Bull. Environ. Contam. Toxicol. (1993) 50:377–384
© 1993 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Use of Life-Tables and Application Factors for Evaluating Chronic Toxicity of Kraft Mill Wastes on *Daphnia magna*

Fernando Martínez-Jerónimo, Rafael Villaseñor, Félix Espinosa, and Guillermo Rios

I. P. N., Escuela Nacional de Ciencias Biológicas, Apdo. Postal 32-034, México D.F. 06030, Mexico

In México the pulp and paper manufacturing industry is one of the most important industrial activities, and holds the second place in the amount of wastewater discharges generated, accounting for about 12 % of the total (Poggi *et al.* 1989). It is well known that raw pulp and paper wastes are toxic to aquatic fauna, although most of the studies have been based on acute, short-term bioassays (McKean 1980). Acute toxicity tests are preferred as they are simple, inexpensive, and can be done with limited resources (Peltier and Weber 1985), but information obtained in this way often is insufficient to determine acceptable concentrations that ensure the protection of aquatic biota from chronic effects on growth, survival, and reproduction (Gentile *et al.* 1982; van Leeuwen *et al.* 1985). Because of the time, high cost, and complex equipment required to perform chronic toxicity tests, sometimes it is possible to predict a concentration that presumably has no sublethal or chronic effects from acute toxicity data, by using an Application Factor (AF), which is the ratio of the "Maximum Acceptable Toxicant Concentration" (MATC) to the Acute Median Lethal Concentration (LC50) value, for a given species and toxicant (Kenaga 1981; Adams and Heidolph 1985). The AF is used to obtain a safe level from a LC50, by dividing the LC50 by a value ranging from 10 to 20, for non-persistent pollutants, and from 10 to 100, for persistent chemicals and pesticides. Although these ranges are assigned based on the judgment of scientists, they often fall between the limits of 10 and 25 (Sprague 1971). This paper discusses the usefulness of AF's for determine "Safe Concentrations" of black liquor wastes from a paper kraft mill process for *Daphnia magna*, and experimentally assess their validity through life-table analyses of chronic bioassays.

Send reprint requests to F. Martínez-Jerónimo to the above address.

MATERIALS AND METHODS

The freshwater cladoceran *Daphnia magna* was used as test organism. The LC50 (48-hr) of a kraft mill effluent was estimated by means of an acute static toxicity bioassay, conducted according with the methodology suggested by Peltier and Weber (1985), but using 30 neonates per dilution, distributed in three replicates. A total of six effluent dilutions, with a 100-mL test volume, were applied in the definitive test. Reconstituted hard water (Peltier and Weber 1985) was used as dilution water in all the experiments.

For the chronic toxicity bioassay three dilutions of the LC50 were tested, corresponding to Application Factors of 3, 10 and 25. Each treatment had ten replicates and they were conducted in 125-mL glass vessels, with 80 mL of total test solution; an additional control series, without toxicant, was simultaneously carried out. One parthenogenetic female *Daphnia magna* neonate (less than 24-hr old) was randomly assigned to each replicate. The green microalgae *Ankistrodesmus falcatus* in a constant concentration of 250,000 cells/mL, was used as food; the medium (reconstituted hard water), with toxicant and food, was completely renewed twice a week. Survival was daily assessed. Upon reproduction, brood size was determined and neonates were separated from the mother thenceforth. Time to first reproduction, inter-brood time, variations in clutch size, total fecundity, and longevity were determined for all the treatments and the control. A life-table approach was used in order to calculate age-specific survivorship, net reproductive rate (R_o), and reproductive value (V_x); these demographic statistics were compared with traditionally-used measures of acute and chronic toxicity. Experiments were carried out over one full life-cycle, *i. e.*, until all the individuals died in all the replicates. Dissolved oxygen concentration and pH were recorded daily; temperature was 20±1 °C.

RESULTS AND DISCUSSION

The LC50 for the kraft mill effluent was estimated by means of the probit method (Peltier and Weber 1985), being 13.4 %. The dilutions applied for the chronic bioassay were: 4.47 % (AF=3), 1.34 % (AF=10), and 0.54 % (AF=25). Figure 1 shows the survival curves for *Daphnia magna*, up to the 40th day. Survival drops abruptly after the 9th day in the AF=3; although this effluent concentration did not produce acute toxicity effects, test organisms were in poor condition and all died by the 11th day. A similar response was observed with AF=10 (Fig. 1), but in this dilution, mortality increased steeply after the 8th day.

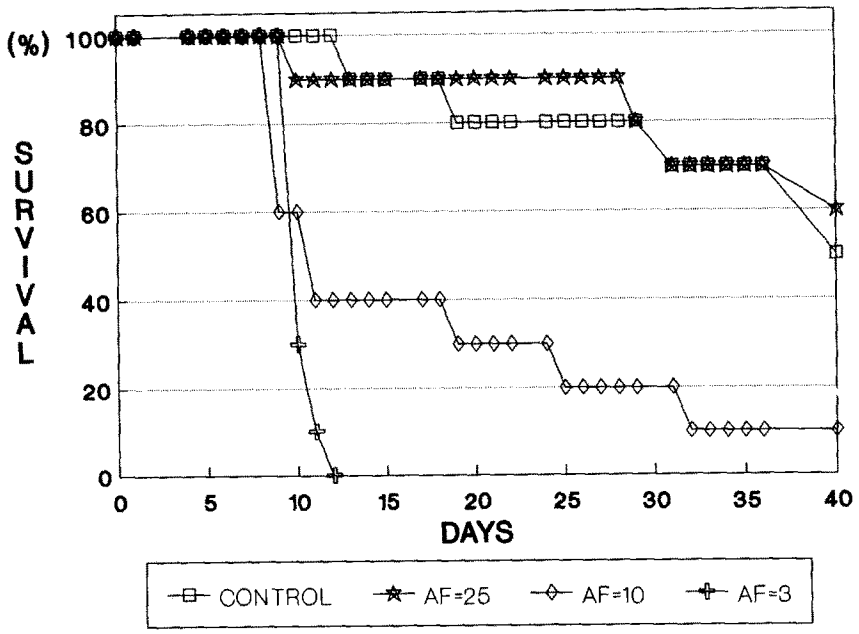

Figure 1.- Survival curves for *Daphnia magna* with different application factors for dilutions of Kraft mill wastes.

Survival curves for control and AF=25 are very similar up to the 39th day, with test organisms in AF=25 having a slightly higher survival. The mortality observed in the control is acceptable, as it is in accordance with the normal variation recorded for this trait in *Daphnia magna* (Adams and Heidolph 1985); in fact, the lack of consistent and sustained reproduction, as well as inadequate survival over the 21-day period, has been strongly criticized as one of the most frequent problems in chronic bioassays with *D. magna*. As can be seen in Fig. 1, from the 20th and 30th day, and up to the 39th day, survival curves show the greatest differences in treatment's effect, with respect to the control.

Clutch sizes in consecutive reproduction until the 40th day, are shown in Figure 2. Ninety percent of the test organisms in AF=3 had only one reproduction, but most of the offspring were dead or in poor condition; this effect was produced by the toxicant concentration in the dilution applied. Reproduction, as the only one endpoint, is not enough to assess chronic toxicity effects, because, as can be seen in Fig. 2, treatments

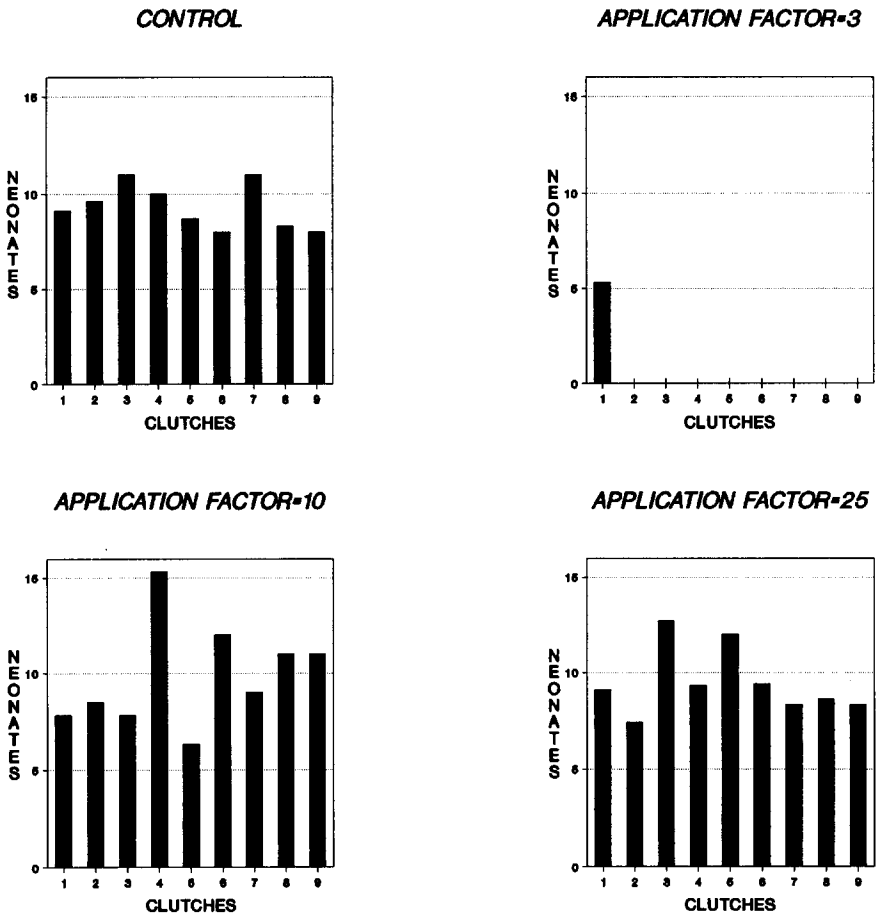

Figure 2.- Average live offspring in consecutive clutches for *Daphnia magna* with different AF for dilutions of Kraft mill wastes.

like AF=10, which actually produced chronic toxicity (Fig. 1), on average had similar reproduction and even larger clutch sizes than the control. Inter-brood times are shown in Table 1; although the average inter-brood time for the control is slightly shorter than those of the treatments, a one-way ANOVA indicates that there were not statistically significant differences among them ($P>0.05$).

For the life-table approach, organisms were classified into 5-day age-classes, and the age-specific survivorship (l_x) and fertility values (m_x) were calculated according to Krebs (1985). Reproductive

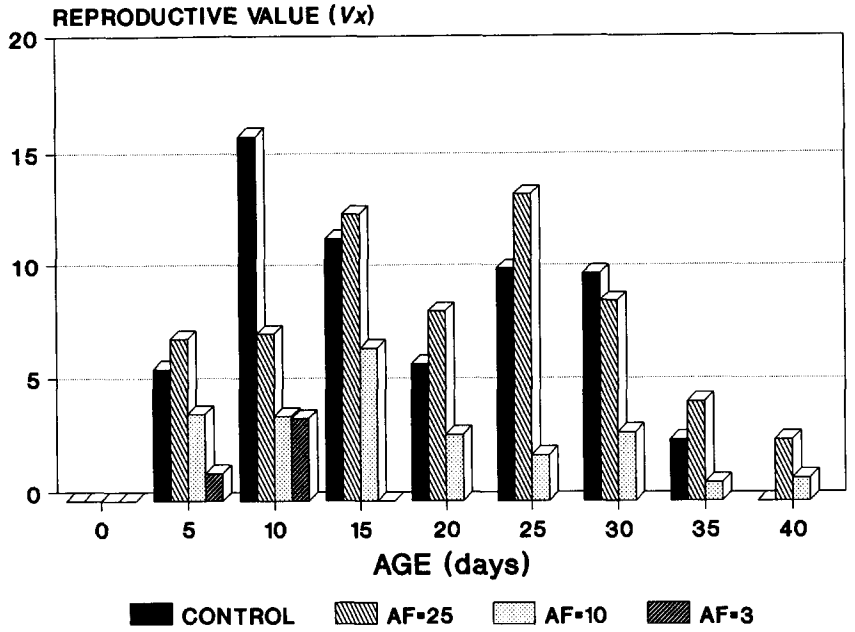

Figure 3.- Reproductive values for *Daphnia magna* with different AF for dilutions of Kraft mill wastes.

values (V_x) were calculated as $l_x \cdot m_x$, and these data, up to the 40th day, are shown in Figure 3. As can be seen, the trend followed by reproductive values for the AF=25 is very similar to that of the control and, even though organisms with AF=10 had a longer survival, their V_x's were always smaller than the control. Figure 3 shows clearly that the only concentration of Kraft mill wastes with no chronic effects, is that corresponding to a AF=25. Net reproductive rates (R_o) were calculated as the sum of all the V_x's, separately for each treatment and for the control; these values are shown in Table 2. An one-way ANOVA, followed by a

Table 1.- Inter-brood time (days) for *Daphnia magna* with different AF for dilutions of Kraft mill wastes.

APPLICATION FACTOR DILUTION (%)	Control	25 0.54	10 1.34
Mean	3.39	4.24	4.04
Standard deviation	1.42	2.25	1.57
n	42	84	25

n: number of total observations

Gabriel's multiple comparisons test (Sokal and Rohlf 1981) indicated that there were no significant differences in R_o for the control and AF=25, whereas R_o's for AF=3 and AF=10 significantly differ from that of the control ($P<0.05$). According to these results, a "safe concentration" for the kraft mill waste could be estimated from the LC50 value previously established, by the use of an Application Factor of 25. It has been demonstrated that an AF=10 does not ensure the protection of the population against chronic effects in prolongated exposures and, although some organisms in this concentration can survive and reproduce apparently without any damage, at the population level they were in fact affected, showing low reproductive values and a definitively lower net reproductive rate (Table 2).

Table 2.- Net reproductive rates (R_o) for *Daphnia magna* with different AF for dilutions of Kraft mill wastes

A F DILUTION (%)	Control	3 4.47	10 1.34	25 0.54
R_o	62.33	5.8	29.80	84.93

Analyzing the results as a whole, most of the conclusions could have been obtained by the 20th day. Longer experiments (like those covering the whole life-cycle) have, besides their higher cost, some inconsistencies in the reproduction and survival of test organisms (Adams and Heidolph 1985). This conclusion is in agreement with the general tendency observed in modern aquatic toxicology, in the sense of reducing the time to obtain results to match a constantly increasing demand for assessing and preventing damages to aquatic ecosystems, and in addition to this, to bring down analysis cost. There are many papers dealing with this subject, but most of them are related to the use of other species with shorter life-cycles (*e.g.*, *Ceriodaphnia*); nevertheless, as Lewis and Horning (1988) have pointed out, *Ceriodaphnia* tests are labor intensive and counting of the neonates requires the use of a stereomicroscope. Adams and Heidolph (1985) carried out 7-, 14- and 21-d sub-chronic tests, concluding that there are only minor differences between 14- and 21-d MATC's; as they stated, the beneficial impact of reducing the exposure period would be to reduce costs, increase confidence in the daphnids data base, and increase the utility of this species.

In the same way, Lewis and Horning (1988) put forward a

partial life-cycle test with *D. magna*, which requires only 7 d and is carried out with 10-d-old organisms, assessing as end-points survival and reproduction. They conclude that, for the reference toxicant sodium pentachlorophenate, the No Observed Effect Concentration (NOEC) for this 7-d bioassay is very similar to that in the frequently recommended 21-d test. Our results support the use of short tests for assessing chronic effects of toxicants and toxic effluents, and suggest using of demographic parameters as additional criteria in aquatic toxicology. Application Factors could be also a way to reduce time and costs in the determination of safe concentrations for effluents.

Acknowledgments. This work was supported by a DEPI-IPN grant. Thanks to Dr. S. Sánchez-Colón for his review and comments of the manuscript.

REFERENCES

Adams WJ, Heidolph BH (1985) Short-cut chronic toxicity estimates using *Daphnia magna*. In: Cardwell RD, R Purdy, RC Bahner (eds) Aquatic toxicology and hazard assessment: Seventh symposium, ASTM STP 854, American Society for Testing and Materials, Philadelphia, pp 87-103

Gentile JH, Gentile SM, Hairston NG, Sullivan BK (1982) The use of life-tables for evaluating the chronic toxicity of pollutants to *Mysidopsis bahia*. Hydrobiologia 93:179-187

Kenaga EE (1981) Aquatic test organisms and methods useful for assessment of chronic toxicity of chemicals. Reg Toxicol Pharmacol 1:277-292

Krebs CJ (1985) Ecology, 3 ed. Harper & Row, New York

Lewis PA, Horning WB (1988) A short-term chronic toxicity test using *Daphnia magna*. In: Adams WJ, Chapman GA, Landis WG (eds) Aquatic toxicology and hazard assessment: Tenth symposium, ASTM STP 971, American Society for Testing and Materials, Philadelphia, pp 548-555

McKean WT (1980) Pulp and paper industry. In: Guthrie FE and Perry JJ (eds) Introduction to environmental toxicology. Elsevier, New York, pp 210-226

Peltier WH, Weber CI (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3 ed. EPA/600/4-85/013, U.S. Environmental Protection Agency, Cincinnati, Ohio

Poggy HM, Hernández R, Rinderknecht N, Calzada JF (1989) Supplemented Kraft condensate treatment in high rate anaerobic processes. 44th Annual Purdue Industrial Waste Conference, Purdue University, Indiana, U.S.A., pp 1-25

Sokal RR, Rohlf FJ (1981) Biometry, 2 ed. W. H. Freeman and Co., San Francisco

Sprague JB (1971) Measurement of pollutant toxicity to fish. III Sublethal effects and "safe" concentrations. Water Res 5:245-266

van Leeuwen CJ, Luttmer WJ, Griffioen PS (1985) The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. Ecotoxicol Environ Saf 9:26-39

Received April 1, 1992; accepted September 15, 1992.