Original Article

Sewage Effluent Toxicity Assessment: Comparison between Whole Effluent Toxicity (WET) and the Japanese Pollutant Release and Transfer Register (PRTR) Data

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Sewage effluent plays an important role in regional water cycles and is gaining attention. Because a wide variety of chemicals are used in industry and daily life, the environmental impacts of trace chemicals in sewage effluents are a source of concern. Whole effluent toxicity (WET) testing uses biological responses to directly evaluate the toxicity of chemical substances in effluent. In contrast, the Japanese Pollutant Release and Transfer Register (PRTR) system provides estimated amounts of potentially hazardous chemicals released in effluent from factories and sewage treatment plants. The objectives of this study were to evaluate the ecological impacts of sewage effluent using WET tests and PRTR data and to compare the results from the two approaches. Of seven effluent samples tested, four demonstrated toxicities to aquatic organisms. Water analyses suggest that residual chlorine is responsible for toxicity to algae, while linear alkyl benzene sulfonates do not contribute to toxicity. The PRTR data tended to overestimate toxicity in comparison to the observed toxicity. The causes of this discrepancy are discussed, such as insufficient data. Due to the difference in calculation method, PRTR data of sewerage tends to be overestimated.

Keywords: sewage effluent, whole effluent toxicity (WET), ecotoxicological effects, residual chlorine, PRTR system

INTRODUCTION

Sewage effluent plays an important role in regional water cycles and is gaining attention [1,2]. Because a wide variety of chemicals are used in industry and daily life, the environmental impacts of trace chemicals in sewage effluents are a source of concern. In Japan, current effluent standards aim to protect human health and the environments. As for the protection of the aquatic organisms, the only effluent standard for zinc (or discharge limits) has been established so far. However, one standard item is not enough to protect aquatic life from the stresses posed by many chemical substances present in water environment. Therefore, it is necessary to develop a more effective and dependable management system for effluent chemicals. The whole effluent toxicity (WET) approach, which was established in the USA and Canada and

uses biological responses to evaluate the comprehensive effect of chemicals in effluent, overcomes the shortcomings of traditional effluent regulation based on individual chemical compounds [3]. The introduction of this approach has been discussed since 2009 in Japan, and a draft of test guidelines was released in March 2013. However, there have been few reports regarding WET results for sewage effluent in Japan [4–7]. In contrast, the Japanese Pollutant Release and Transfer Register (PRTR) system promotes voluntary improvement of chemical management by business operators, who notify the government of the amounts of specified chemical substances released into the environment [8]. The PRTR law applies only to companies with more than 20 workers that handle certain chemicals over a specified amount; smaller companies aren't required to submit data even if they handle large amounts of toxic chemicals [6]. Previous research has

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Fig. 1 Research schematic showing how the ecotoxicity of final effluents from studied sewage treatment plants was determined. PRTR: Pollutant Release and Transfer Register; WET: whole effluent toxicity

shown that PRTR data are useful for effluent toxicity estimation in industrial effluents for screening purposes [9].

Previous studies have suggested that the ecological risk of sewage might be assessed via PRTR substances and pharmaceuticals [10]. However, direct relationships between toxicity and chemical substances were not demonstrated for the final effluent from specific treatment plants, as the study used only national average values. If the final effluent ecotoxicity can be predicted using PRTR data, this method could be useful for screening purposes. However, the toxic effects of specific chemicals not included in the PRTR list, such as residual chlorine and detergents, must not be ignored.

The overall objective of this study was to assess the ecotoxicity of the final effluent from seven sewage treatment plants using short-term chronic tests for three types of aquatic organisms. The ecotoxicity of each effluent was also predicted based on PRTR data and a toxicity database. Finally, the results of both approaches were compared (Fig. 1).

MATERIALS AND METHODS

Sampling and preparation of sewage effluent

During November 2013 to December 2014, seven final effluent samples were collected from seven sewage treatment plants located in Toyama, Japan (Table 1). The sewage treatment methods consisted of conventional activated sludge processes at five plants and oxidation ditches at the remaining two plants. At one treatment plant (A), which has two discharge outlets, grab samples of each effluent were mixed in proportion to discharge in order to prepare a composite sample. At the other six plants, single grab samples were collected. The samples (3.8 L) were immediately transported to the laboratory in amber glass bottles in a lightproof ice box, then filtered through a 60 µm nylon strainer, and stored in a dark refrigerator (4) until analysis and bioassay; biological tests were conducted within 36 h of sampling.

Table 1 Sewage treatment plants used in this study.

Plant	Process	Sewage collection	Daily average flow (m ³ /d)	Industrial wastewater (%)	Disinfectant
A	AS	Separate	53,000	3	Sodium hypochlorite
B	AS	Separate	71,000	27	Sodium hypochlorite
C	AS	Combined	100,000	17	Sodium hypochlorite
D	AS	Combined	50,000	62	Sodium hypochlorite
E	AS	Separate	5,000	4	Sodium hypochlorite
F	POD	Separate	350	0	Iso cyanoric ester
G	OD	Separate	13,000	12	Sodium hypochlorite

AS: activated sludge system, POD: prefabricated oxidation ditch, OD: oxidation ditch

Analysis of water parameters

Water temperature, pH, electrical conductivity, and dissolved oxygen (DO) were recorded at each sampling site using a pH-DO meter (pH/COND meter D-54, Horiba, Kyoto, Japan) and DO meter (ProODO, YSI Inc., Yellow Springs, USA). As soon as the samples arrived at the laboratory, total hardness was measured according to the standard method [11]; EDTA was used as the titrant to complex Mg²⁺ and Ca²⁺ ions. Total and free residual chlorine were measured using a commercially available test kit based on the diethyl-*p*-phenylenediamine method (DR2800, Hach Co., Loveland, USA). Molybdenum blue absorption [12] was used to measure total phosphorus after decomposition by potassium peroxodisulfate. Total nitrogen was determined via UV spectrophotometry [12] after decomposition by alkaline potassium persulfate. The multi N/C2100S meter (Analytik Jena AG, Jena, Germany) was used to measure total organic carbon (TOC). The total concentration of metals was detected using inductively coupled plasma mass spectrometry (ICP-MS) [13]. Briefly, 50 mL of each filtered sample was placed in a polypropylene tube (DigPREP; SCP SCIENCE, Quebec, Canada) with 5 mL concentrated nitric acid (EL grade, Kanto Chemicals, Tokyo, Japan), digested on a hot plate, and adjusted to 50 mL using ultra-pure water (Milli-Q) before ICP-MS analysis on an Agilent 7700e spectrophotometer (Agilent Technologies, Palo Alto, USA). For the measurement of linear alkylbenzene sulfonate (LAS), samples for LAS measurement were analyzed by high performance liquid chromatography (HPLC) using fluorescence detection in accordance with the 2000 Ministry of the Environment Ministry Survey Item Survey Manual [14]. After conditioning using a column of Sep-pak Plus C₁₈, the column was attached with a concentrator (AQUA, Loader III SPL 798C, GL Sciences Inc., Tokyo, Japan), and 1 L of sample was flowed through. After dehydrating the column through

air by use of a glass syringe, it was attached to a manifold and extracted by flowing 5 mL of methanol. The extract was placed in a glass tube and refrigerated. After extracting all the samples, the extract was evaporated to dryness with nitrogen gas (Turbo Vap II, Zymark Corp., Hopkinton, USA). After drying and solidification, the volume was adjusted to 2 mL with acetonitrile / water (65:35) to prepare a test solution. The test solution was separated by high performance liquid chromatography (CLASS-VP, Shimadzu, Kyoto, Japan) and quantified with the fluorescence detector. The LAS standard (C₁₂H₂₅C₆H₄SO₃Na for ABS Analysis, FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) was used as standard.

Whole effluent toxicity tests

In this study, the three short-term chronic biological tests proposed in the Japanese WET testing guidelines draft [15] were performed. Final effluent samples were serially diluted (0, 5, 10, 20, 40, and 80%) to a range of concentrations. The green microalga *Raphidocelis subcapitata* (NIES-35) was used for algal growth inhibition tests as per Organisation for Economic Co-operation and Development (OECD) testing guideline TG201 with downgraded minor modifications [16]. Precultured algae were inoculated at 5×10^3 cell/mL in an Erlenmeyer flask (200 mL capacity) and exposed to a range of effluent concentrations prepared with an OECD medium (60 mL). Six replicates were used for the control and three replicates were prepared for each effluent concentration. The algal cell density was determined after 0, 24, 48, and 72 h with an electronic particle counter (detection range 3–12 μ m; CDA-500, Sysmex, Kobe, Japan) in a 72 h growth test. The median effective concentration (EC₅₀) and no observed effect concentration (NOEC) values were determined for the growth rate using analytical software (Ecotox-Statics ver. 2.6, Japanese Society of Environmental Toxicology, Tsukuba, Japan).

Crustacean reproduction tests, which consisted of three-brood renewal toxicity tests, were conducted with *Ceriodaphnia dubia* according to the Environment Canada method [17]. First, neonate daphnia (< 24 h old) were transferred to glass vessels containing 15 mL dilute effluent. Each dilute effluent required appropriate daily volumes of food, which consisted of yeast, Cerophyll, and trout chow (Recenttec K. K., Tokyo, Japan) and algae (*Chlorella*, Recenttec K. K.). Each test solution was renewed three times per week, and 10 replicates were performed per effluent concentration. EC₅₀ and NOEC values were determined for fecundity using analytical software (Ecotox-Statics ver. 2.6).

Short-term toxicity tests were performed on *Danio rerio* fish embryo and sac-fry stages according to OECD TG212 [18]. Briefly, 10 fertilized eggs (< 4 h) were placed into a glass vessel containing 50 mL test solution. Each dilute effluent concentration comprised four replicates and was observed daily during the test period (9 d). The NOEC value was calculated using the survival and hatching rates with analytical software (Excel Tokei ver. 6.0, Esumi Inc., Tokyo, Japan).

The results of the toxicity tests were compared using chronic toxicity units (TUC), which were calculated as follows:

$$\text{TUC} = 100/\text{NOEC} (\%) \quad (1)$$

TUC values of > 1 for a given effluent indicate that aquatic organisms might be adversely affected by the effluent; larger TUC values indicate higher toxicity. If the maximum concentration (80%) in this study has no toxic effect, the NOEC is 80% or more, and as a result, the TUC value is 1.25 or less, which is considered to have virtually no toxic effect.

Hazard quotient calculated from PRTR data

Figure 2 shows the method for calculating the hazard quotient (HQ). First, using PRTR data [8], the average annual emissions (kg/y) from each sewage treatment plant was divided by the number of annual days of operation (365 d/y) and then divided by the average effluent discharge (m³/d) to estimate the effluent concentration (*C_i*). The HQ_{*i*} for chemical *i* was then calculated using the following formula:

$$\text{HQ}_i = C_i/\text{NOEC}_i \quad (2)$$

The x% effect concentration (EC_x) does not depend on the choice of test concentrations and number of replications, which might be more accurate than NOEC to evaluate to toxic effect [19]. However, the NOECs have been widely used for statistical analysis of chronic ecotoxicity data, and are more accessible in terms of literature search from vari-

Fig. 2 Schematic showing how the hazard quotient (HQ) was calculated. NOEC: no observed effect concentration; PRTR: Pollutant Release and Transfer Register.

ous risk assessment reports and toxicity databases [20–22]. Therefore, we used NOECs in this study. In adopting toxicity values, values from tests using the same methods and types of test organisms as those used in the WET tests were prioritized. If more than one NOEC value was available, the lowest value (which would produce the most sensitive result) was used. Data were available for the *R. subcapitata* algal growth inhibition test. However, data that exactly matched the other two tests were not readily available. Thus, the toxicity value was selected using the following procedure: The lowest chronic value from the same test (same end point, same exposure period) and same family of freshwater species was prioritized. If no chronic data were available, an acute chronic ratio (ACR) of 10 [23,24] was applied to estimate the chronic value from the acute value for the same or a similar organism. In cases featuring more than one PRTR chemical in a single effluent, the toxic effects were assumed to be additive [25,26], and the sum of the HQ_{*i*} values was calculated. Toxicity due to PRTR substances might be expected when the sum of these ratios exceeds 1.0. The NOECs of the PRTR substances are shown in the Supplementary Materials (Table S1).

Fig. 3 Chronic toxicity units (TUC) values for sewage effluent samples. NM: not measured.

RESULTS AND DISCUSSION

Effects on aquatic organisms

Figure 3 shows the TUC values of the seven effluents (detailed results are shown in the **Figs. S1-S3**). Toxicity to crustacean and algae was highest in effluent B. In particular, the TUC of algae measured 20; in other words, it would be necessary to dilute this effluent at least twenty times to avoid statistically significant toxic effects. The second-highest toxicity to algae was observed in effluents F and G, which had TUC values of 10, followed by effluent D with a TUC value of 2.5. Of the seven effluents, effluent B, with a TUC of 2.5, was the most toxic to crustaceans. Only effluents A and F were subjected to the fish test, because conditions were not appropriate for fish spawning at the time of collection of other effluents. Effluents with TUC values of less than 1.25 were considered non-toxic. In this study, algae were the most sensitive to sewage effluent. However, perhaps due to the differences in water quality, daphnia were the most sensitive in previous studies on sewage effluent [5] and industrial effluent [13].

Water parameters

Table 2 shows the water quality in the final effluent samples. The contributions of various parameters to observed toxicity are discussed below. The measurement result of the water temperature is greatly different. Removal of algae growth inhibitory factors in sewage effluent is reported to be significant in the summer and differences in removal efficiency depending on the season [27]. In this study, we compared the results

of samples taken at different seasons as the representative value of each treatment facility, and there is a possibility that it is affected by the seasonal variation. In order to evaluate the average environmental impact of effluent at each treatment plant, it is necessary to conduct similar evaluation of sewage effluent at different seasons. The highest LAS concentration observed in the effluent samples was ~ 100 $\mu\text{g/L}$. The LAS NOECs of C_{11} - C_{13} for *R. subcapitata* (72 h-NOEC), *C. dubia* (8 d-NOEC), and *D. rerio* (9 d-Hatching NOEC) were 1.8–11 mg/L, 0.07–1.9 mg/L and 0.28–4.5 mg/L, respectively [28]. It is revealed that the longer the chain length is, the higher the toxicity is. The concentrations of alkyl chains numbers C_{10} – C_{14} were measured for LAS concentrations in this study. Any of these concentrations is 0.05 mg/L or less. It was compared with the actually measured literature toxicity values of LAS concentration, the total toxicity values of LAS were the 12% of NOEC at maximum. However, PRTR data as of fiscal year 2015 [8] indicate that ~ 12 t LAS has been discharged directly into aquatic environments and ~ 43 t has been discharged into sewage treatment plants in Japan. Due to the large amounts of discharge into aquatic environments and toxicity to aquatic organisms, LAS was added to the Japanese water environmental standards in 2013 for the protection of aquatic life. Although LAS likely did not contribute to effluent toxicity in this study, the highest ratio of the predicted environmental concentration to the predicted no effect concentration (PEC/PNEC) was as high as 300 in the freshwater area in 2000 [29]. Therefore, this compound must be monitored due to its high risk to aquatic organisms.

The highest concentration of total residual chlorine was

Table 2 Water quality of final effluents from the sewage treatment plants (A–G).

Effluent	pH*	DO*	Temp.*	Hardness	TOC	T-N	T-P	LAS	Residual chlorine (mg/L)	
		(mg/L)	(°C)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(µg/L)	Free	Combined
A	6.51	8.17	22.5	72.5	5.2	4.70	0.86	0.10	0.02	0.00
B	6.75	8.25	17.0	52.5	NM	6.13	0.84	0.15	0.04	0.35
C	6.95	5.62	15.9	98.0	NM	NM	NM	0.14	0.02	0.04
D	7.36	8.19	8.1	72.5	NM	NM	NM	0.07	0.04	0.17
E	7.26	6.05	26.2	57.0	7.9	15.0	16.1	116	0.03	0.01
F	6.68	5.56	20.1	46.0	3.7	4.76	4.27	70	0.03	0.06
G	7.12	5.79	18.6	73.0	4.7	NM	NM	50	0.04	0.35

*measured on site; NM: not measured; DO: dissolved oxygen; TOC: total organic carbon; T-N: total nitrogen; T-P: total phosphorus; LAS: linear alkyl benzene sulfonates

Fig. 4 Effect of effluent G on algae. 80#: 80% dechlorinated effluent; average $\pm$ standard error; ** $p < 0.01$, compared with control.

0.39 mg/L in effluents B and G, followed by 0.21 mg/L in effluent D. The highest concentration of free chlorine was 0.04 mg/L in effluents B, D, and G. For combined residual chlorine (NH_2Cl , monochloramine), a 96 h lowest observed effect concentration (LOEC) of 0.01 mg/L has been reported for *R. subcapitata* [30]. However, the 96 h NOEC value was not determined in this study. Herein, monochloramine was prepared and subjected to a *R. subcapitata* 72 h algae growth inhibition test using a previously reported method [30]. The resultant NOEC was 0.01 mg/L, which is the same as the reported 96 h LOEC. Monochloramine may be the main ingredient inhibiting algal growth in effluents B, D, and G.

Fig. 5 Concentration–response relationship between combined chlorine and algae growth inhibition at sewage plants B, D, F, and G; open squares and circles represent monochloramine results.

NH_2Cl :A NH_2Cl :B: We conducted the test with NH_2Cl twice and added A and B to distinguish it.

The addition of a reducing agent, sodium thiosulfate, to effluents F and G prevented the inhibitory effect, supporting this hypothesis (Fig. 4).

Figure 5 shows the relationship between the residual combined chlorine concentration and algal growth inhibition. Growth inhibition developed at combined chlorine concentrations of more than 0.01 mg/L in all cases except for effluent F. However, growth inhibition appeared at higher combined chlorine concentrations in effluents B, D, and G than in pure compound experiments, suggesting that reducing agents in the sewage effluent may have reacted with combined chloramines, including monochloramine, during the experiment.

Table 3 Metal analysis results (unit: $\mu\text{g/L}$) from the sewage treatment plants (A–G).

	Cr	Ni	Cu	Zn	Cd	Pb
A	0.3	1.8	6.5	62	0.1	0.7
B	2.1	4.5	12	84	0.3	2.8
C	0.2	9.5	3.7	26	<0.01	0.2
D	0.2	0.8	3.2	33	<0.01	0.1
E	0.7	2.4	8.7	63	0.1	41.8
F	<0.01	<0.01	7.0	27	<0.01	<0.01
G	<0.01	<0.01	21	52	<0.01	<0.01

In effluent D, which showed growth enhancement by nutrients at lower concentrations, the effect of chloramine may have been offset. The toxicity of residual chlorine to aquatic organisms is widely known [5,6], and dechlorination [7] and alternative disinfection methods, such as UV disinfection, have been adopted to reduce the toxicity of residual chlorine.

Heavy metals such as Ni, Cu, and Zn, are known to be toxic to aquatic organisms at relatively low concentrations (Table S1). As shown in Table 3, Ni, which has a NOEC of algae value of $40 \mu\text{g/L}$, featured concentrations of less than $10 \mu\text{g/L}$ at all plants. Therefore, Ni likely did not contribute to algae toxicity. The Cu concentrations were generally small, only the Cu concentration of plant B and plant G were higher than the NOEC value of $10 \mu\text{g/L}$, suggesting that Cu might be contribute to toxicity in these plants. Several tens of $\mu\text{g/L}$ Zn were detected at all plants. The algae toxicity NOEC value of this metal is $53 \mu\text{g/L}$, and that for crustaceans is $25 \mu\text{g/L}$, suggesting that Zn contributions to toxicity should be considered. However, because toxic effects were not observed even in effluent of plants C and E, where the concentration was higher than the reported NOEC, it was inferred that Zn did not contribute to toxicity. This reduction in metal toxicity might be explained by the coexisting organic matter and cations in the effluents, which have been reported to counteract the influence of metals [31,32].

In this study, there is a possibility that the toxicity to algae was likely derived from a combination of chlorine and other compounds, such as ammonia and triclosan, which have been reported to cause chronic sewage effluent toxicity in previous studies [33,34], but no measured in this study. This might explain why algae was most sensitive among three test organisms in this study. However, daphnia was reported to be the most sensitive in another study on sewage effluents although causative substances were not identified [5]. As for the previous study on industrial effluent [13], Ni of which concentration was higher than those of sewage effluents, was suspected to the cause of toxicity on *C. dubia* that is more

Fig. 6 Number of Pollutant Release and Transfer Register (PRTR) substances released from each sewage treatment plant in 2013 and 2014.

sensitive to this metal than algae. In this way, the type of wastewater affects the most affected species and their degree, so a test battery using different species is necessary for WET tests.

Further analysis and toxicity identification evaluation [35,36] are necessary to estimate the contributions of these compounds to toxicity. Moreover, emerging pollutants, such as pharmaceuticals and personal care products, in final effluents may pose risks to aquatic organisms [10,37]. Further monitoring is therefore necessary to evaluate environmental concentration trends for these chemicals.

Hazard quotient based on PRTR data

Figure 6 shows the number of PRTR substances discharged from each sewage treatment plant to aquatic environments in 2013 and 2014. In addition to the 28 substances reported at plant F, an average of three substances per treatment plant

(min. = 0, max. = 9) were reported. The six most abundant PRTR substances released from multiple sewage treatment plants were boron compounds, zinc compounds (water-soluble), arsenic and inorganic arsenic compounds, copper salts (water-soluble except for complex salts), dioxins, and manganese and its compounds (**Fig. S4** in Supplementary Materials).

NOECs exist for 30 PRTR substances, though dioxin NOEC values were not available for algae and crustaceans. Results from same species–same method tests (compared to the WET tests) from the database were divided by estimated NOECs; these ratios were 53.3, 20.0, and 13.3% for algae, crustaceans, and fish, respectively. To improve the accuracy of effluent risk assessments based on PRTR data, it is essential to enrich the chronic NOEC dataset for *C. dubia* and zebrafish (**Fig. S5** in Supplementary Materials).

The crustacean HQ for *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN) at treatment plant F was large (6,780). EPN is an organophosphorus insecticide and exerts insecticidal effects by inhibiting acetylcholinesterase activity in the central nervous system [38]. In this study, the NOEC value for EPN was small at 0.015 µg/L (calculated by dividing the acute toxicity value for crustaceans by the ACR of 10). Although EPN has a large influence on crustaceans even at low concentrations, the effluent from treatment plant F did not demonstrate toxicity toward these organisms (**Fig. 3**). Therefore, it is possible that the effluent concentration was overestimated or the toxicity value underestimated. Regarding the later, the possibility of underestimating the toxicity value is low even when adopting the acute value as-is, as the HQ value was large (680). On the other hand, the PRTR annual release data for sewage treatment plants are usually calculated as the product of the annual average concentration and the annual discharged water volume [39]. Therefore, unless there is a large fluctuation in the amount of final effluent, the annual average concentration should be nearly equal to the estimated emissions concentration. The EPN emissions from plant F were nearly constant from 2013 to 2016 at 12–14 kg/y, and the annual average concentration back-calculated from these data was approximately 0.1 mg/L as follows.

$$\text{Daily emissions} = (12\text{--}14) / 365 = (0.033\text{--}0.038) \text{ kg/day}$$

Daily emissions concentration

$$= \text{Daily emissions } (0.033\text{--}0.038) \text{ kg} / \text{drainage volume } 350 \text{ m}^3 \text{ (Table 1)}$$

$$= 0.09\text{--}0.11 \text{ mg/L}$$

This value corresponds to about one-tenth of the EPN

Fig. 7 Hazard quotient for sewage treatment plants calculated using (a) average of Pollutant Release and Transfer Register (PRTR) data from 2013 and 2014 and (b) PRTR data from the sampling year.

effluent standard of 1 mg/L. Based on the annual average water quality values, most of other PRTR substances from treatment plant F were also around one-tenth of the effluent standard. If the measured values are between the limit of quantification (LOQ) and the limit of detection, the guideline recommends using half of the LOQ as the annual average concentration [39]. For plant F, the annual average concentration was assumed to be one-tenth of effluent standard. However, when the ratio of the annual average concentration to the toxicity value is large, as for EPN, PRTR method seems to overestimate the annual concentration, leading to a false positive inference of risk. Due to these issues, EPN was not used in the crustacean HQ calculation at treatment plant F. Emissions of EPN were not reported at the other treatment plants.

The HQ results are shown in **Fig. 7**. Although slight variances are observed due to differences in calculation methods, HQ was predicted to exceed 10 at three treatment plants. In

Fig. 8 Chronic toxicity units (TUc) and hazard quotient (HQ) calculated using (a) average of Pollutant Release and Transfer Register (PRTR) data from 2013 and 2014 and (b) PRTR data from the sampling year.

particular, relatively high HQ values were observed for all organisms at plant F, which also featured the largest number of PRTR substances (Fig. 6). Of the three types of test organisms, the HQ values were largest in crustaceans, followed by algae. This finding differs from the results of the biological tests, in which algae were the most affected and featured TUc values of 10 or more (Fig. 3).

Relationship between TUc and HQ

The relationship between TUc and HQ is shown in Fig. 8. As shown in the figure, since the measured TU value is higher than the predicted HQ value for algae, it is considered to be the effect of substances not subject to PRTR such as monochloramine mentioned above. Both an overestimation of effluent concentration and/or an underestimation of toxicity are possible causes for this discrepancy. Zinc compounds were responsible for most of the high HQ values for algae and crustaceans at treatment plants A and B. At plant F, zinc compounds, chromium (III) compounds, and copper salts were equally responsible for high HQ values. In contrast, thiuram and inorganic cyanide compounds showed larger contributions to HQ for fish at treatment plant F (Fig. 9). The average ratios of estimated concentrations (from PRTR data) to measured values (by ICP-MS) were 4.2, 3.0, 4.6, and 9.6 for copper, zinc, manganese, and arsenic, respectively, suggesting that the estimated concentrations were larger than the measured values (Fig. S6 in Supplementary Materials).

The influence of seasonal and diurnal fluctuations in water quality should not be neglected. The annual average water concentration, which is used in the PRTR data calculations mentioned above, is inaccurate, and the same problem might occur for other PRTR substances, such as thiuram and inorganic cyanide compounds. Furthermore, the toxicity of heavy metals, such as copper, can reportedly be decreased by coexisting substances, such as organic substances and cations [31,32]. In addition, more than 55% of the dissolved metals in sewage effluent are complexed with dissolved organic matter [40], which should reduce the metal toxicity compared to that predicted for free ions.

From the discussion above, we conclude that HQ values based on PRTR data likely overestimate toxicity, and that metal toxicity tends to be lower than that predicted by the measured concentration. Thus, results from both methods are inconsistent. Unlike industrial factories, where chemical substances in wastewater are measured appropriately, it is necessary to examine the accuracy of effluent PRTR data at sewage treatment plants that accept discharge from various sources.

CONCLUSIONS

To evaluate the ecotoxicological effects of effluent from seven sewage treatment plants in Toyama, we conducted WET tests, analyzed water quality, and predicted effluent toxicity using PRTR data. Toxic effects on aquatic organisms were observed in four samples; four samples were toxic to

Fig. 9 Contribution of each Pollutant Release and Transfer Register substance to hazard quotient (HQ) values for each organism group during the sampling year.

algae, and one was toxic to crustaceans. According to chemical analyses and monochloramine experiments, combined residual chlorine was the dominant cause of algae toxicity. The toxicity predicted using PRTR data was likely to be overestimated for daphnia and fish. There are two related factors that may help explain the overestimation. As for metals, there is a possibility that bio availability might be reduced by coexisting matters. The other factor might be the calculation method of PRTR, when the measured values of the target substance are between LOQ and LOD. Therefore, it is necessary to examine the accuracy of PRTR data when evaluating sewage effluent toxicity based on these data.

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SUPPLEMENTARY MATERIALS

Supplementary Materials for this article are available on the journal website in a separate file.

https://www.jstage.jst.go.jp/article/jwet/17/4/17_18-028/supplement/_download/17_18-028_1.pdf

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