ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2007 Vol. 50, Št. 2: 103–112

Sprejeto (accepted): 2008-01-31

Toxicity of effluents

Strupenost iztokov

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Abstract. Reliable characterisation of the hazardous properties of effluents is an important step in water quality management. A "chemical-specific" approach, based on measurements of individual chemicals or chemical groups, has been shown to have limitations particularly in complex mixtures of chemicals such as effluents. This study shows the importance of direct toxicity assessment of wastewater samples using a toxicity test. Data obtained by direct testing were compared to the toxicity predicted using key toxic compounds data. Toxicity of wastewater samples from tannery and chemical industries as well as key toxic chemicals were assessed using the water flea *Daphnia magna*. The toxicity predicted from the levels and toxicities of wastewater components was found to be unreliable due to under- or over-estimation of the experimentally determined toxicity. For this reason, direct toxicity measurement is more appropriate for the effective and reliable assessment of effluent quality.
Keywords: toxicity, *Daphnia magna*, direct toxicity assessment, chemical industry, tannery wastewater, toxic unit.

Izvleček. Učinkovito gospodarjenje z vodami zahteva zanesljivo in realno ovrednotenje potencialno škodljivih lastnosti iztokov. Uporaba kemijsko-specifičnega pristopa, ki temelji na meritvah posameznih kemikalij in kemijskih skupin, je pokazala številne pomanjkljivosti posebno v kompleksnih mešanicah kot so iztoki iz industrijskih obratov in čistilnih naprav. V prispevku smo prikazali pomembnost neposrednega določanja strupenosti odpadnih vod z uporabo strupenostnega testa. Primerjali smo dejansko izmerjeno strupenost vzorcev odpadnih vod iz različnih industrij z napovedano strupenostjo, ki smo jo izračunali na osnovi poznavanja ključnih strupenih kemikalij v vzorcih in njihovih izmerjenih koncentracijah. Napovedana strupenost se v večini primerov ni ujemala z dejansko izmerjeno strupenostjo. Zato je neposredno določanje strupenosti iztokov z izpostavitvijo vodnih organizmov primernejše in pripomore k bolj zanesljivi in pravilnejši karakterizaciji iztokov.
Ključne besede: strupenost, *Daphnia magna*, neposredno določanje strupenosti, kemijska industrija, usnjarske odpadne vode, enota toksičnosti.

Introduction

Over one thousand new chemicals are produced every year and concerns about the fate of chemicals in the environment and their impacts on ecosystems have risen over the last decades. The environmental fate of many commercially available chemicals is not well known; for most of them there is little or no toxicity data, biodegradability or bioaccumulation data. In spite of the fact that most chemicals are released to the environment in complex mixtures, international water quality policy continues to rely on a "chemical-specific" approach based on measurements of individual substances (Power & Boumphrey 2004). In the USA and Canada a "whole-effluent" toxicity (WET) approach has been established to overcome many of deficiencies of the "chemical-specific" approach and in the UK, a direct toxicity assessment (DTA) was subsequently developed from the WET (Wharfe 2004). The WET approach is based on the fact that chemicals can behave differently when in a mixture, and exposure of aquatic organisms in a toxicity test to a whole effluent sample provides direct information about the cumulative toxicity of the effluent (Tonkes et al. 1998).

The aim of the present study was to determine the importance of direct toxicity assessment in the characterisation of wastewater quality. Predicted toxicities based on the sum of toxic units of individual key toxic chemicals were compared to toxicities measured directly by assessment of wastewater samples originating from tannery and chemical industrial plants. An acute toxicity measurement using water flea *Daphnia magna* is a part of the Slovenian monitoring programme for toxic effluents discharged into receiving streams (Official Gazette of the Republic of Slovenia, 1996). Accordingly, *D. magna* was used as a test organism with which to assess the toxicity of key toxic chemicals and wastewater samples.

Material and methods

Wastewater samples and standard solutions of chemicals

The 6 h flow proportional wastewater samples, stored in the dark at 4^{o}C, were analysed immediately upon receipt in the laboratory. Untreated wastewater samples were used for chemical analysis and toxicity testing.

The wastewater samples from a leather-processing tannery were collected after physico-chemical pre-treatment including coagulation and flocculation with aluminium sulphate and anionic polyelectrolyte. The wastewater samples from chemical plant 1, which produces raw materials such as laminates, melamine, urea-formaldehyde resins, and materials for textile impregnation were tested before treatment. The wastewater sample from chemical plant 2, manufacturing white pigments, was polluted with metals and discharged directly to a nearby river.

Chemical analyses were performed according to standard procedures (APHA, AWWA, WEF, 2005). Ammonia was measured by the macro-Kjeldahl method and sulphate by chemically suppressed ion chromatography (DIONEX 4000) in the filtered samples using 0.2 µm filter. Sulphide was measured in the fixed samples upon receipt of the sample by the spectrophotometric methylene blue method. Aluminium, barium, chromium, cadmium and zinc were measured by ICP-AES (Thermo Jarrell Ash). Formaldehyde was determined spectrophotometrically; methanol and butanol were analysed by gas chromatography.

Appropriate volumes of standard stock solutions of the following key toxic chemicals were diluted with water to obtain the desired concentrations and tested for toxicity; ammonium (NH_4Cl, p.a., Merck), aluminium [$Al_2(SO_4)_3.18H_2O$, extra pure, Merck], sulphide ($Na_2S.8H_2O$ p.a., Merck), chromium [$KCr(SO_4)_2.12H_2O$ p.a., Fluka], formaldehyde (37 % v/v, Kemika), zinc [$(CH_3COO)_2Zn.2H_2O$ p.a., Merck], and cadmium ($CdCl_2.5H_2O$, Sigma).

Toxicity testing

Five concentrations and a control were tested in each experiment. A preliminary test and two definitive trials were conducted for each chemical and wastewater sample.

Water flea *Daphnia magna* Straus 1820 were obtained from the Institut für Wasser, Boden und Lufthygiene des Umweltbundesamtes, Berlin. Water fleas were cultured in 3-L aquariums containing

2.5 L of modified M4 medium (Kühn et al. 1989) in a temperature – controlled room at 21±1°C and illuminated with fluorescent lamps for 12 h per day at a light intensity of approximately 1800 lx. They were fed a diet of green algae *Desmodesmus subspicatus* corresponding to 0.13 mg carbon/daphnia per day. Neonates (age < 24 h) were used in acute and chronic toxicity tests.

In the acute toxicity tests, water fleas were exposed to different concentrations of chemicals and wastewater samples and the immobile organisms were counted after a 24h exposure period as required in the Slovenian legislation (Official Gazette of the Republic of Slovenia, 2001); water fleas not able to swim within 15s after gentle agitation of the test container were considered to be immobile (International Organization for Standardization, 1996). The obtained results were analysed using probit analysis to calculate the 24h EC50 values with corresponding 95% confidence limits (US EPA, 1994a).

In the chronic toxicity tests, water fleas were exposed in a semi-static exposure system as described in the OECD Guideline (Organisation for Economic Cooperation and Development, 1998). An individual water flea was exposed in a 100 ml beaker containing 50 ml of solution; ten replicates were used for each concentration and a control. The solutions were renewed on Mondays, Wednesdays, and Fridays and at the same time the pH, temperature, and dissolved oxygen concentration were checked. Each water flea was fed a diet of the algae *D. subspicatus* at a ratio of 0.15 mg carbon/day per water flea. The endpoints of the chronic toxicity test were survival and reproduction of water fleas after a 21d of exposure. The reproduction results were analysed by the one-tailed Dunnett's test to calculate the NOEC and LOEC values (US EPA, 1994a).

Toxicity unit approach

The acute toxicity (24h EC_{50}) and the chronic toxicity endpoints (21d NOEC) of wastewater samples and individual chemicals were converted to toxic units (TU) to compare the toxicity of individual compounds in the wastewater and the actual toxicity of the wastewater samples (Doi 1994, Guerra 2001, US EPA 1994b). A measured toxicity of wastewater was defined as 100 divided by the 24h EC_{50} or the 21d NOEC to obtain an acute toxic unit (ATUm) and a chronic toxic unit (CTUm), respectively:

ATUm = 100/24h EC_{50}

CTUm = 100/21d NOEC

The predicted acute toxic unit (ATUi) and the predicted chronic toxic unit (CTUi) of a key toxic compound (TUi) was obtained by dividing the measured toxicant concentration (ci) in the wastewater sample by the 24h EC50 or 21d NOEC determined for an individual chemical:

ATUi = Ci/24h EC50i

CTUi = Ci/21d NOEC

The predicted acute toxic unit (ATUp) and chronic toxic unit (CTUp) of wastewater samples are the sum of the toxic units of individual chemicals (Doi, 1994):

ATUp = ΣATUi

CTUp = ΣCTUi

Results and discussion

Tannery wastewater

That wastewater samples from the tannery are heavily polluted with organic compounds is indicated by high concentrations of COD (S1 = 2227 mg/L, S2 = 1224 mg/L) and BOD_5 (S1 = 1800 mg/L, S2 = 940 mg/L). Information about the tannery process revealed that aluminium, ammonium, chromium, and sulphide ions are the key toxic compounds used in the technology of leather processing and the pre-treatment of the wastewater. In this case ammonium ion was determined at a pH of 7.5, which is similar to the pH of the wastewater samples. Ammonia toxicity depends highly on pH; the concentration of unionised ammonia, which is toxic to aquatic organisms, increases with pH (Helawell 1986). The acute and chronic toxicity to daphnids of key toxic chemicals and the wastewater samples from tannery are given in Table 1.

Table 1: Acute and chronic toxicity to *Daphnia magna* of key toxic chemicals and tannery wastewater samples (S1 and S2) with the concentrations of chemicals measured in samples.
Tabela 1: Akutna in kronična strupenost ključnih strupenih kemikalij in vzorcev usnjarskih odpadnih vod (S1 in S2) na vodne bolhe *Daphnia magna* in izmerjene koncentracije kemikalij.

Key toxic chemicals	Wastewater concentrations S 1	S 2	Acute toxicity 24h EC50 (mg/L) (95 % CL)	Chronic toxicity NOEC (mg/L)	Toxic units S 1 ATUi	S 1 CTUi	S 2 ATUi	S 2 CTUi
Ammonium (mg/L)	136.5	122.6	173 (156 – 191)	7.0	0.79	19.5	0.71	17.5
Aluminium (mg/L)	11.9	3.0	5.4 (5.2 – 5.6)	0.13	2.20	91.5	0.56	23.1
Chromium (mg/L)	1.9	0.4	10.7 (10.2 – 11.7)	0.31	0.18	6.1	0.04	1.3
Sulphide (mg/L)	1.72	6.98	6.7 (6.0 – 7.4)	3.13	0.26	0.55	1.0	2.2
					ATUm		CTUm	
Sample S1 (v/v %)	/	/	28.3 (25.4 – 31.1)	1.9	3.5		52.6	
Sample S2 (v/v %)	/	/	13.8 (10.6 – 16.3)	1.7	7.3		58.8	

In acute exposure, the ions most toxic to daphnids are aluminium and sulphide; the 24h EC_{50} values obtained were 5.4 mg/L and 6.7 mg/L respectively. In chronic exposure the aluminium and chromium ions are the most toxic, with NOEC values lower than 1 mg/L in both cases. Both wastewater samples were acutely toxic to daphnids; the toxicity of the sample S1 (24h EC_{50} of 28.3 v/v %) was lower than that of sample S2 (24h EC50 13.8 v/v %). Similar chronic toxicity was obtained for both tested samples. However, sample S2 was generally less contaminated than sample S1 although the concentration of sulphide was higher (Tab. 1).

The predicted acute (ATUi) and chronic (CTUi) toxicity derived from the 24h EC_{50} values and 21d NOEC and expressed in toxic units (Tab. 1) was compared to the measured toxicity of the wastewater samples (Fig. 1).

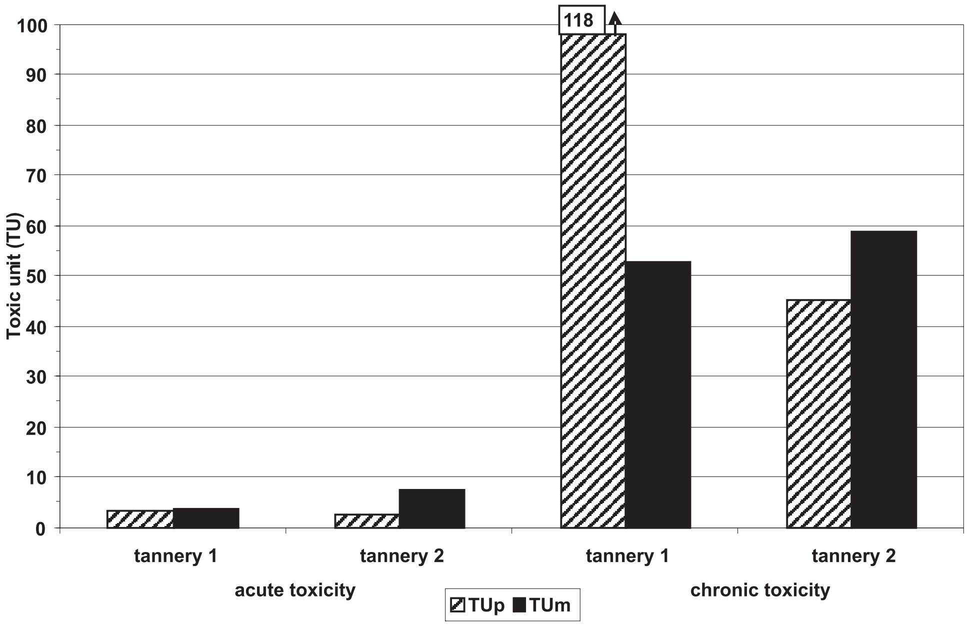

Figure 1: Predicted (TUp) and measured toxicity (TUm) of wastewater samples from tannery based on the acute toxicity (24h EC_{50}) and chronic toxicity (21d NOEC).
Slika 1: Napovedana (TUp) in izmerjena strupenost (TUm) usnjarskih odpadnih vod na osnovi akutne (24h EC_{50}) in kronične strupenosti (21d NOEC).

In the first sample of tannery wastewater the predicted acute toxicity agreed well with the measured toxicity but such agreement was not found in the case of chronic toxicity where the measured toxicity was significantly over-estimated. In the second sample the measured acute and chronic toxicities were slightly under-estimated (Fig. 1).

Wastewater from chemical industries

Information from the process operators concerning the technological process revealed that formaldehyde, methanol, butanol and metal ions (zinc, barium, cadmium) were the key toxic chemicals of the wastewater samples from chemical industries 1 and 2. The acute toxicities of wastewater samples from different chemical industries and key toxic chemicals were determined (Tabs. 2 – 3). The wastewater samples obtained from chemical industry 1 had a pH of 4.8 – 7.2 and in this case the toxicity of ammonia was studied at pH 6.4.

The wastewater samples obtained from the chemical industry 1, which produces raw materials for various industries, contained high concentrations of organic substances (the average COD was 2013 mg/L, the average BOD_5 1078 mg/L) and the samples were acutely toxic to daphnids. The acute toxicities of all samples (A, B, C) were similar despite different concentrations of chemicals in the samples analysed. The most toxic chemical to daphnids was formaldehyde. This contributed most to the predicted toxicity followed by ammonium ion (Tab. 2). The concentrations of methanol and butanol were high (the average methanol was 291 mg/L, the average butanol 89 mg/L), but their contribution to the predicted toxicity is insignificant as methanol and butanol are essentially non-toxic to water fleas according to the literature data (Lilius et al. 1994, Material Safety Data Sheet 2004).

Table 2: Acute toxicity to *Daphnia magna* of key toxic chemicals and wastewater samples from chemical industry 1 with the concentrations of chemicals measured in samples.
Tabela 2: Akutna strupenost ključnih strupenih kemikalij in vzorcev odpadnih vod iz kemijske industrije 1 na vodne bolhe *Daphnia magna* in izmerjene koncentracije kemikalij.

	Wastewater concentrations			Acute toxicity	Toxic units ATUi		
Sample	A	B	C	24h EC50 (mg/L) (95 % CL)	A	B	C
Formaldehyde (mg/L)	299	921	412	17.6 (16.1 – 19.1)	17	52.3	23.4
Ammonium (mg/L)	116	30	87	262 (249 – 291)	0.44	0.11	0.33
					ATUm		
24h EC50 (v/v %) (95 % CL)	1.9 (1.2 –3.0)	2.7 (2.6–2.8)	4.5 (3.7 –5.4)	/	52.6	37.0	22.2

Table 3: Acute toxicity to *Daphnia magna* of key toxic chemicals and wastewater samples from chemical industry 2 with concentrations of chemicals measured in samples.
Tabela 3: Akutna strupenost ključnih strupenih kemikalij in vzorcev odpadnih vod iz kemijske industrije 2 na vodne bolhe *Daphnia magna* in izmerjene koncentracije kemikalij.

Key toxic chemical	Wastewater concentrations	Acute toxicity 24h EC50 (mg/L) (95 % CL)	Toxic units ATUi
Zinc (mg/L)	39.5	7.6 (5.8 – 9.7)	5.2
Cadmium (µg/L)	0.7	0.74 (0.63 – 0.88)	0.001
Barium (mg/L)	172	530 ‡	0.32
Wastewater			ATUm
24h EC50 (v/v %) (95 % CL)	/	0.54 (0.38 – 0.72)	185

‡ Le Blanc, 1980.

The wastewater sample from chemical industry 2 showed significant toxicity to daphnids as the obtained 24 h EC_{50} was low (Tab. 3). The highest toxicity was observed for cadmium with an observed EC_{50} below 1 mg/L. Daphnids are the most sensitive invertebrate organisms to zinc after 2 – 4 d of exposure (Moore & Ramamoorthy 1984) but we found that zinc was not highly toxic to daphnids after 24 hours. Zinc however, makes a major contribution to the predicted toxicity. The measured toxicity of the wastewater sample was far higher than the predicted toxicity and synergism between metals in the sample is a probable reason.

The predicted acute toxicity derived for wastewater samples from chemical industries was compared to the measured acute toxicity of the wastewater samples (Fig. 2).

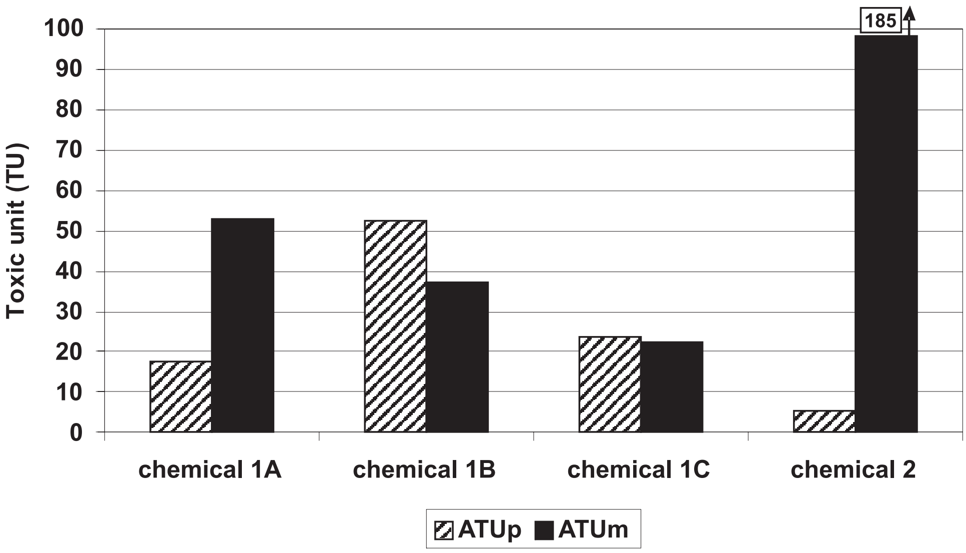

Figure 2: Predicted (ATUp) and measured toxicity (ATUm) of wastewater samples from chemical industry 1 (Samples A, B, C) and chemical industry 2 based on the 24h EC50 values.
Slika 2: Napovedana (TUp) in izmerjena strupenost (TUm) vzorcev odpadnih vod iz kemijske industrije 1 (Vzorci A, B, C) in kemijske industrije 2 na osnovi 24h EC50 vrednosti.

Analysis of the successive samples of the chemical industry 1 revealed a quite different situation regarding the predicted toxicities; in the first sample, under-estimation was observed but in the second sample, over-estimation of the measured toxicity was detected, and in the third sample, good agreement between the predicted and the measured values was found. In the sample obtained from chemical industry 2 the measured toxicity was significantly (more than 7 times) under-estimated (Fig. 2).

The results obtained showed that the prediction of toxicity of effluents based on chemical analyses and toxicity of key chemicals is not a reliable way to assess the actual toxicity. In general under- or over-estimation of the actual toxicity were found when the toxicity of effluent samples was predicted from the toxicities of the components. Prediction of toxicity by this additive method gave satisfactory results in only two cases of all cases studied. In the successive samples of wastewater from chemical industry 1 completely different results were observed. Ratios of the measured and predicted toxicities of successive samples of the same wastewater were not constant. This may be because variability of chemical composition of wastewater samples during time due to changes in the plant's process which could lead to presence of some toxic unknown chemical in the wastewater (Tišler et al. 2004).

In recent years, a lot of effort has been put into development of QSAR (quantitative structure-activity relationship) models with the aim to predict toxicities of individual chemicals and their mixtures (Zhang et al 2007, Lin et al. 2002, Xu & Nirmalakhandan 1998, Niederlehner et al. 1998, Nirmalakhandan et al. 1994, de Wolf et al. 1988). Complex mixtures like effluents may contain a wide range of pollutants with different modes of action and possible interactions between them could significantly contribute to the effluent toxicity. Furthermore, detailed compositions of effluents are very often unknown. For these reasons the use of QSAR approach is not suitable as it does not give reliable information about the actual effluent toxicity.

In a long term exposure of daphnids the prediction of toxicity from that of the components was also an unreliable way in which to estimate the wastewater toxicity. Literature data show that a prediction of wastewater toxicity from the toxicity of key chemicals depends on the toxicity data used. Bervoets et al. (1996) evaluated the impacts of effluent toxicity on a nearby river using an effluent toxicity test with *D. magna*, an ambient toxicity test and an ecological survey. Guerra (2001) characterised the industrial effluents using chemical analyses and a battery of toxicity tests and found the relationship between the measured and predicted toxicities depends on which toxicity data and test organisms were used. He proposed a battery of toxicity tests and measurement of specific chemical parameters in a monitoring programme of effluents. It is well known that a single bioassay can not detect every different mode of action of toxic effects caused by effluents. Data obtained by the genus *Daphnia* are very useful for screening a toxicity of effluents and seeking identification of cause and source of toxicity but a battery of species representing different trophic levels (decomposers, primary producers, primary and secondary consumers) is required to assess the potential adverse impacts of effluents on receiving streams (Johnson et al. 2004). Consequently, additional toxicity tests are necessary in monitoring programmes designed to assess the impacts of effluents on receiving streams as prescribed by Slovenian law.

Conclusions

The results obtained clearly demonstrate the importance of using a direct toxicity assessment approach for a reliable assessment of wastewater quality. Prediction of toxicity based on chemical analyses and toxicity of key chemicals fails to provide an accurate assessment of the toxicity of wastewater. The predicted and measured toxicities showed an acceptable agreement in only two of all the wastewater samples studied. The relationship between the predicted and measured toxicities was not constant even in the successive samples of the same wastewater and moreover varied over time in the same effluent, as a result perhaps, of changes in the technological process. Moreover, interactions between the compounds in the wastewater sample can not be detected with the prediction toxicity principle using key toxic chemicals and chemical analyses. For these reasons, exposure of organisms in a toxicity test provides more relevant measure of actual toxicity of the effluent sample because the response of the exposed organism will reflect the effects of all known and unknown chemicals in the samples.

Acknowledgement

We gratefully acknowledge Prof. Dr. Bill Milne whose suggestions and comments greatly improved the manuscript.

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