evaluated by comparison with pesticide monitoring data (columns E–H in Table 2). For all the data in the table, the groups of selected pesticides for which the PV_i/GV_i values were >0.01 included all the pesticide with DV_i/GV_i values of ≥0.01. There was no pesticide with a PV_i/GV_i value of <0.01 and a DV_i/GV_i value of ≥0.01. As shown in Column H of Table 2, 26–75% of the selected pesticides with PV_i/GV_i values of ≥0.01 had DV_i/GV_i values of ≥0.01 (the success rate in selecting pesticide candidates for monitoring was 26–75%). We feel these percentages constitute successful first efforts. It is notable that the pesticides with PV_i/GV_i values of <0.01 were all (100% in Column H of Table 2) with DV_i/GV_i values of <0.01 (the success rate in removing pesticides unnecessary for monitoring was 100%).

The three rivers we evaluated in the current study are currently monitored for pesticides selected on the basis of previous experience by the local water supply authorities rather than by means of our proposed method. However, for rivers where pesticides are not monitored or have no basis for determining the necessity of monitoring, our proposed method could save time and expense in identifying monitoring needs. The use of this methodology would also help determining the necessity of monitoring metabolites and degrades of pesticides if reaction pathway and kinetics of pesticide degradation are known and incorporated into the diffuse pollution hydrologic model.

A considerable number of water supply authorities in Japan monitor all or most of the 102 pesticides that are listed in Pesticide Group 1 of JDWQG without conducting any risk assessment to properly select the mentoring pesticides. Under such circumstances, our proposed new score tables and method could assist in the selection of pesticides to be monitored and the determination of which pesticides require no monitoring. No such decision could be reached by means of any other

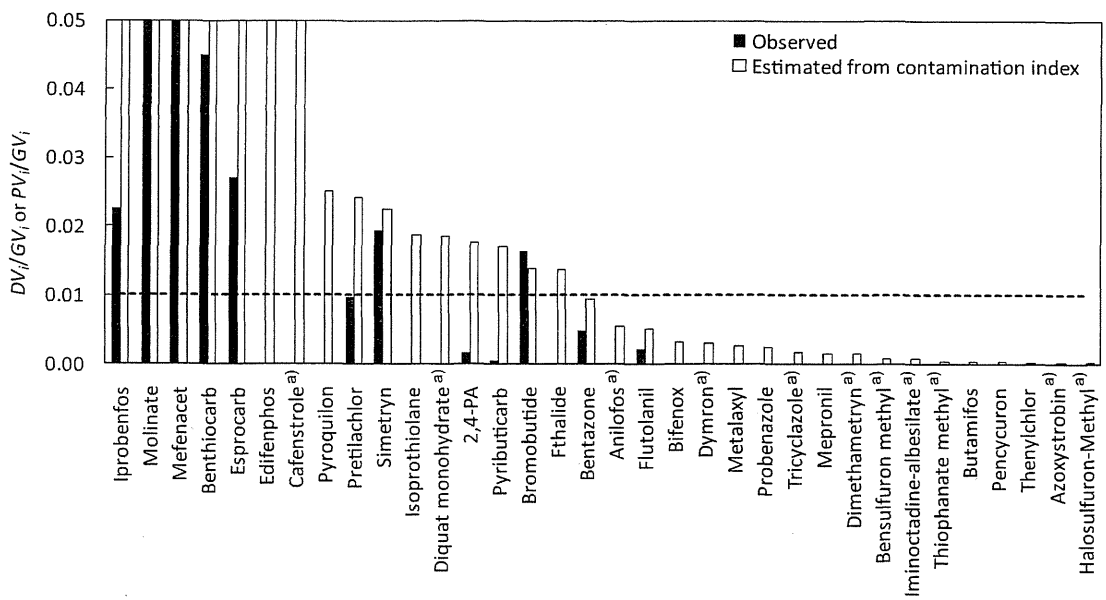

Fig. 4 – PV_i/GV_i (open bars) and DV_i/GV_i (solid bars) values for pesticides in the Sagami river basin in 2004. a) the concentrations were not measured (PV_i is the possible maximum predicted concentrations of pesticide i, GV_i is the reference concentration of pesticide i in JDWQG, and DV_i is the observed concentration of pesticide i).

Table 2 – Performance of new score tables for selecting pesticides (PV_i is the possible maximum predicted concentrations of pesticide i, GV_i is the reference concentration of pesticide i in JDWQG, and DV_i is the observed concentration of pesticide i).

Column A	Column B	Column C	Column D	Column E	Column F	Column G	Column H
	Year		Applied in rice-paddy	With measured concentration	With DVi/GVi < 0.01	With DVi/GVi ≥ 0.01	Success ratio (%)
Mabechi River	2004	Number of pesticides	34	18	13	5	
		With $PV_i/GV_i \geq 0.01$	21	14	9	5	36[a]
		With $PV_i/GV_i < 0.01$	13	4	4	0	100[b]
	2005	Number of pesticides	34	22	17	5	
		With $PV_i/GV_i \geq 0.01$	20	16	11	5	31[a]
		With $PV_i/GV_i < 0.01$	14	6	6	0	100[b]
	2006	Number of pesticides	33	18	12	6	
		With $PV_i/GV_i \geq 0.01$	19	14	8	6	43[a]
		With $PV_i/GV_i < 0.01$	14	4	4	0	100[b]
	2007	Number of pesticides	30	18	12	6	
		With $PV_i/GV_i \geq 0.01$	18	13	7	6	46[a]
		With $PV_i/GV_i < 0.01$	12	5	5	0	100[b]
	2008	Number of pesticides	30	18	9	9	
		With $PV_i/GV_i \geq 0.01$	16	12	3	9	75[a]
		With $PV_i/GV_i < 0.01$	14	6	6	0	100[b]
Sagami River	2004	Number of pesticides	34	23	16	7	
		With $PV_i/GV_i \geq 0.01$	16	14	7	7	50[a]
		With $PV_i/GV_i < 0.01$	18	9	9	0	100[b]
	2005	Number of pesticides	31	26	20	6	
		With $PV_i/GV_i \geq 0.01$	17	15	9	6	40[a]
		With $PV_i/GV_i < 0.01$	14	11	11	0	100[b]
	2006	Number of pesticides	32	30	23	7	
		With $PV_i/GV_i \geq 0.01$	18	17	10	7	41[a]
		With $PV_i/GV_i < 0.01$	14	13	13	0	100[b]
	2007	Number of pesticides	31	28	22	6	
		With $PV_i/GV_i \geq 0.01$	17	16	10	6	38[a]
		With $PV_i/GV_i < 0.01$	14	12	12	0	100[b]
Chikugo River	2004	Number of pesticides	32	23	16	7	
		With $PV_i/GV_i \geq 0.01$	23	20	13	7	35[a]
		With $PV_i/GV_i < 0.01$	9	3	3	0	100[b]
	2005	Number of pesticides	32	29	23	6	
		With $PV_i/GV_i \geq 0.01$	24	23	17	6	26[a]
		With $PV_i/GV_i < 0.01$	8	6	6	0	100[b]
	2006	Number of pesticides	33	29	23	6	
		With $PV_i/GV_i \geq 0.01$	22	21	15	6	29[a]
		With $PV_i/GV_i < 0.01$	11	8	8	0	100[b]
	2007	Number of pesticides	32	28	21	7	
		With $PV_i/GV_i \geq 0.01$	25	22	15	7	32[a]
		With $PV_i/GV_i < 0.01$	7	6	6	0	100[b]

a Number of pesticides with $DV_i/GV_i \geq 0.01$/number of pesticides with $PV_i/GV_i \geq 0.01$.
b Number of pesticides with $DV_i/GV_i < 0.01$/number of pesticides with $PV_i/GV_i < 0.01$.

ranking tool, such as the old score table, which prioritizes pesticides but does not have an absolute criterion for selection. Pesticide compounds with a similar property can be analyzed with the same multi-residue method. The monitoring costs thus do not simply depend on the mere number of compounds to analyze, but on the number of analytical methods to run and the individual costs of these methods. However, the analysis requires the standard solution and the accuracy control of analysis for each compound, and therefore the proper selection of monitoring pesticides has a strong merit.

4. Conclusions

1) New, improved score tables designed for selecting pesticides on the basis of their properties were prepared by restructuring and refinement based on sensitivity analyses conducted with a pesticide diffuse pollution model. The correlations between observed pesticide concentrations and contamination index values calculated with the new score tables were greatly improved over correlations obtained with the old score table.
2) Possible maximum concentrations of pesticides (PV_i) were estimated from the one-sided 95% upper confidence limit for the regression line for the contamination index. The number of pesticide candidates for monitoring selected on the basis of the threshold PV_i/GV_i quotient of 0.01 was roughly two-thirds of the number of pesticides applied. All the pesticides that actually detected in the river waters with DV_i/GV_i quotients larger than 0.01 were included in the list of selected pesticide candidates. The new score tables give contamination index values and then PV_i/GV_i values,

which can be expected to be useful criteria for determining whether or not a pesticide should be monitored on the basis of a threshold value of 0.01.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2011.11.036.

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Chlorine Demands of Amino Acids and Amino Sugars in Water

Gebhard B. LUILO*, Koji KOSAKA, Mari ASAMI****

**Department of Chemistry and Chemical Biology, University of New Mexico, MSC03 2060, 1-University of New Mexico, Albuquerque, NM 87131-0001, USA*

***Department of Environmental Health, National Institute of Public Health, 2-3-6 Minami, Wako, Saitama 351-0197, Japan*

ABSTRACT

Nitrogenous compounds are ubiquitous in drinking water sources and they increase the chlorine demand of water, shift the breaking point, and also cause strong odors. The present study was performed to investigate the chlorine demands of selected amino acids and amino sugars in water. The results indicated that the chlorine demands of the precursors were in the order: aromatic amino acids (except histidine) > S-amino acids > non-S-amino acids and amino sugars. Aromatic amino acids were expected to have the highest chlorine demand due to chlorine substitution in both the aliphatic and aromatic parts of these molecules. However, the chlorine demand of histidine was threefold lower than those reported previously, which may be attributed to the experimental conditions or shorter contact time used in this study. The chlorine demands of most compounds did not show marked differences at contact times of 15 - 96 h, although some of the nitrogenous organic compounds showed an increasing trend in chlorine demand with time when our 24 h study was compared with previous studies conducted at 72 and 96 h. Chlorine demand in 24 h showed a good correlation with predicted data. Kinetic studies are required to understand how fast the precursors can react with chlorine in typical water treatment contact times and chlorine doses. The information presented here will be useful in controlling disinfection byproducts.

Keywords: amino acids, amino sugars, chlorine demand, QSPR model

INTRODUCTION

Microbial contamination of water is not simply a problem of the past, and the morbidity and mortality rates associated with waterborne diseases are highly related to poverty or the lack of access to sanitary and safe potable water supply systems (Galal-Gorchev, 1996; Hermer, 1999; Ballester and Sunyer, 2000). Epidemiological data have shown that cases, outbreaks, and mortality rates of cholera are higher in developing countries than in developed countries (Swerdlow *et al.*, 1997; WHO, 1998; Andersson and Bohan, 2001; Lee *et al.*, 2002; Hutin *et al.*, 2003; Griffith *et al.*, 2006). Thus, potable water must be routinely disinfected using chlorine (chlorine gas and hypochlorites) or alternative disinfectants to eliminate pathogens (USEPA, 1999). While developed countries can afford both chlorine and alternative disinfectants, such as ozone or chlorine dioxide, chlorine is the only reliable, affordable, and convenient disinfectant in many countries (Galal-Gorchev, 1996; Chaidou *et al.*, 1999; WQHC, 2002). The advantages of chlorine over alternative disinfectants are that it has a wide biocidal spectrum, does not require high levels of technical skill to use, and has low investment and operation costs (Galal-Gorchev, 1996; PNL, 1998; USEPA, 1999; ACC 2008).

While chlorine has played a major role in reducing waterborne diseases worldwide (Galal-Gorchev, 1996; Lee *et al.*, 2002), it has posed a new challenge to potable water

Address correspondence to Mari Asami, Department of Environmental Health, National Institute of Public Health, Email: asami@niph.go.jp

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supply authorities because chlorine has high oxidizing power (Larson and Weber, 1994). It reacts with traces of different classes of naturally occurring and anthropogenic organic compounds, which are collectively called dissolved organic matter, to form chlorinated organic compounds (Crittenden *et al.*, 2005). This is because water treatment processes do not completely remove components of dissolved organic matter (Ribas *et al.*, 1991; Volk *et al.*, 2002; Volk *et al.*, 2005), and dissolved organic matter is a complex mixture of various natural and anthropogenic organic molecules of unknown structures (Clesceri *et al.*, 1998; Peuravuori and Pihlaja, 2007).

Nitrogenous organic compounds are one of the classes of dissolved organic matter present in water, and the most abundant forms are amino acids, amino sugars, nucleic acids, and proteins derived from natural and anthropogenic sources (NRC, 1987). Although water treatment can remove most nitrogenous organic compounds, it may still leave trace amounts of organic compounds at the point of chlorination (Pietsch *et al.*, 2001; Dotson and Westerhoff, 2009). For example, the reaction of chlorine with amino acids produces trihalomethanes and haloacetic acids (Hureiki *et al.*, 1994; Bull *et al.*, 2006; Hong *et al.*, 2009) as well as odorous chloroaldimines (Brosillon *et al.*, 2009). Although all nitrogenous organic compounds have reduced nitrogen (amine, amide, imine) with a lone pair of electrons in their structure, they are not equally reactive toward chlorine. The reactivity of organic compounds with chlorine at a given pH may be influenced by a number of factors, such as the number and oxidation states of amine groups, number and relative positions of OH and NH_2 in aromatic rings, and number of aliphatic sulfur atoms (Hureiki *et al.*, 1994; Bull *et al.*, 2006; Arnold *et al.*, 2008; Hong *et al.*, 2009; Luilo and Cabaniss, 2010). Some studies have indicated that activated aromatic amino acids had higher chlorine demands than sulfur-containing amino acids (hereafter denoted as S-amino acids), followed by non-sulfur-containing amino acids (hereafter denoted as non-S-amino acids) after an incubation period of 72 - 96 h at pH 7 - 8 (Hureiki *et al.*, 1994; Hong *et al.*, 2009). However, the typical potable water treatment contact time is much less than 72 h. Thus, the chlorine demands reported over such a long contact time scale represent those that occur both at the treatment plant and in the distribution system due to the reaction of residual chlorine with traces of amino acids. Another study investigated the chlorine demands of five amino acids with a contact time of 15 h at pH 7, and their results were comparable to those determined in other studies performed over longer time scales (de Laat *et al.*, 1982; Hureiki *et al.*, 1994; Hong *et al.*, 2009). These observations suggest that these amino acids are mostly depleted between 15 and 72 h. There are gaps in chlorine demand data for all amino acids and amino sugars at contact times of 15, 24 and 48 h. There is also a lack of kinetic data important for estimating chlorine dose sufficient to sustain chlorine residuals in distribution systems. As it is still difficult to deduce these figures from the literature due to the lack of consistency between studies, our experiments obtained data under consistent conditions to allow comparison, especially with regard to contact time.

This study was performed to determine the chlorine consumptions of amino acids and amino sugars as representative nitrogenous organic compounds in water with a contact time of 24 h. The results were compared to those obtained using other contact times to determine the impact of time on chlorine demands, to predict which amino acids are most likely to be depleted at water treatment plants and which will be depleted in storage tanks or distribution systems.

MATERIALS AND METHODS

Model compounds and reagents

Pure reagents, nitrogenous organic compounds, and ammonium chloride were used in this study for the bench-scale chlorination experiments. All reagents used were of analytical grade unless otherwise noted. The 20 amino acids were purchased from Wako Pure Chemicals (Osaka, Japan); three amino sugars and ammonium chloride were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water purified with Gradient A10 (Millipore, Bedford, MA, USA) was used for all experiments. Phosphate buffer was prepared with potassium dihydrogen phosphate and disodium hydrogen phosphate obtained from Wako Pure Chemicals (Osaka, Japan). Hypochlorite (10% - 15%) used as the chlorine source was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Chlorination and chlorine demand

The solutions of model nitrogenous organic compounds (5 μM) and ammonia (5 μM) in glass flasks were buffered at pH 7 using phosphate buffer solution (1 mM), and one buffer solution was used as a control. Each buffered solution was dosed with a small amount of hypochlorite solution while stirring using a magnetic stirrer. The control was subjected to the same reaction conditions except no nitrogenous organic compound or ammonia was present. The sample solutions were added to the flasks to minimize the headspace. After mixing for about 1 min, all flasks were placed in the dark at 20°C for 24 h without mixing. Chlorine residual was set at 1.0 ± 0.2 mg Cl_2/L after 24 h. To obtain the samples at 1.0 ± 0.2 mg Cl_2/L after 24 h, several flasks dosed with different amounts of chlorine were prepared for each compound. That is, chlorine doses were different among the nitrogenous organic compounds and ammonia solutions (i.e., 1.1 - 5.3 mg Cl_2/L). The chlorine residuals for control, nitrogenous organic compounds, and ammonia solutions at 24 h were determined using the standard *N*, *N*-diethyl-*p*-phenylenediamine (DPD) titration method (Eaton *et al.*, 2005).

Prediction of chlorine demand

Models are valuable tools for validating experimental data. Although there are many empirical models for predicting chlorine demand and disinfection byproduct formation in drinking water, there has been only one previous report of a Quantitative Structure-Property Relationship (QSPR) model (Luilo and Cabaniss, 2010). In this study, the QSPR was used as a model because the structure of a compound is the determining factor in chlorination reaction at optimal contact time, pH, and temperature. Here, the QSPR included eight constitutional descriptors mentioned below.

The QSPR model is summarized by Equation 1. This QSPR was calibrated using 159 compounds and was validated using 42 external data. The QSPR had coefficient of determination of calibration R_c^2 equal to 0.86 and a residual standard deviation (SDE) of 1.24 mol-Cl_2/mol-Cp (Luilo and Cabaniss 2010). The brief description of each descriptor in the model is described below.

$$Cl_2\ demand = 7.61 \times RAI + 1.16 \times ArOH + 3.00 \times ACN + 1.23 \times CI + 2.37 \times AS + 1.01 \times O:C + 0.49 \times ArORact - 0.72 \times ArORnact \quad \text{.... (Eq. 1)}$$

The ring activation index (RAI) is the ratio of the sum of strong aromatic ring activators

(NH_2 and OH) to the number of rings. Carbonyl index (CI) is motivated by the observation that carbonyl compounds undergo chlorine substitution reaction via keto-enol tautomerization and that β-dicarbonyl compounds (e.g., 3-oxopentanedioic acid), consume more chlorine than simple ketones (e.g., 2-propanone). The symbol ArOH is the number of phenolic groups in aromatic ring, AS is the number aliphatic sulphur, ACN is the number of aliphatic carbon bonded to amines, O : C is the ratio of atomic oxygen to carbon, ArORact is the number of ring-activating alkoxy groups in aromatic ring, i.e., alkoxy groups (weak ring activators) attached to aromatic rings that have no strong ring activators (NH_2 and OH) on them; and ArORnact is the number of non-activating groups in aromatic ring, i.e., alkoxy groups attached to aromatic rings that have strong ring activators (NH_2 and OH) on them. A value of zero was given to a molecule that did not have any one of the descriptors. The predictive power of the QSPR model for chlorine demand was evaluated using external validation data (any sets of data that were not used in calibration of the model). The q^2 (coefficient of determination of validation) can be calculated using Equation 3 below (Golbraikh and Tropsha, 2002; Tropsha *et al.*, 2003) and is calculated differently from R_c^2, which can easily be calculated manually using Equation 2. However, most statistical software can generate R_c^2 and residual SDE (how far each data point is from the best fitted line or model) when performing simple or multiple linear regression between response and one variable or a list variables.

$$R_c^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y})^2} \quad \text{..... (Eq. 2)}$$

where, y_i and $\hat{y}_i$ are experimental and predicted values respectively and $\bar{y}$ is the mean of experimental data in calibration dataset. On the other hand the q^2 for external validation, q^2_{ext}, was calculated using Equation 3 (Golbraikh and Tropsha, 2002; Tropsha *et al.*, 2003).

$$q^2_{ext} = 1 - \frac{\sum_{i=1}^{test} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{test} (y_i - \bar{y}_{tr})^2} \quad \text{..... (Eq. 3)}$$

where y_i and $\hat{y}_i$ are experimental and predicted Cl_2 demands (from external validation data) respectively; $\bar{y}_{tr}$ is the average of the experimental Cl_2 demands in the entire calibration data (Golbraikh and Tropsha, 2002; Tropsha *et al.*, 2003). Since the q^2 may be influenced by the outliers in training and external data sets (Eq. 2), Golbraikh and Tropsha (2002) recommended using slope, k and R^2 of regression line obtainable from the plot of predicted values against the experimental values (normal regression) or vice versa (reverse regression) in addition to q^2. The symbols, k_i and R_i^2 are slope and R^2, respectively, and are obtained from the regression plot of predicted Cl_2 demand and experimental Cl_2 demand with y-intercept. Whereas k_o and R_o^2 are slope and R^2, respectively, when the y-intercept in the regression in the same plot is set to zero (regression through origin). The essence of this test is that if the QSAR/QSPR model is close to perfection, k_i and k_o, and R_i^2 and R_o^2 should not be significantly different from

each other and both should be closer to 1. Thus, the QSAR/QSPR model is regarded to have high predictive power if it meets the following criteria: $R_c^2 > 0.6$, $q^2 > 0.5$; $(R_i^2 - R_o^2)/R_i^2 < 0.1$; $0.85 \leq k \leq 1.15$ (Golbraikh and Tropsha, 2002; Tropsha *et al.*, 2003).

The root mean square error (RMSE) and model bias deviation (MBD) are also useful in checking the predictive power of the model and were computed using Equations 4 and 5,

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}} \quad \text{..... (Eq. 4)}$$

$$MBD = \frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)}{\sum_{i=1}^{N} y_i} \times 100\% \quad \text{..... (Eq. 5)}$$

where y_i and $\hat{y}_i$ are experimental and predicted Cl_2 demand and N is the total number of observations. The RMSE of external validation is expected to be closer to the residual (SDE) of model calibration for a model with high predictive power. The MBD provides a qualitative diagnosis of the predictive power of the model. A MBD of zero indicates that the model has no prediction bias. However, a negative MBD indicates that the model predicts lower than the experimental value and positive MBD indicates that the model predicts higher than the experimental value. However, the magnitude of MBD does not necessarily indicate how many data were biased higher or lower than expected because one data point (compound) that is either over-predicted or under-predicted may drive total residuals (numerator in the Equation 5) up or down, respectively.

Therefore, in this work the eight descriptors represented in Equation 1 were calculated from the structure of each amino acid and amino sugar in accordance with the procedure described in the literature (Luilo and Cabaniss, 2010). A value of zero was given to any of the eight descriptors if a molecule lacked functional groups needed to calculate it. The descriptors for each molecule were substituted in the QSPR (Equation 1) to estimate the chlorine demand while the predictive power of the QSPR on the model compounds were determined in accordance with the methods described in literature (Golbraikh and Tropsha, 2002; Tropsha *et al.*, 2003).

RESULTS AND DISCUSSION

Chlorine demand of nitrogenous compounds

The chlorine demands (mg Cl_2/L) for 20 amino acids, 3 amino sugars, ammonia and ultrapure water (blank) were determined from the difference between chlorine dose and chlorine residual. The chlorine demand of each model compound was corrected by subtracting the chlorine demand of the blank (0.1 mg Cl_2/L). The net chlorine demand of each compound was further transformed into a unit of mole of chlorine per mole of compound (mol-Cl_2/mol-Cp) (Table 1). The results showed that tyrosine and tryptophan had chlorine demands of 10.99 mol-Cl_2/mol-Cp and 12.54 mol-Cl_2/mol-Cp, respectively, whereas that of histidine was 4.54 mol-Cl_2/mol-Cp.

Table 1 - Chlorine demands of amino acids and amino sugars.

Compounds	Cl_2 demand (mg/L)	Compound (μmol/L)	MW (g/mol)	Cl_2 demand (μmol/L)	Cl_2 demand (mol/mol)
Amino acid					
Glycine	1.65	5	75.07	23.24	4.65
Alanine	0.90	5	89.09	12.68	2.54
Valine	0.85	5	117.15	11.97	2.39
Isoleucine	0.90	5	131.17	12.68	2.54
Leucine	0.90	5	131.17	12.68	2.54
Serine	1.20	5	105.09	16.90	3.38
Threonine	1.40	5	76.12	19.72	3.94
Methionine	2.35	5	149.21	33.10	6.62
Cysteine	2.65	5	121.16	37.32	7.46
Aspartic acid	1.40	5	133.10	19.72	3.94
Glutamic acid	0.95	5	147.13	13.38	2.68
Lysine	1.60	5	146.19	22.54	4.51
Arginine	2.00	5	174.20	28.17	5.63
Histidine	1.50	5	155.15	21.13	4.23
Asparagine	2.05	5	132.12	28.87	5.77
Glutamine	1.30	5	146.14	18.31	3.66
Proline	1.20	5	115.13	16.90	3.38
Tryptophan	4.45	5	204.23	62.68	12.54
Phenylalanine	1.00	5	165.19	14.08	2.82
Tyrosine	3.90	5	181.19	54.93	10.99
Amino sugar					
Glucosamine	1.60	5	179.19	22.54	4.51
Galactosamine	1.89	5	179.19	26.62	5.32
Mannosamine	1.50	5	179.19	21.13	4.23
Ammonia	0.85	5	17.03	11.97	2.39

*Amino acids are listed from simple to complex.

The low chlorine demand for histidine determined in the present study relative to previous 72 h and 96 h studies may be attributable to the shorter contact time (Hureiki *et al.*, 1994; Hong *et al.*, 2009). However, the chlorine demand for tyrosine in the present study, 10.99 mol-Cl_2/mol-Cp, was similar to 11.40 mol-Cl_2/mol-Cp reported in a previous 15-h study (de Laat *et al.*, 1982). As these two data for tyrosine were not significantly different from each other despite the 9 h difference, it is likely that most of the tyrosine reacts in less than 15 h. However, contact time longer than 24 h may increase the chlorine demand of tyrosine. Thus, the chlorine demand of tyrosine in the present study was lower than 13.40 and 13.20 mol-Cl_2/mol-Cp reported previously (Hureiki *et al.*, 1994; Hong *et al.*, 2009). There were no significant differences in chlorine demands between 72 and 96 h, indicating that tyrosine is depleted in less than 72 h. Figure 1 shows a comparison of the chlorine demands in the present study with those reported in the literature.

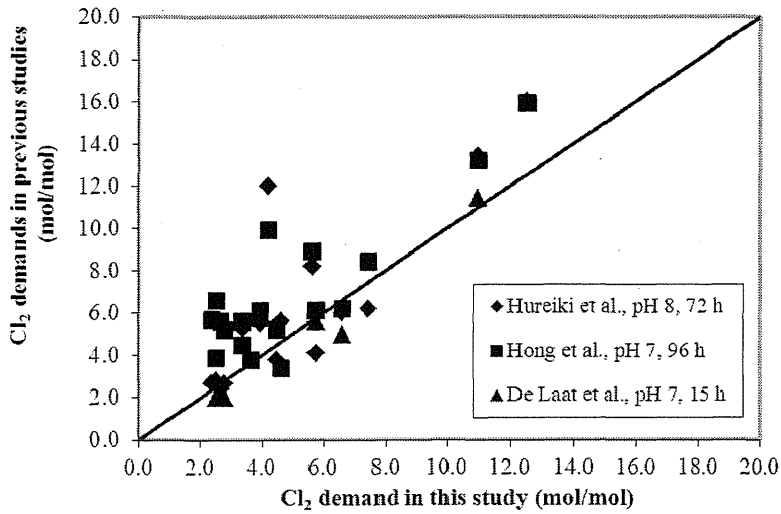

Fig. 1 - Chlorine demands in the present study and those reported in the literature.

The high chorine demand of aromatic amino acids is generally related to the structure because each has an aromatic ring with an alanyl substituent. The contribution of the alanyl substituent is expected to be around 3 mol-Cl_2/mol-Cp and the rest should be contributed by the aromatic ring through electrophilic substitution reaction. Tyrosine has a phenol group that activates the ring favoring electrophilic substitution reaction. The chlorine demand of the 4-hydroxyphenyl ring is expected to contribute about 9 mol-Cl_2/mol-Cp based on the results of previous studies for phenol or 4-hydroxytoluene (de Laat *et al.*, 1982; Gallard and von Gunten, 2002). Similarly, tryptophan is expected to undergo electrophilic substitution reaction in the aromatic heterocyclic indole moiety, and its contribution is expected to be around 10 - 13 mol-Cl_2/mol-Cp depending on the contact time (de Laat *et al.*, 1982; Hureiki *et al.*, 1994; Hong *et al.*, 2009).

The chlorine demand of histidine, which has imidazole and alanyl moieties, was 4.23 mol-Cl_2/mol-Cp. Our study also showed that alanine had a chlorine demand of 2.5 mol-Cl_2/mol-Cp. If the alanyl substituent in the histidine is taken into account, the imidazole moiety contributed only 1.3 mol-Cl_2/mol-Cp. This contribution is lower than expected from the electrophilic substitution in histidine, which has two endocyclic amines. Other studies indicated that the chlorine demands of histidine at 72 and 96 h were 12.0 and 9.9 mol-Cl_2/mol-Cp, respectively (de Laat *et al.*, 1982; Hureiki *et al.*, 1994; Hong *et al.*, 2009). However, it is difficult to clarify the reason why the 96-h study had lower chlorine demand than the 72 h study. If the chlorine demands of alanine at 72 and 96 h were taken into account in each study, the imidazole moiety is expected to contribute about 6 and 9 mol-Cl_2/mol-Cp, respectively. This suggests that there is either an error in our data or that histidine undergoes electrophilic substitution very slowly in the first 24 h as studies with longer contact times indicated higher chlorine demands than observed in our experiments (Hureiki *et al.*, 1994; Hong *et al.*, 2009).

S-amino acids had intermediate chlorine demands, with values of 7.46 and 6.62 mol-Cl_2/mol-Cp for cysteine and methionine, respectively. These results are not markedly different from those reported in previous studies performed with contact times

of 72 and 96 h (Hureiki *et al.*, 1994; Hong *et al.*, 2009). S-amino acids have two basic groups (sulfur and amine) at which chlorine substitution takes place. Aside from these two common amino acids, water may be contaminated with traces of cystine, thiamine, and biotin, all of which have sulfur in their structures. These contaminants and any other sulfur-containing organic molecules may contribute to higher chlorine demand in water treatment plants. Another group of amino acids with relatively high chlorine demand closer to those of the S-amino acids are arginine and asparagine. These amino acids possess amide and imine groups that can undergo chlorine substitution, and they have chlorine demands of 5.63 and 5.77 mol-Cl_2/mol-Cp, respectively. However, this study showed that chlorine demand for arginine was about 3 units lower than those reported in previous studies with longer reaction times (Hureiki *et al.*, 1994; Hong *et al.*, 2009). It was found from the structure that there are 3 amines and 1 imine groups in arginine. However, 2 amines and 1 imine that are connected to the carbon-6 may not be as basic as the α-amine group due to resonance stabilization in the former. Thus, the reactions of these groups in arginine (or its byproducts) with chlorine is slow and it may most likely be incomplete in the 24 h contact time used in this study. On the other hand, the chlorine demand of asparagine was comparable to or higher than those described in previous studies (Hureiki *et al.*, 1994; Hong *et al.*, 2009). Similarly, the chlorine demand of glutamine was similar to those in previous studies. Thus, the reactions of these compounds or their byproducts with chlorine were completed in less than 24 h.

The chlorine demand of asparagine was larger than that of glutamine. Although glutamine has an amide group, similar to asparagine, the two are structurally different since asparagine has one methylene bridging amine and amide groups, while glutamine has an ethylene group bridging the amine and amides. The difference in their chlorine demands was considered to be due to the difference in the methylene and ethylene groups. It should be noted that the pKa of the methylene group is lower than that of the ethylene group, but the difference is small.

Six amino acids have chlorine demands ranging between 3 and 4, i.e., proline, serine, threonine, lysine, glycine, and aspartic acid (Table 1). These values were generally lower than those reported in previous studies conducted with longer contact times (Hureiki *et al.*, 1994; Hong *et al.*, 2009). This suggests that these amino acids may continue reacting even in distribution systems, because it is expected that treated water may still be in the distribution system after 24 h. While glutamic acid showed a chlorine demand of 2.68 mol-Cl_2/mol-Cp, both leucine and isoleucine, which isomers, showed the same chlorine demand of 2.54 mol-Cl_2/mol-Cp (Table 1). These results for the latter are similar to those of the 72 h study (Hureiki *et al.*, 1994) but lower by about 3 units compared to the 96-h study (Hong *et al.*, 2009).

Phenylalanine and alanine consumed 2.82 and 2.54 mol-Cl_2/mol-Cp, respectively, and the results are not significantly different from the previous 15 h and 72 h studies (de Laat *et al.*, 1982; Hureiki *et al.*, 1994). However, Hong *et al.* (2009) reported chlorine demands of 3.90 and 5.2 mol-Cl_2/mol-Cp for alanine and phenylalanine, respectively, which are higher than those in our experiments and in the other two studies mentioned above. The chlorine demands were increased by 1 and 2 units from those in the 15 h and 72 h studies, respectively. These observations indicated that alanine and phenylalanine would have reacted with chlorine if the contact time was increased to 96 h. Although

phenylalanine is an aromatic amino acid, it is not as reactive as tyrosine. The difference is that phenylalanine lacks strong ring-activating substituents, such as OH and NH_2. Therefore, electrophilic substitution may occur very slowly in the phenyl ring, which is why the chlorine demand of phenylalanine is expected to be slightly higher than that of alanine and less than that of tyrosine (Hong *et al.*, 2009).

Amino sugars are nitrogenous organic compounds that have amine groups, and can react with chlorine similar to amino acids. In this study, glucosamine, galactosamine, and mannosamine were tested for chlorine consumption under the same conditions as those used for the amino acids. The results indicated that the chlorine demands were very close to each other and were also similar to those of some amino acids despite the differences in their structures (Table 1). These results also indicated that high levels of amino sugars in water sources may contribute significantly to the total chlorine demand of water. Finally, all nitrogenous compounds may contain amine, amide, or imine groups in their structures. In the present study, ammonia was used as the simplest nitrogenous compound in water. Chloramines are formed after the reaction of ammonia and chlorine. In the case of the nitrogenous organic compounds used in the present study, *N*-chloramines were considered to be formed by chlorination. This was supported by the frontier electron density (FED) calculation, which showed that the nitrogen in the amine group had the highest electron density of all atoms in alanine (Chu *et al.*, 2009). Thus, it was considered that the amine group was an important site for chlorine substitution in nitrogenous compounds, as discussed previously (Bull *et al.*, 2006). However, chlorination contact time at water treatment plants and distribution systems may not be sufficient to allow complete reaction with some compounds in water. Although there have been few reports of amino acids in tap water, an unpleasant smell in tap water indicates the formation of odorous compounds (e.g., chloroaldimines). Thus, the presence of such odorous compounds was considered to indicate the importance of determining the chlorine demand under the conditions used for chlorination in actual distribution systems.

Prediction of chlorine demand

Further analysis of the data reported in this work has shown that there is a weak linear relationship (R^2 = 0.265, F = 7.93, P = 0.01, N = 24) between the molecular weight (MW) and chlorine demand at 95% confidence level (Fig. 2). The weak relationship may be attributed to the fact that only certain sites of the molecule (functional groups) are involved in chemical reaction with chlorine and not the whole molecule.

Thus, the number of functional groups and relative position in the molecule are highly important in chemical reaction. That is why two molecules with different molecular weights may have the same chlorine demands as shown by the following pairs of molecules: lysine vs. glucosamine, alanine vs. leucine or serine vs. proline (Table 1). Although the MW of tyrosine differs from that of amino sugars by 2 units, its chlorine demand was 2 times higher than that of amino sugars (Table 1). Thus, models based on structure of molecules may be more useful for predicting chlorine demands than those models that were derived using bulk water parameters (e.g., pH, turbidity, UV-absorption, temperature) or molecular properties (e.g., MW, molecular volume, molecular surface area).