Bull. Environ. Contam. Toxicol. (2002) 69:719–726

DOI: 10.1007/s00128-002-0120-8

Bulletin of Environmental Contamination and Toxicology

Incorporation of Toxicity Tests to the Discharges of Pulp Paper Industry in Turkey

D. T. Sponza

Dokuz Eylul University, Engineering, Faculty, and Environmental Engineering Department, Buca-Kaynaklar Campus, Izmir, Turkey

Received: 13 June 2001/Accepted: 12 July 2002

Although in some cases the effluent quality of wastewater does not actually violate the discharge limits, toxicity could be present in the wastewater. Throughout the world, where industrial effluent and hazardous waste are posing a growing problem, a number of biological assays have been developed and evaluated for aquatic toxicity testing (Sponza, 1999; Sponza, 2002). The incorporation of acute and complementary chronic toxicity tests into the wastewater discharge standards with various organisms is necessary in order to protect aquatic ecosystems (Slabbert, 1996). Enrichment toxicity tests containing bacteria could be valuable screening tools for identifying and categorizing toxic effluents together with acute toxicity tests (Wharfe and Tinsley, 1995; Slabbert and Venter, 1999). The assessment of whether a complex substance is a risk to organisms in the environment is only possible by acute and enrichment toxicity tests (Schowanek et al., 2001). These tests are novel applications and give an idea whether there is potential toxicity or growth stimulation conditions. International wastewater discharges policies are focused on the precautionary principle i.e. the reduction of specific pollutants in order to reduce their emissions on the receiving ecosystems (Kinnersley, 1990). In Turkey the receiving ecosystem discharge standards given in the wastewater regulation include chemical and biochemical parameters such as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), NH_4-N, phenol, sulfur, Fe, Cr, oil and grease, for discharges have been from different industries such as the leather, dye and textile industries. Only the fish toxicity test, based on the toxicity dilution factor (TDF), which indicates the toxicity, was included in the Turkish Water Pollution and Control Regulation (Turkish Wat. Pollut. Control Reg, 1992). Although these Regulations were revised on 1 July 1999, none of the toxicity tests were incorporated into them. In other words, the standards limiting the toxicity have not yet been incorporated into the Turkish Water Pollution Control Regulations.

The chlorinated organics present in effluent from kraft mills employing either chlorine or chlorine dioxide in the bleaching process have become substances resistant to biological degradation, toxic to aquatic species, genotoxic and accumulate in the body of a variety of organisms (Sun et al., 1989). Low absorbable organic halogens (AOX) removal has been reported such as 30 and 35 % in the aerobic and anaerobic treatment systems (Ferguson, 1994; Haggblom and Salkinoja-Solonen, 1991). Chlorine bleach effluent are considered toxic to the environment due to their highly structured composition. Exposure to bleached kraft pulp mill effluents can cause various disorders in fish, including sublethal chronic responses (Dube and Culp,1997).

The aim of this study was the toxicity evaluation of wastewater effluents from the pulp and paper industry. As part of the monitoring program, enrichment toxicity tests and conventional toxicity tests were performed to assess and categorize the toxic discharges. Chemical analysis results were compared to traditional and enrichment test results.

MATERIALS AND METHODS

Samples taken from the effluent of the secondary settling tank, which follow the biological activated sludge treatment plant in the pulp and paper industry, were analysed for chemical, biochemical and toxicity parameters during the eight months from March 1998 to January 1999. The conventional toxicity tests consisted of tests with a protozoan (*Vorticella sp.*), algae (*Chlorella sp.*), fish (*Lepistes sp.*) and bacteria (Coliform and Floc bacteria). All test results are expressed in EC_{50}, which means that this is the concentration which affected 50% of the organisms tested in different volumes of effluent. All concentrations are given in volume percentage of the effluents. The higher this result, the less acutely toxic the sample. For example, one hundred percent effluent is undiluted effluent. When the effluent is diluted ten times, the expression is 10%. If this is the result of the test, the effluent is acutely toxic for 50 % of the test organisms at a dilution of 10.

In the determination of conventional toxicity, the initial organism concentrations were measured ($25*10^4$ colonies per ml for coliform and floc bacteria, 250 cells in 100 ml for both *Vorticella sp.* and *Chlorella sp.* and 10 *Lepistes sp.* in 5 liter), then the numbers of organism were monitored for 24 and 48 hours of exposure period with different volume percentage of effluents and EC_{50} values were calculated for every microorganism. All tests were performed in triplicate.

The Total Coliforms and Floc forming bacteria were isolated by membrane filtration on *mEndo* broth and on FF *Protease-peptone yeast* broth as described in 922 B by following Standard Methods (APHA-AWWA, 1992) and the Dugan and Lundgren (1968) method, respectively. In order to determine the fish toxicity, effluent wastewater taken from the treatment plants was diluted to a precise volume and the percent EC_{50} values of *Lepistes sp.* were monitored after 48 hours of incubation period in a 5 liter aquarium at ambient temperature (APHA-AWWA, 1992). For algae toxicity, the identification and enumeration of *Chlorella sp.* were done under a microscope on filtered and immersion coated membranes at the end of the incubation period at 21 ^{o}C within three days in algae medium absorbed membranes (Pelezar and Chan, 1972). *Vorticella sp.* was inoculated into the diluted effluent wastewater and incubated for 24 hours at

21°C. Motility or viable cells of *Vorticella sp.* were assessed as vitality (Pelezar and Chan, 1972).

Assessment of conventional toxicity tests, done by taking the effective concentration of the effluent sample as causing mortality of 50 percent of the organisms tested, is shown as follows for different volume percentages of effluents (Canton, 1991; Tonkes *et al.*, 1999): If the volume percentages of the effluent wastewater varied between 1 – 10 %, 10 – 100%, < 1 % and > 100 % the acute toxicity evaluations were as follows: "Moderately acutely toxic", "Slightly acutely toxic", "Acutely toxic" and "Not acutely toxic", respectively.

Enrichment toxicity tests are based on the growth of *Enterobacter aerogenes* in a chemically defined minimal growth medium. The presence of a toxic agent or a growth promoting substance will alter the 48 hour population by an increase or decrease of 20% or more when compared to the control (APHA-AWWA, 1992). Twenty-one ml of wastewater samples taken from the effluent of the secondary settling tank was added to flasks B, C, D, E containing different type of substrate medium. The liquid volume of the flasks were 30 ml. Flask A; was the control and contained a sufficient amount of nitrogen (2.5 ml of ammonium sulfate at a concentration of 1.20 g l^{-1}), carbon (2.5 ml of sodium citrate at a concentration of 0.58 g l^{-1}), phosphorus (1.5 ml of KH_2PO_4 at a concentration of 2.70 g l^{-1}) sources and distilled water without effluent wastewater sample. Flask B contained the nitrogen, carbon and phosphorus sources as above and an effluent wastewater sample. Flask C didn't contain carbon or nitrogen sources. Flask D contained carbon and phosphorus sources with effluent wastewater sample but didn't contain the nitrogen source. Flask E contained nitrogen and phosphorus sources with effluent wastewater sample but didn't contain a carbon source (APHA-AWWA, 1992). All flasks were inoculated with 1 ml of *E. aerogenes*. Dilutions varying between 1: 10 and 1: 10^6 were carried out to arrive at a countable density range between 30 and 100 viable cells per ml in every flask. At the end of the incubation period at 27 °C, the number of *E. aerogenes* was counted by the membrane filtering technique. Assessment of enrichment toxicity test results was done based on the ratio of *E. aerogenes* numbers (B, D and E) to control (A) as follows: If the B/A ratio varies between 0.8 and 1.2 this shows that the effluent contains no toxic substances, but shows the presence of growth-limiting substrates in effluent samples. If B/A ratio is less than 0.8 this shows that the effluent wastewater contains toxic substances. The B/A ratio could go as high as 3.0 from 1.2. For ratios C/A, D/A, E/A a value in excess of 1.2 indicate the presence of available nitrogen or carbon sources or both of them for bacterial growth (APHA-AWWA, 1992). Fishes (*Lepistes sp.*) were used to determine toxicity dilution factor. At the end of 24 and 48 hours, the dilution in which all fishes were alive was accepted as the appropriate dilution ratio (Turkish Wat. Pollut. Control Reg, 1992).

BOD_5, TSS, and total chromium were measured as described in 5210 B, 2540 D and 3500 D, respectively by following Standard Methods (APHA-AWWA, 1992). COD and phenol concentrations were determined on filtered samples by using

Spectroquant Kits numbered 014541 and 014551. Color was measured in filtered samples as spectral absorption intensity by a Unicam spectrophotometer at 597 and 254 nm. The AOX measurements were carried out in AOX analyzer MT-20. The values reported in the figures were the averages of the measurements.

Differences of sensitivity score between microorganisms determined in conventional acute toxicity test were examined by performing a non-parametric Kruskal-Wallis test followed by a Mann-Whitney U test between paired microorganism groups (Conover, 1971). All results are reported at a significance level of $p \leq 0.10$ (Zar, 1984). The s tatistical package used for analysis was SPSSWIN in Windows (Norussis, 1986).

RESULTS AND DISCUSSION

The pulp-paper industry treatment plant consists of a bar screening unit, an equalization tank, a primary settling tank, a biological treatment plant following the chemical treatment and a secondary settling tank. The treated chlorinated effluent is discharged in İzmir Bay. The Turkish Receiving Water Discharge Standards for pulp-paper industry effluents were as follows: COD, 800 $mg.l^{-1}$; BOD_5, 300 $mg.l^{-1}$; TSS, 50 $mg.l^{-1}$; pH, 6-9; T.Cr, 2 $mg.l^{-1}$ and TDF, 8 (Turkish Wat. Pollut. Cont. Reg., 1988).

The water quality analysis results showed that the sampled effluents were violating the discharging limits on days 1, 10, 80, 90, 100, 110, 170, 180 and 190 in the pulp-paper industry (Figures 1 and 2). Little work has been carried out on bleaching and kraft-mill products using conventional toxicity tests in Turkey. A severe problem with the decolorisation of dye containing bleaching process discharges is that this would pose a serious hazard if released into the aquatic environment untreated. If the COD/BOD_5 ratio is between 3 and 5, the wastewater is industrial and can be degraded slowly. If COD/BOD_5 ratios vary between 3 and 9, the wastewater is toxic (Tchobanoglous and Burton, 1991). Significantly, many of the effluent samples also exhibited high COD/BOD_5 ratios such as 3 and 6 on days 1, 10, 80, 90, 100, 170, 180 and 190. Throughout these experiments high COD concentrations in the effluent was accompanied by high levels in TDF (r^2=0.94, p= 0.005). The same results were observed for phenol and the permissible levels were also exceeded. The color and AOX in the effluent samples were measured at high levels on days 1, 10, 80, 90, 180 and 190 on which acute toxicity was observed (See Figures 1 and 2). The multiple regression analysis between TDF, COD, phenol, AOX and color showed that a very strong linear correlation exists between y (TDF) and x (COD, phenol, AOX and color) variables in the effluent samples (r^2=0.94, p= 0.005).

Table 1 shows all acute toxicity and enrichment test results carried out during the nine months of the sampling period in the effluent samples. Throughout these analyses the EC_{50} values measured for protozoan differed significantly from statistics showed that protozoan had higher EC_{50} values and lower bacteria, fish showed that protozoan had higher EC_{50} values and lower sensitivity scores than

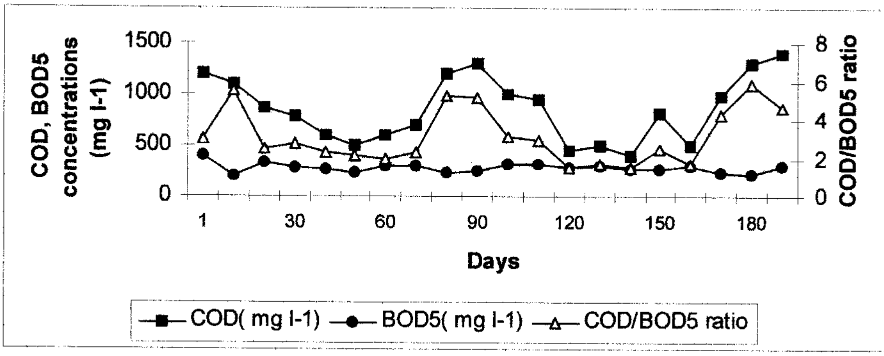

Figure 1. Variation of COD and BOD concentrations in the effluents

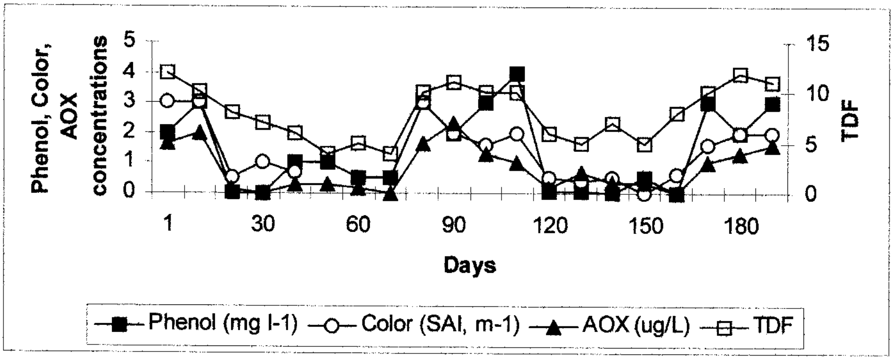

Figure 2. Variation of chemical parameters and TDF in the effluents

the other remaining microbial groups. Definitive acute tests in the pulp-paper industry showed that EC_{50} (%) value was between 70-100% for protozoan in the effluents. Similar results were obtained by Slabbert and Venter (1999).

The coliforms and floc bacteria had similar toxicity response and mortalities to the paper industry effluents (Mann-Whitney U-test statistic=0.345, p=0.10). The coliform bacteria had lower EC_{50} values than protozoa and these differences were significant (Mann-Whitney U-test statistic=8.540, p<0.10) The toxicity response of coliform and algae was found to be different. Nevertheless, these differences were not significant (Mann-Whitney U-test statistic=0.194, p=0.10)

All the results obtained from the acute definitive toxicity tests were confirmed by the results obtained from the enrichment tests in which *E. aerogenes* was used instead of coliform, floc bacteria, algae, fish and protozoan. The results of the enrichment tests showed that the bacteria *E. aerogenes* was a reliable indicator in the assessment of toxicity for pulp and paper industry effluents. The B/A ratios obtained from the enrichment toxicity tests gave detailed information about properties of wastewater effluents such as toxic, limiting or stimulating substrate

Table 1. Toxicity test results in pulp-paper industry effluents (Mean; n= 3)

Days	EC_{50} Values (% V/V)					Enrichment Toxicity Tests Remarks of B/A ratio° *general results**	
	Coli form	Floc Bacteria	Fish	Algae	Proto zoan	(B/A) Ratio	
1	0.6	0.17	1.0	0.19	12	0.26	**Potential Toxicity°** ***Acutely Toxic****
10	0.5	0.19	1.0	0.22	> 100	0.19	**Potential Toxicity°** ***Acutely Toxic****
20	40	88	90	98	> 100	2.34	Growth Stimulation° *Not acutely Toxic**
30	40	63	79	68	> 100	1.8	Growth Limit.Nutr.° ***Moderately Toxic****
40	39	58	40	56	87	1. 24	Growth Limit. Nutr.° ***Slightly Acut. Toxic****
50	12	9	22	34	87	1.04	Growth Limit. Nutr.° ***Moderately Toxic****
60	99	88	77	> 100	89	1.98	Growth Stimulation° *Not Acutely Toxic**
70	> 100	> 100	> 100	> 100	> 100	2.01	Growth Stimulation° *Not Acutely Toxic**
80	0.5	0.2	0.7	0.5	87	0.3	**Potential Toxicity°** ***Acutely Toxic****
90	0.3	0.05	0.1	0.42	0.56	0.4	**Potential Toxicity°** ***Acutely Toxic****
100	0.3	0.3	0.05	23	85	0.45	**Potential Toxicity°** ***Acutely Toxic****
110	6	4	3	8	100	0.9	Growth Limit. Nutr.° ***Moderately Toxic****
120	89	70	100	100	89	2.19	Growth Stimulation° ***Not Acutely Toxic****
130	98	97	86	95	> 100	2.65	Growth Stimulation° *Not Acutely Toxic**
140	69	89	> 100	34	459	0.88	Growth Limit. Nutr.° ***Slightly Acut. Toxic****
150	98	99	90	99	100	2.58	Growth Stimulation° *Not Acutely Toxic**
160	> 100	> 100	99	70	75	1.97	Growth Stimulation° *Not Acutely Toxic**
170	0.40	0.12	0.8	0.5	90	0.40	**Potential Toxicity°** ***Acutely Toxic****
180	0.14	0.6	0.7	0.12	> 100	0.83	Growth Limit. Nutr.° ***Slightly Acut. Toxic****
190	67	27	18	19	99	0.88	Growth Limit. Nutr.° ***Slightly* Acut. Toxic***

contents. The data generated by the samples indicate that all the samples tested were not toxic to all test organisms. Overall toxicity for the aforementioned days was to be expected for most of the samples which are associated with high COD, AOX, phenol and colors. The precise chemistry of effluents are not known very well except for COD, AOX, phenol and color. Therefore it is not possible to speculate on the mode of toxic action due to the difficulties involved in the characterization of the chemical content of wastewater.

Although in some cases the effluent wastewater characteristics have ensured the prescribed effluent quality in standards given for the paper industry; the toxicity test results indicates a potential toxicity. For instance although the COD, phenol concentrations and TDF values were lower than the permissible discharge limits in the paper industry on some days; the toxicity test results showed the presence of acute toxicity. It is to be concluded that the reasons for this are a lack of knowledge of the composition, and a lack of acute toxicity data for the substances which are known to be present. The general classification of all effluents based on both conventional and enrichment tests can be summarised as follows for the eight months of the sampling period: 5 effluents are acutely toxic, 3 effluents are moderately acutely toxic, 4 effluents are slightly acutely toxic and 8 effluents are not acutely toxic. Moderate and slight acute toxicity were observed on certain days depending on AOX and color concentrations in effluents. The effluent toxicity could not be explained by using physicochemical tests. Acute toxicity was observed for at least two organism in 6 out of 20 effluent analyses

The results of this study showed that the enrichment toxicity tests are practical, cost effective and accurate tests in the determination of potential effects. The toxicity of effluents discharged into the environment should be effectively monitored and a toxicity test should be included in water quality legislation to give recommendations concerning a strategy for regulatory toxicity control of different industrial branches. For environmentally significant discharges where not all important constituents can be individually identified, a clearly defined toxicity limit should be specified, along with the appropriate toxicity test to be used. The authorities should take urgent measures to reduce the quantity of dangerous substances before they reach the aquatic environment. Toxicity tests should be incorporated into receiving water discharge standards and the existing discharge standards should be reviewed to include the toxicity tests in Turkey.

Acknowledgments The financial assistance from Dokuz Eylül University AIF grant no ***0 922 98 01 34*** is acknowledged.

REFERENCES

APHA, AWWA, WPC (1992) Standard Methods for the examination of Water and Wastewater. 16 th edition, Washington D.C., USA.

Canton JW (1991) Catch-up operation on old pesticides: an integration. Rijksinstituut voor Volksgezondheid en milieu (RIVM) report no: 80 1002

Conover WJ (1971) Practical non-parametric statistics. Wiley, New York.

Dube MG, Culp JM (1997) Growth responses of periphyton and chronids exposed to biologically treated kraft pulp mill effluent. Wat Sci Techol 35 :339-345.

Dugan PR., Lundgren DC (1968) Microbiological analysis. Appl Microbiol 8: 357-361.

EPA (1991) Technical support document for water quality based toxic control. EPA 505/2-90-001.

Ferguson JF(1994) Anaerobic and aerobic treatment for AOX removal. Wat Sci Technol 29: 149-162

Haggblom M, Salkinoja-Solonen M(1991) Biodegradability of chlorinated organic compounds in pulp bleaching effluents. Wat Sci Technol 24: 161-170

Kinnersley D (1990) Discharge consent and compliance policy: a blueprint for the future, NRA water quality series, No. 1.

Norussis MJ (1986) SPSS/PC for the IBM/P , XT/AT, SPSS Inc., Chicago.

Pelezar MT, Chan ECS (1972) Laboratory experiences in microbiology. third edition, Mc Graw-Hill Book Company, USA.

Schowanek D, Fox K, Holt M, Schroeder F, Koch V (2001) GREAT-ER: a new tool for management and risk assessment of chemicals in river basin. Wat Sci Technol 43: 179-185

Slabbert JL (1996) Guidelines for toxicity bioassaying of water and effluents in South Africa, Contact report for the water research commission, project no. K5/358/0/1, Division of Water Technology, Pretoria, South Africa.

Slabbert JL, Venter EA (1999) Biological assay for aquatic toxicity testing. Wat Sci Technol 39: 367-373.

Sponza D (2002) Necessity of toxicity assessment in turkish industrial discharges (Examples from metal and textile industry effluents). Environ Monit Asssess 73: 41-66.

Sponza D (1999) Assessment of biotoxicity analysis for different industrial effluent. In Karpuzcu M (ed) Priorities in the Environmental Pollution. vol 1. 18-19 November, Gebze, Kocaeli, İstanbul, p.578.

Sun YB, Chang HM, Joyce TW (1989) Dechlorination and decolorization of high molecular-weight chlorolignin from bleach plant effluents by an oxidation process. Textile Product Eng 72: 209-213

Tchobanoglous G, Burton FL (1991) Wastewater Engineering Treatment Disposal Reuse. Third ed., Metcalf and Eddy Inc., USA.

Tonkes M, de Graaf JJ, Graansma, J (1999) Assessment of complex industrial effluents in the Netherlands using a whole effluent toxicity approach. Wat Sci Technol 39: 55-61.

Turkish Water Pollution Control Regulation (1988) Receiving water discharge standards for different industries, In official newspaper numbered 19269 and 2 November, Volume 2, Lebib publications, Ankara Turkey.

Turkish Water Pollution Control Regulation (1992) Receiving water discharge standards for different industries In official newspaper numbered 19900 and 10 September (revised), Volume 2, Lebib publications, Ankara Turkey.

Wharfe JR, Tinsley D (1995) The toxicity based and the wider application of direct toxicity assessment to protect aquatic life. J Chart Inst Water Environ Manag 5: 526-530.

Zar JH (1984) Biostatistical analysis , 2nd ed., Prentice Hall, Englemoood Cliffs.