Quantification of *umu* genotoxicity level of urban river water

T. Kameya, T. Nagato, K. Nakagawa, D. Yamashita, T. Kobayashi and K. Fujie

**ABSTRACT**

In recent years, the request of environmental safety management for carcinogenic substances, mutagenic substances and/or reproductive toxicity substances (CMR) has increased. This study focused on clarifying the genotoxicity level of environmental water and its release source by using the *umu* test provided in ISO13829. Although a genotoxicity index “induction ratio (IR)” is used in ISO13829, we normalised it to make it possible to compare various environmental water quantitatively to each other as a new index “genotoxic activity (GA = (IR-1)/Dose)”. Sample water was collected and concentrated to 100 times or 1,000 times by a solid phase extraction method. As the test results, it was found that GA level in actual river water varied widely from less than the determination limit of 23 [1/L] to 1,100 [1/L] by quantitative comparison, and the value was also equivalent to more than 50 times the level of tap water. The GA level of household wastewater was not so high, but the levels of treated water from wastewater treatment plant (WTP) were from 220 [1/L] to 3,200 [1/L]. Raw sewage of some WTP shows high level genotoxicity. A part of genotoxicity substances, for example 50%, could be removed by conventional wastewater treatment, but it was not enough to reduce the water environmental load of genotoxicity.

**Key words** | CMR, genotoxicity, solid phase extraction, *umu* test, water quality

**INTRODUCTION**

In recent years, several new schemes of hazardous chemicals management, such as the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (United Nations 2003) and the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (European Chemicals Agency: ECHA 2007), have entered into force. In each scheme, genotoxic substances have been focused as substances with high concern. In Japan, 218 carcinogenic substances, mutagenic substances and/or reproductive toxicity substances (CMR) are required to conform to the Pollutant Release and Transfer Register (PRTR) and/or the Material Safety Data Sheet (MSDS) systems. However, little water environmental monitoring of CMR substances has been done, and it may cause the delay of understanding about environmental pollution and of safety management for them. Therefore, it is considered that some effective tools of water environmental monitoring and the monitoring data storage for CMR substances are necessary.

The *umu* test, which was used for the detection of mutagens and carcinogens, was modified and established as a new evaluation system of environmental quality of water by Oda *et al*. 1985. The important advantages of the *umu* test are the detection of synergistic effects, compounds the genotoxicity of which is activated in vivo, and unknown genotoxicity compounds especially in environment without individual identification. The test method is based on the SOS-inducing activity. “SOS” in the context concerned refers to a warning of the presence of genotoxicity substances as detected by an elevated rate of genotoxicity in genetically modified tester strains of *Salmonella typhimurium* TA1535/pSK1002. Then, the β-galactosidase inducing activity is measured by the colorimetry method using o-nitrophenyl-β-D-galactopyranoside (ONPG).
They also developed a microplate method of the umu test for rapid detection of genotoxins and genotoxic potentials of environmental samples (Reifferscheid et al. 1991). This method was accepted to the international standard method as the ISO 13829 “Water quality – Determination of the genotoxicity of water and wastewater using the umu-test” in 2000 (International Organization for Standardization (ISO) 2000). Using and/or applying this method, the SOS-inducing activity of many chemicals and environmental genotoxicity potentials of many environmental water samples were investigated (Nakamura et al. 1987; Ono et al. 1992, 1996, 2000; Reifferscheid & Heil 1996; Vahl et al. 1997; Dizer et al. 2002; Shen et al. 2003; Yasunaga et al. 2004; Crebelli et al. 2005; Nakajima et al. 2006; Ito et al. 2008).

For the determination of SOS/umu-inducing activity of environmental water sample, there are two technical challenges. One is the low concentration in water samples, because of very low concentration of genotoxic substances in environmental waters. For this reason, some concentration extractions had been done, such as Soxhlet extraction with toluene and methanol (Vahl et al. 1997), solid phase extraction with XAD-7 resin (Reifferscheid et al. 1991), Sep-Pak® C18 resin (Ono et al. 1996; Crebelli et al. 2005), Blue-rayon (Ono et al. 2000), XAD-2 resin (Shen et al. 2003), Sep-Pak® Plus PS-2 resin (Ito et al. 2008). In these methods using the hydrophobic adsorption resins, only hydrophobic substances were concentrated and extracted, and the genotoxicity tests were done. Here, it was thought that hydrophilic substances such as metal compounds could be evaluated by another method such as inductively-coupled plasma mass spectrometry (ICP-MS). On the other hand, hydrophobic substances that can be concentrated are different based on each adsorption resin type. Ishii et al. (2000) investigated the applicable condition of the solid phase extraction (SPE) with adsorption resin PS-2 cartridge, which was used in this study, for 44 kinds of chemicals, and showed the effects of water solubility and chemical concentration. According to these results, the relation among concentration available water volume \( v_c \) [L/cartridge], water solubility \( S_w \) [mg/L] and chemical concentration \( C \) [g/L] was shown as the equation (1). By this method, various compounds such as water solubility from 0.6 mg/L to 408,000 mg/L except hydrophilic weak acids could be applied to the SPE.

\[
v_c = 1.3 \times 10^3 \times S_w^{-0.7} C^{-0.2}
\]

The other one is the test condition of dilution or concentration factor of water samples. We know that it is often difficult to obtain the quantitative relation in a bioassay, but some good dose-responses are also obtained for genotoxicity substances (Oda et al. 1985; Yasunaga et al. 2004; Nakajima et al. 2006) and environmental water samples (Ono et al. 1996, 2000; Ito et al. 2008). On that basis, normalisation of dilution or concentration factor is important to compare the level of water quality of each sample.

Furthermore, we understand that identification of genotoxic substances in environmental water is also very difficult and is a challenging issue in future research. It is also correct that genotoxicity detected by the umu test on different water environments may be caused by different compounds. However, umu genotoxicity is an overall index to evaluate water environment and/or water treatment, like biochemical oxygen demand (BOD) that was measured with a specific condition.

Based on this above background, the aim of this study is, at first, the quantification of umu genotoxicity level of urban river water through a concentration procedure and a normalisation of genotoxicity index. Sample water was pretreated by a solid phase extraction using adsorption resin Sep-Pak® Plus PS-2, and the quantitative dose-response was investigated for all water samples. The genotoxicity level per unit volume of sample water was evaluated as a new index that did not depend on sample dose. In addition, this study aimed to measure and discuss genotoxicity level of various sources from which genotoxic substances were discharged.

**METHODS**

**Sample water**

River water samples were collected at 23 points; those were the official water environment monitoring points in Kanagawa Prefecture, Japan. In addition, sample waters were also collected from public wastewater treatment plants (public WTPs) and household wastewater treatment plants (household WTPs). In Japan, the public WTP accepts both household sewage and industrial sewage. Household WTPs (called Jyokaso) are used in a local community area, and accept just about household sewage such as night soil and grey water. From public WTPs, 19 samples of effluent water were collected, and six samples of process waters, in which four influent waters were included, were also collected. Each three samples of raw waters and treated waters were collected from household wastewater treatment plants, respectively.

**Preparation of test sample**

All sample waters were, at first, filtrated by using a 1μm-glass fibre filter, and then concentrated by a method of solid phase...
Determination of genotoxicity level of various environmental water samples

In the ISO 13829 method, the SOS-inducing activity is determined at each test concentration (or dilution level) using the inducing ratio (IR). In this study, a new index “genotoxic activity (GA)” was used to determine the genotoxicity level of various environmental waters. Because GA is a value for each unit volume of the sample water, the quantitative comparison of genotoxicity level of different waters becomes possible to each other.

\[
GA \, (1/L) = \frac{IR - 1}{Dose \, volume \, of \, sample \, water \, (L)} \quad (2)
\]

\[
IR = \frac{Cf}{Gf} \quad (3)
\]

\[
Cf = \frac{A_{415,T} - A_{415,B}}{A_{415,S} - A_{415,B}} \quad (4)
\]

\[
Gf = \frac{A_{595,T} - A_{595,B}}{A_{595,S} - A_{595,B}} \quad (5)
\]
RESULTS AND DISCUSSION

Genotoxic activity level and distribution of urban river water

The sampling sites of urban rivers in this study are shown in Figure 3. Sample waters were collected from 23 monitoring sites of seven river basins. The results of the *umu* tests for the water samples are shown in Figure 4. The tests had been done three times for each site in two years. It was found that the levels of GA were from less than 23 [1/L], which was the quantification limit, to 1,100 [1/L], and that there were large differences among the sites. These values of GA were significantly larger than the values of 20 [1/L] for tap water (Kubo et al. 2006). Especially, larger GA value, for example more than 500, was shown from the river water of east region, which is a heavily populated area, and an artificial influence of concern may be indicated. A large amount of treated water from public WTPs has been discharged to the rivers in which the high level of GA was observed. In these rivers, the GA values at the monitoring points of downstream of public WTPs were all higher. It is necessary to research the reasons, because various genotoxic substances may flow into public WTPs in Japan. Here, the fact that there was large difference of genotoxicity level in urban rivers is a novel new finding, and this is the first report of the comparable quantification of genotoxicity level in Japan, although regional variation, seasonal variation, statistical significance and so on of the results are not enough. Besides, hydrophobic genotoxic substances which could be concentrated by using the hydrophobic adsorption resin PS-2 were detected in this study. But the indication of them was very hard at this time, although there are data on what known potential carcinogens are detected by the *umu* test, and what known potential carcinogens are not detected by the *umu* test (Oda et al. 1985; Nakamura et al. 1987; Ono et al. 1992; Reifferscheid & Heil 1996; Yasunaga et al. 2004). Therefore, the determination of risk level from the difference of GA level is now an impossible issue, because the study on toxicity...
strength and effect level is not established. However, the difference of GA level means the difference of the control level of hydrophobic genotoxic substances in water environment, and the reduction of GA is necessary as well as BOD.

Genotoxic activity level of various sources and the comparison with river water

The genotoxic activity (GA) levels of the treated effluent waters from household WTPs are shown in Figure 5. The tests had been done two times for each plant effluent. The level of GA for effluent water from household WTP ranged from 150 [1/L] to 710 [1/L]. Those values were not so high compared with those from public WTP and were equivalent to the GA value of small public WTP, but were significantly larger than the GA values of 160 [1/L] (mean value) of the river water in Kanagawa Prefecture.

The GA level of the treated effluent water which was discharged from public WTPs into the river in Kanagawa Prefecture was also shown in Figure 5. The tests had been done three times for each plant effluent in three years. There was observed the wide range distribution of the GA value from 85 [1/L] up to 3,200 [1/L] among each plant mainly in the east region of Kanagawa Prefecture. On the other hand, the value of GA less than a quantitative limit of 220 [1/L] was also observed in some plants. In addition, the GA values of effluent water from the small public WTP were relatively lower. It seemed that there were a lot of influents of household sewage and a few influents of industrial sewage in small public WTPs, while industrial wastewater was often accepted by public sewerage system in Japan. Genotoxic substances may be included in some specific industrial sewage.

From these results, we are not surprised that the level of the genotoxicity of public wastewater is higher than that of household wastewater. However, it was confirmed as a new finding that the contribution by public WTPs and household WTPs was not enough to remove genotoxic substances from sewage, and it was also considered that some WTPs had a potential to become a main emission source of genotoxic substances in urban river water environment.

Genotoxic activity level in WTPs and its change

In six public WTPs and three household WTPs, influent waters were collected and tested as well as effluent water. The *umu* test results are shown in Figure 6. From the results, at first, it was confirmed that the GA levels of influent waters in public WTPs were much higher, from 4,000 [1/L] up to 11,000 [1/L], than the GA levels of river water. On the other hand, the GA levels of influent waters in household WTPs were about 2,000 [1/L], but those were also higher than the GA levels of river water. Therefore, it was confirmed that genotoxic substances were also included in household sewage (night soil and grey water), although industrial sewage includes the higher GA level of genotoxic substances.

By the way, it seemed that the removal ratios of genotoxic activity were about 50% or more in the WTPs, but it was not enough to reduce genotoxicity to the levels in river water not exposed to effluents from wastewater treatment plants. In general, public and household WTPs are optimally designed and operated to remove BOD, suspended solid, nitrogen and phosphate mainly. However, unfortunately, the genotoxic activity levels of effluent water from WTPs were sometimes high, shown in Figure 5. Namely, it is considered that there is a limitation of genotoxic activity removal for the conventional WTPs, because of the higher genotoxic activity level of influents. It was confirmed that reduction of genotoxic substances at each origin source was very important.
CONCLUSIONS

In this study, we focused on clarifying the genotoxicity level of environmental water by using the umu test. In addition, we focused on release sources of genotoxicity to water environment. At first, hydrophobic fractions in water samples were concentrated by using the adsorption resin and tested by the umu test without S9 activation. Although the index of the “induction ratio (IR)” is used to evaluate the genotoxicity in the ISO13829 method, we normalised it to a new index of the genotoxic activity (GA = (IR-1)/Dose volume of water sample) to make it possible to compare various environmental waters quantitatively to each other. As the results, it was found that GA level in actual river waters varied widely from less than the determination limit of 23 [1/L] to 1,100 [1/L]. The value is equivalent to more than 50 times the level of tap water. The GA level of household wastewater was not so high. But the GA levels of treated water from wastewater treatment plant (WTP) were from 220 [1/L] to 3,200 [1/L]. Raw sewage of some WTP shows high level genotoxicity. A part of genotoxic substances, for example 50%, could be removed by conventional wastewater treatment, but it was not enough to reduce the water environmental load of genotoxicity. Therefore, some WTPs may become one of the significant releasing sources of genotoxicity for water environment, and it is considered that the reduction of genotoxicity at each origin source is very important.

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