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Project Summary

Statistical Approach to Predicting Chronic Toxicity of Chemicals to Fishes from Acute Toxicity Test Data

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A methodology and a computer program were developed cooperatively by the U.S. Environmental Protection Agency (U.S. EPA) Ecological Risk Assessment Research Program and the University of Missouri-Columbia to predict chronic toxicity of chemicals from acute toxicity test data. A comprehensive approach to predicting chronic toxicity from acute toxicity data was derived in which simultaneous consideration was given to concentration, degree of response, and time course of effect. A consistent endpoint (lethality) and degree of response (0%) were used to compare acute and chronic tests.

The software, Multifactor Probit Analysis (MPA), calculates the lethal concentration of a chemical for expected effect, P (probability of response), for extended periods of exposure time. The MPA software is versatile, and the user can choose from several probit models and seven different transformation combinations of the independent variables. This software is entirely menu driven, allows the user to predict concentration of a toxicant at any time and any percent effect, and calculates a point estimate and a measure of dispersion (95% approximate confidence limits).

Predicted no-effect concentrations were highly accurate 92% of the time (within a factor of 2.0 of the limits of the maximum acceptable toxicant concentrations for lethality) when the technique was applied to a data base of 18 chemicals and 7 fish species. Predictions were also quite accurate for a pond study and two quail tests. Growth effects can be estimated from predicted chronic lethality, but reproductive effects should not be.

This Project Summary was developed by EPA's Environmental Research Laboratory, Gulf Breeze, FL, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Using acute lethality data to estimate chronic toxicity to fishes customarily involves deriving an application factor or an acute-chronic ratio, both of which require acute and chronic toxicity testing. The application factor is derived by dividing the MATC for a compound, as determined in a chronic toxicity test with a given fish species, by the acute flow-through LC50 for the same compound tested with the same species. The acute-chronic ratio (ACR) is the inverse of AF. The AF or ACR is then used to estimate chronic no-effect concentrations for other species for which only acute toxicity data exist. Both approaches have limitations in using these ratios to estimate chronic toxicity.

One limitation is that biological endpoints and degrees of response are often not comparable between acute and chronic toxicity data. When one uses either the AF or ACR, the acute median lethal concentration (LC50) is compared with the MATC, often derived from an endpoint other than lethality. Even though the mode of action for lethality is often assumed to be the same under acute and chronic exposures, the mode of action may not be

the same for different endpoints (e.g., growth or reproduction compared with lethality). Although different degrees of response (acute 50% versus chronic no-effect or 0%) could be used when response slopes are similar, the slopes may be different. Additionally, the use of the AF or ACR method does not take into consideration the progression of lethality through time that is observed in acute toxicity tests. The concentration-time-response interaction has been addressed previously, but it has been directed toward better defining the LC50. The acute toxicity value represents only one point in time (96-h LC50), and the progression of degree of response with duration of exposure should be essential when one predicts chronic toxicity from acute toxicity data.

A more comprehensive, alternative approach is proposed here in which simultaneous consideration is given to concentration, degree of response, and time course of effect, all of which are usually included in the results of an acute test, but seldom used. A consistent endpoint (lethality) and degree of response (0%) are used to predict chronic lethality from acute toxicity tests. Two assumptions may be required: (1) concentration-response is a continuum in time, and (2) the mode of action for lethality is similar under acute and chronic exposures.

Methods

Simple linear regression (Y = a+bX) was used to derive lethal concentrations of no effect (LC0 = 0.01%) for each observation time in an acute toxicity test and to predict the chronic no-effect concentration for lethality from those LC0s.

Degree of Response

In chronic toxicity tests, we are most often interested in the no-effect concentration (e.g., that concentration causing 0% effect), whereas in acute tests, the degree of response usually used is 50%. Although a probit value does not exist for 0% or 100%, an approximate value can be derived. In the use of probit analysis of acute toxicity data, the probit value used for 100% mortality is actually the probit value for 99.99%. An approximate value for LC0 can thus be derived by subtracting the probit value for 99.99% (8.7190) from 10 to provide a probit value of 1.2810 for 0.01% mortality.

Time Course of Effect

Predicting chronic toxicity from acute toxicity data requires a means of estimating the LC0 for an indefinite period of time (chronic) from an acute toxicity test conducted over a finite period of time (96-h LC50). Approaches to the problem of estimating tolerance over an indefinite time period have been developed by other researchers, although it was with the LD50 or LC50. They noted that as the time of exposure becomes sufficiently long, the LD50 or LC50 approaches an asymptotic value. A hyperbola describes this relationship and can be expressed as a straight line by using the reciprocal of time (t) as the independent variable. The equation becomes LD50 = a+b(1/t). Since 1/t approaches zero as t approaches infinity, the intercept (a) represents the LD50 over an indefinite time of exposure.

Technique

The acute toxicity test must be conducted with strict adherence to standard test methods to obtain estimates of LC0 over time. The times of 24, 48, 72, and 96 h were selected because observations in standard acute toxicity tests are usually made at these time periods. Less than 24-h observations were used when available. Inclusion of these observations is very important when most toxicity occurs during the early part of a 96-h test. The greatest concentration that causes no mortality and the least concentration that causes complete mortality were used for 0% and 100% responses. All concentrations causing mortality (0%≤x≤100%) were also included in our calculations. When regression analysis could not be conducted (less than 3 observations), the highest nonlethal concentration was used as the estimate of LC0 for that observation time. Having a range of mortalities for all time periods is best; although observation times with only 0 and 100% mortalities are acceptable if a concentration-response is evident in time.

Linear regression analysis was used to calculate the estimated LC0 at all observation times from acute flow-through tests (Figure 1) as probit % mortality = a+b(log concentration). The LC0's at each time period were then regressed against the reciprocal of time (Figure 2) where LC0 = a+b(1/t). The intercept (a) of this regression is the predicted no-effect concentration for chronic lethality. Log transformations, log LCO = a+b (l/t) or log LCO = a+b log (l/t), were required for ten tests because of negative intercepts and/or curvilinear nature of the data.

When test data permits, response-surface models (multiple regression) for analyzing all data from an acute toxicity test simultaneously (Figure 3) are preferable to the two-step simple linear regression approach described above. We therefore developed a probit surface methodology and a user-friendly software program for simple linear and multiple regression models to predict chronic toxicity based on acute time-exposure-effect data. This method is called Multifactor Probit Analysis (MPA) and uses the iterative reweighed least squares method to estimate the pa-

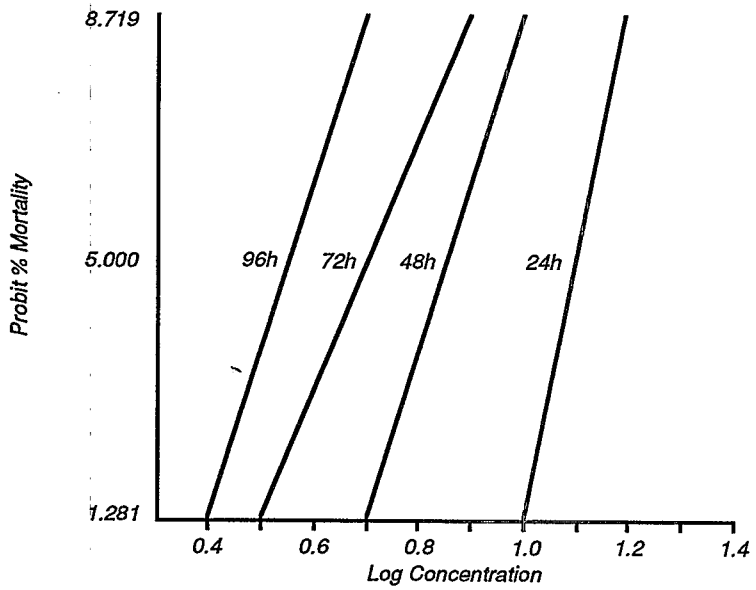

Figure 1. Dose-response curves used to derive the LCO (0.01%) for various observation times in acute toxicity tests (1.281 = a+bX). Probit % mortality: 1.281 = 0.01%, 5.000 = 50%, and 8.719 = 99.99%.

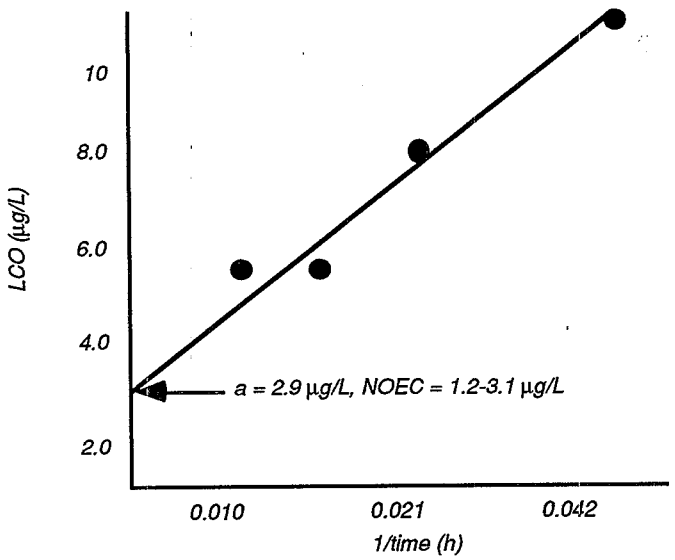

Figure 2. *Prediction of the chronic no-effect value for lethality from acute toxicity test data with Kepone and fathead minnows (LCO = a+b [1/t]). The intercept (a) represents the LCO (2.9 µg/L) over an indefinite exposure time, and the maximum acceptable toxicant concentration (MATC) for chronic lethality was between 1.2 and 3.1 µg/L.*

rameters of the probit surface. The independent variables consist of time of exposure and concentration of the toxicant. The dependent variable is the probit of the proportion responding to an exposure concentration. MPA allows the user to predict the concentration of a toxicant at any time and percent mortality as well as calculate a measure of variability (95% confidence limits). MPA has two primary functions. The first function is for entering and editing data not only for the MPA subroutine, but also for other software. Data files already prepared in ASCII format can be retrieved using this function. The second function is statistical analysis. Once a data set has been entered, a selected MPA subroutine executes an analysis. The output which is produced depends on the analysis option chosen. Chi-square and r^2 values are used for selecting the best model. The analysis options are:

A. Simple probit analysis using one independent variable, which is concentration. A single exposure time is assumed.

B. Multifactor probit analysis which includes two independent variables; concentration and exposure time. This option assumes parallel probit regression lines at each exposure time.

C. Multifactor probit analysis using concentration, time, and interaction as independent variables. This option is different than B in that non-parallel probit lines over time are assumed.

D. Multifactor probit analysis with three independent variables (concentration of toxicant 1, exposure time, and a third variable which could be a second toxicant).

E. Simple probit analysis, using concentration as the independent variable, at each level of exposure time.

F. Simple probit analysis where the independent variable representing exposure time is the reciprocal of time (1/time).

G. Multifactor probit analysis where the independent variable representing exposure time is 1/time.

The calculation of LC0s is dependent on slope and time course of effect, both of which are influenced by sample size (number of fish per concentration) and dose separation (dilution factor among concentrations). In this study, sample sizes ranged from 10 to 30 organisms and dilution factors ranged from 50 to 75%.

Data Base

The acute and chronic tests selected for analyses were conducted at the Columbia National Fisheries Contaminant Research Center (U.S. Fish and Wildlife Service, Columbia, MO) and the U.S. EPA Environmental Research Laboratory, Gulf Breeze, FL, on seven fish species: rainbow trout, *Oncorhynchus mykiss*; cutthroat

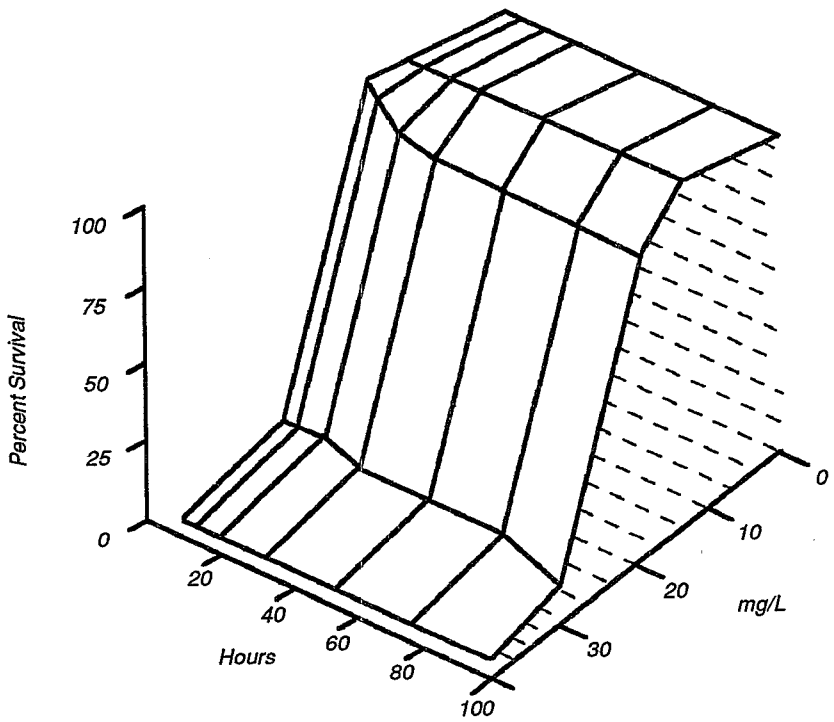

Figure 3. *Acute flow-through toxicity test results with carbon tetrachloride and sheepshead minnows demonstrating dose-response data in time (96-h LC50 = 19 mg/L).*

trout, *O. clarki*; brook trout, *Salvelinus fontinalis*; lake trout, *S. namaycush*; fathead minnow, *Pimephales promelas*; channel catfish, *Ictalurus punctatus*; and sheepshead minnow, *Cyprinodon variegatus*. With the exception of a few static acute tests used, acute and chronic tests were conducted in flow-through diluter systems. Each diluter delivered four to seven concentrations of toxicant and a control. Water temperature was maintained within ±1° C of the desired temperature, and day length was regulated. Acute and chronic tests were conducted in accordance with standard procedures, and concentrations of all chemicals were measured.

Acute and chronic flow-through tests were also conducted with carbon tetrachloride and sheepshead minnows as another test of the LC0 method, because carbon tetrachloride is considered to have different modes of action between acute and chronic exposures with mammals.

Two additional types of data sets having available and acceptable acute toxicity tests were analyzed — a pond study with bluegills, *Lepomis macrochirus*, and fluorene, a component of petroleum, and a study with coturnix quail, *Coturnix japonica*, and mercuric or methyl mercuric chloride. Fourteen 0.08 ha ponds were treated with various concentrations of fluorene (July 26, 1982). Ponds were drained approximately 70 days after exposure (early October 1982), and fish were counted, measured, and weighed to determine survival, growth, and production of recruits. Fluorene exposures in the ponds were based on average measured concentrations following treatment on days 1, 3, and 7. Acute toxicity tests with bluegills were conducted in the laboratory under static conditions to simulate pond exposures. Five-day acute dietary tests were conducted with coturnix quail by presenting the chemicals at various concentrations in turkey starter mash for 5 days. Daily observations for evidence of toxicity were made until clinical signs were no longer detectable (10 days). Chronic toxicity was determined by feeding the mercurials at various concentrations in *ad libitum* diets from hatching to adulthood (9 weeks).

Results and Discussion

Predicted values were compared with the observed values of chronic tests (early life-stage and partial and full life cycle toxicity tests) and proved highly accurate for a variety of chemicals and fish species (Table 1). The predicted no-effect concentrations (PNEC) were very close to or within the limits (highest concentration with-

Table 1. *Comparison of Observed Maximum Acceptable Toxicant Concentrations (MATC) and Predicted No-Effect Concentrations (PNEC) for Lethality Based on Flow-Through Acute Tests.*

Chemical and species	Log Kow	MATC (µg/L)	PNEC (µg/L)
Butyl benzyl phthalate	*4.44*	*>360*	*635* [a]
Fathead minnows			
Carbon tetrachloride	*2.64*		*10,427*
Sheepshead minnows		*4,500≤x≤11,200*	
Chlordane	*5.80*		
Sheepshead minnows		*7.1≤x≤17*	*14* [a]
Complex effluent			
Fathead minnows		*2.0≤x≤3.5%*	*5.2%*
2,4-D Butyl ester	*2.81*		
Cutthroat trout		*24≤x≤44*	*112*
Lake trout		*33≤x≤60*	*67* [a]
2,4-D PGBEE	*4.88*		
Cutthroat trout		*31≤x≤60*	*59*
Lake trout		*52≤x≤100*	*74*
Endosulfan	*4.90-6.00* [b]		
Sheepshead minnows		*1.1≤x≤2.5,0.92≤x≤2.1*	*1.4*
Endrin	*4.56-5.30*		
Sheepshead minnows		*0.12≤x≤0.31*	*0.12*
EPN	*4.80*		
Sheepshead minnows		*4.1≤x≤7.9*	*3.9* [a]
Fluridone	*1.87*		
Channel catfish		*1,000≤x≤2,000*	*1,182* [c]
Heptachlor	*5.44*		
Sheepshead minnows		*1.9≤x≤2.8,2.2≤x≤3.5*	*2.6*
Kepone	*6.08*		
Fathead minnows		*1.2≤x≤3.1*	*2.9*
Methoxychlor	*4.20*		
Rainbow trout		*1.1≤x≤3.1*	*0.94* [a,d]
Sheepshead minnows		*12≤x≤23,23≤x≤48*	*12, 12, 17* [a]
Pentachlorophenol	*5.01*		
Fathead minnows		*>142*	*240*
Phorate	*3.50*		
Sheepshead minnows		*0.24≤x≤0.41*	*0.15* [a]
Pydraul 50E	*4.62-6.08* [e]		
Fathead minnows		*317≤x≤752*	*592*
TFM			
Brook trout		*4,000≤x≤8,800*	*4,311*
Toxaphene	*4.83*		
Brook trout		*0.068≤x≤0.14*	*0.041*
Brook trout (adult)		*0.14≤x≤0.29*	*1.4* [a]
Fathead minnows		*0.62≤x≤1.3*	*1.7* [a]
Channel catfish		*0.07≤x≤0.13*	*0.057*
Sheepshead minnows		*1.1≤x≤2.5*	*0.77* [a]

[a] Log transformation of LCO's required.
[b] Endosulfan I = 4.90, Endsulfan II = 6.0.
[c] Based on static test.
[d] Acute toxicity test for rainbow trout was not available and PNEC was based on brook trout test because of similarity in response to toxicants.
[e] Pydraul 50E is a hydraulic fluid consisting of three components; triphenyl phosphate = 4.62, nonylphenyl diphenyl phosphate = 5.93, cumylphenyl diphenyl phosphate =6.08.

out effect on survival and the next higher concentration with a significant effect) of the maximum acceptable toxicant concentrations (MATC) for lethality and varied by less than a factor of two from an MATC 92% of the time. The other 18% of the predictions (two observations) consisted of factors of 2.5 and 4.8 of the observed concentrations.

The technique worked very well in predicting chronic lethality of carbon tetrachloride to sheepshead minnows (PNEC = 10.4 mg/L, observed = 4.5≤x≤11). Although this was only one study with one fish species, it may indicate that one assumption (i.e., the mode of action for lethality is similar under acute and chronic exposures) is not required for the technique. It may also be that carbon tetrachloride does not have different modes of action between acute and chronic exposures in fishes as has been observed for mammals.

The predictive technique was also highly accurate among various single chemicals and mixtures; it seemed representative of a wide range of octanol-water partition coefficients (log K_{ow}). Results of acute static tests might be used when flow-through tests results are not available and the log K_{ow} is low (e.g., fluridone). Chemicals that are highly water soluble will not adsorb to the test container or be taken up by the test organisms as much as with chemicals of low water solubility, and exposure will more closely resemble that for flow-through tests. However, additional research is needed to determine the log K_{ow} below which static test data can be used to predict chronic toxicity.

Although the other studies (pond and quail) analyzed represent a very small data set, it is notable that the PNECs were accurate. The ponds were dosed in a static acute manner (MATC for lethality = 0.0≤x≤67 µg/L) and therefore, static acute toxicity test data were used to determine the PNEC (24 µg/L). With coturnix quail, the observed no-effect dietary concentrations for lethality were >32 ug/g for mercuric chloride and 2.0≤x≤8.0 µg/g for methyl mercuric chloride with PNECs of 226 and 1.3 µg/g, respectively.

The technique for deriving PNECs uses some aspects of concepts developed previously. Acute tests have been conducted until the toxicity curve becomes parallel to the time axis, indicating a threshold concentration. An incipient LC50 is then estimated by selecting an exposure time from the asymptotic part of the toxicity curve. The reciprocal of mean survival times within concentrations was used as early as 1917. Regressing the reciprocal of mean survival time on concentration to derive theoretical thresholds of toxicity was further developed during 1957-67. Although observing survival times in acute tests has merits, it is laborious and is only infrequently done.

Table 2. *Regression Correlations [a] of Survival and Growth No Observed Effect Concentrations Among all Fish Species and Chemicals at Various Time Periods.*

Analysis and days of exposure	n	Intercept (a)	Slope [b] (b)	Coefficient of Determination (r^2)	y±95% C.I.
Weight vs. survival					
30	6	0.395	0.920	0.931	4.35±0.41
60	10	0.682	0.901	0.901	4.63±0.31
90	15	0.194	0.993	0.916	4.33±0.22
Length vs. survival					
30	16	0.284	0.968	0.945	4.64±0.18
60	17	0.263	0.965	0.949	4.60±0.15
90	18	0.275	0.971	0.941	4.41±0.17

[a] Log y = a+b(log x), where y is no-effect concentration for survival and x is no-effect concentration (ng/L) for growth (length or weight).
[b] All slopes were significantly different from 0 (p≤0.01).

The approach of incorporating all data in an acute test (concentration, degree of response, and time course of effect) to predict chronic lethality has a technical basis. During the last 20 years, fish chronic toxicity tests have been shortened from full life cycle tests to 30- to 90-day early life stage or partial life cycle tests, and then to 7-day subchronic tests. Reviews of subchronic, early life stage, partial life cycle, and full life cycle toxicity tests with several fish species demonstrated that the shorter tests are good estimators of chronic toxicity and MATCs observed in the longer life cycle tests. Although the success of developing briefer tests to estimate chronic toxicity is empirically based, it does support the toxicological concept of time course of effect in using acute data to predict chronic lethality.

Another use of acute toxicity data to estimate chronic toxicity is the toxicity threshold value or LC1, which is calculated for 1.0% mortality and at one point in time. This application of acute tests should work well for those chemicals, effluents, and others that differ little in toxicity between acute and chronic effects or where the LC1 is derived at a duration approaching or within chronic exposure conditions. However, the LC1 does not take into account time course of effect, and its use for predictive purposes is limited for a wide range of chemicals; particularly those that bioconcentrate or have cumulative effects.

Relation to Other Endpoints

Chronic toxicity tests commonly include the measurement of long-term effects of a contaminant on the survival, growth, and reproduction of a test organism. Survival and growth are often equally sensitive, and growth may not be of critical importance in establishing no-effect concentrations in most tests. In tests for which growth is the single most sensitive endpoint, survival could be used to estimate the no-effect concentration within a factor of 3.

Growth-related endpoints are highly predictable from survival effects with freshwater fishes (Table 2). Length was less variable than weight, and although all coefficients of determination (r^2) exceeded 0.9, they were slightly higher for length (0.941 to 0.949) than for weight (0.901 to 0.931). Also, no alteration was noted in the intercepts (a) for length versus survival between 30 and 90 days of exposure; the intercepts of weight versus survival varied, without trends, over time. Using these equations (Table 2), estimated no-effect concentrations for growth may be derived from the predicted values for chronic lethality.

No-effect concentrations were always less for reproduction endpoints than for survival, but attempts to relate acute lethality to chronic reproductive effects by regression analysis have not been successful. Because of the likelihood of different modes of action between lethal and reproductive effects, we do not recommend that reproductive effects be predicted using the proposed method. However, the proposed technique is highly beneficial in the preliminary assessment of chronic toxicity of effluents and other chemicals and in predicting chronic no-effect concentrations for survival and growth with fish species that are difficult to culture under chronic testing conditions.

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The complete report consists of paper copy and diskette, entitled "Statistical Approach to Predicting Chronic Toxicity of Chemicals to Fishes from Acute Toxicity Test Data":
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