Effect of Water Hardness on the Tolerance of the Guppy to Beryllium Sulfate

by
CHARLES B. SLONIM
Johns Hopkins University
Baltimore, Md.
and
A. R. SLONIM*
Aerospace Medical Research Laboratory
Wright-Patterson Air Force Base, Ohio 45433

It has been known for a number of years that various salts and metals are more toxic to aquatic organisms in soft water than in hard water, due partly to the buffering capacity and calcium antagonism of hard water solutions (see reviews by DOUDOROFF and KATZ 1950, 1953). Very little data exist, however, regarding the toxicity of beryllium salts to aquatic organisms in spite of the wide industrial usage of beryllium (Be) for over 40 years.

POMELEE in 1953 conducted a small study on the effect of $BeSO_4$ on several aquatic species, but was unable to demonstrate beryllium toxicity because of experimental problems and inadequate numbers of test organisms. In 1956, TARZWELL and HENDERSON reported on the acute toxicity of a variety of metals to the fathead minnow and bluegill. Their brief report included tabulated data on the 96-hour median tolerance limits of the sulfate, nitrate and chloride of beryllium, showing a 10 to 130-fold greater toxicity of beryllium in soft water (20 mg/l as $CaCO_3$) than in hard water (400 mg/l). Recently, SLONIM (1971-1973) reported on the interaction of beryllium sulfate and water of different hardness and on the acute toxicity of this salt to the common guppy as measured in a number of static bioassays. He found mainly that in soft water (20-25 mg/l) $BeSO_4$ was more acidic and about 100 times more toxic to guppies than in hard water ($\geq$400 mg/l). In the case of salamander larvae, $BeSO_4$ was more toxic by just one order of magnitude in soft than in hard water.[1]

This study was undertaken to determine the precise relationship between the median tolerance limit (TL_{50}) for $BeSO_4$ in guppies and water hardness. This paper presents the results of four static bioassays conducted simultaneously, with each bioassay using dilution water at a different level of hardness, respectively. In addition, the results of two subsequent bioassays are reported to assess the hypothetical relationship between the TL_{50} and water hardness.

Experimental

The analytical equipment, reagents and methods used in conjunction with this study have been described previously (SLONIM 1971). The materials, conditions and procedures used in the preparation

* Address for reprints.

[1] SLONIM, A. R., and E. E. RAY: Acute toxicity of beryllium sulfate to salamander larvae (Ambystoma spp). In preparation.

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and conduct of the bioassays were described in detail in a recent report (SLONIM 1973). The beryllium used in the past and present work was in the form of $BeSO_4 \bullet 4H_2O$. The hard water was the supernate of raw ground water that was collected in 9-liter flasks and allowed to stand for one week for sedimentation. This water (approximately 400 mg/l hardness) was diluted with glass distilled water to make up the different dilution waters for the other bioassays; duplicate analysis of the water samples showed the average hardness to be 400, 275, 150 and 22 mg/l, respectively. The fish used were common guppies, Lebistes reticulatus (Peters), and were a healthy Florida strain reared in open lagoons. One week prior to the bioassays, the fish were divided into equal groups and placed in separate aquaria containing water of the same hardness as the bioassay dilution water, respectively.

The bioassays were conducted according to standard procedures (Amer. Public Health Assn. et al. 1965). Two-liter test solutions (control and $BeSO_4$) were placed in one-gallon (3.8 l) widemouthed jars, into each of which five fish were transferred from the respective "acclimatization" aquarium. Duplicate jars were used for each test solution, so that 10 fish were tested at each beryllium level and control (diluent only). The concentration of all $BeSO_4$ solutions is expressed in terms of the beryllium ion, Be^{2+}. The range of Be concentrations selected for this study was extrapolated from Be levels tested in hard and soft water previously (SLONIM and DAMM 1972, SLONIM 1973).

Four bioassays were conducted at the same time, with fish from the same stock, of the same age (3 months) and size (2.4-3.4 cm and 0.10-0.22 g), and under the same environmental conditions, thus keeping the number of variables to a minimum. The response of the fish to the toxic metal was monitored every 1 - 2 hours around the clock, in cooperation with personnel working on other experiments; this continuous monitoring minimized the interaction of dead fish serving as a source of food for the survivors. Various symptoms of toxicity were noted, and the percent of survivors was recorded each day of the bioassay. The median tolerance limits were determined, as previously, for the 24, 48 and 96-hour exposure periods using the graphic interpolation method. This method involves a semilog plot of concentration versus percent survivors; a line is drawn between the two nearest points on each side of the 50% survival line. The concentration value on this line that intersects the 50% survival line is the median tolerance limit, TL_{50}. A log-log plot of the TL_{50} value of each bioassay versus water hardness was used to determine if a regression exists. A regression analysis of this plot was determined by the least squares method along with various statistical parameters (CROW et al. 1960).

One week following the start of the above bioassays, fish from the same stock were taken from two other aquaria and exposed to Be in two bioassays conducted simultaneously at two different hardness levels, 192 and 72 mg/l, respectively, to test the validity of the TL_{50} - water hardness relationship.

Bioassay Results

The chemical/physical characteristics of the different dilution waters alone and with varying amounts of $BeSO_4$ as well as the response of the fish to the test solutions are presented in Table 1. As noted previously, the pH was most affected by increasing amounts of $BeSO_4$; as Be was increased, pH decreased, and the pH level was lower as the water became softer. Similar to previous work (SLONIM 1971), alkalinity and dissolved oxygen did not change with increasing Be levels, although, as expected, alkalinity varied with hardness; only at or above 50 mg/l Be was there a noticeable increase in specific conductance, which reflects the amount of electrolytes present in solution (Table 1). The percent survivors at 24, 48 and 96 hours of beryllium exposure are shown also in Table 1. Note that the toxic effect of Be is not entirely pH-dependent (e.g., the varied response to Be around pH 6.5 in soft water at 96 hours of exposure).

When the percent survivors were plotted versus Be concentration, various curves were obtained for each bioassay per exposure period. The graphic data for the 96-hour period are given in Figure 1. As hardness is lowered, the curves shift downward. The median tolerance limit values for each bioassay are presented in Table 2. In one case, the TL_{50} in soft water (22 mg/l) at 24 hours only was not obtainable since over 50% of the fish survived in each Be concentration tested, including the highest level in soft water (2 mg/l Be). As reported recently (SLONIM 1973), the TL_{50} in hard water did not change after the first 24 hours of exposure, but in soft water became significantly lower from 24 to 96 hours of exposure. At the intermediate levels of hardness (275 and 150 mg/l, respectively), the TL_{50} did not change appreciably after the first day. For each exposure period, as water hardness was lowered, the TL_{50} decreased; thus, as water becomes softer, beryllium sulfate becomes markedly more toxic to guppies.

TABLE 2. Median tolerance limits to guppies of beryllium sulfate in water of varying hardness.

Water Hardness (mg/l)	Median Tolerance Limits, mg/l Be^{2+} *		
	24-hr	48-hr	96-hr
400	22.0	22.0	20.0
275	14.0	13.7	13.7
150	6.8	6.8	6.1
22	>2 †	0.32	0.16

*Determined by the graphic interpolation method.

†See text for explanation.

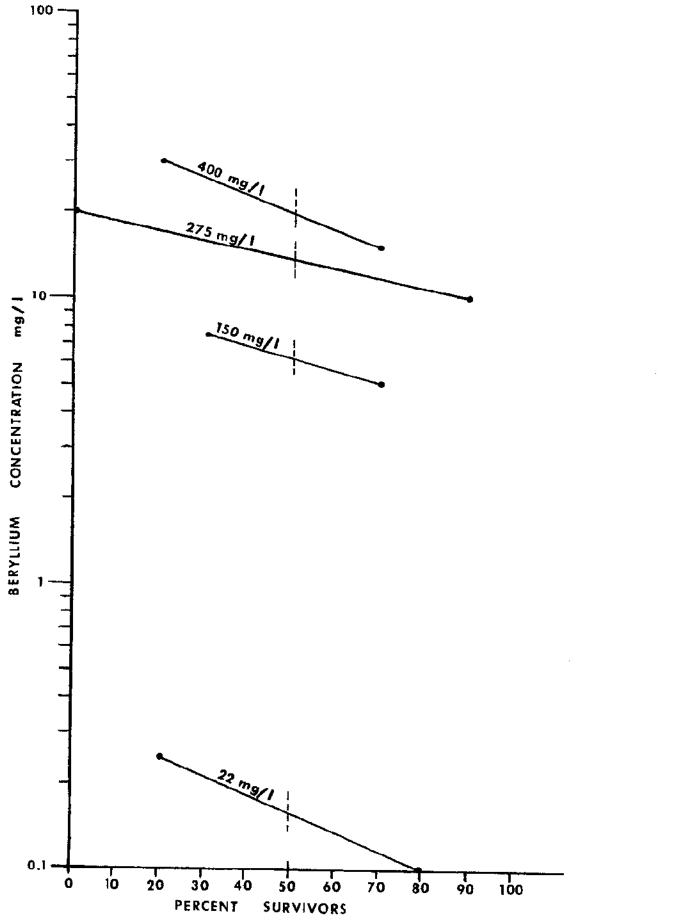

Figure 1. Median tolerance limits at 96 hours of exposure determined by graphic interpolation (values are in Table 2). Hardness value on each curve represents a separate bioassay.

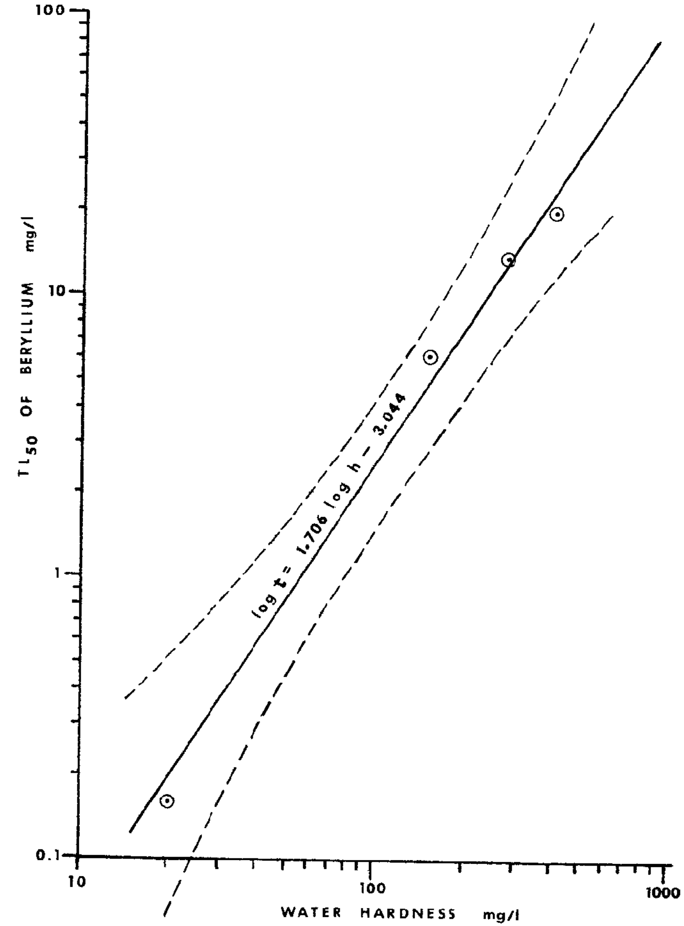

Figure 2. Median tolerance limit as a function of water hardness at 96 hours of exposure. Dotted lines represent 95% confidence range.

TABLE 1. Characteristics and biological effects of beryllium sulfate in water of different hardness.

Water Hardness (mg/l)	Be^{2+} Concn. (mg/l)	Physical Properties				Percent Surviving Guppies		
		pH	Specific Conductance (µmho/cm)	Total Alkalinity (mg/l)	Dissolved Oxygen (mg/l)	24-hr	48-hr	96-hr
400	0*	7.60	730	190	7.7	100	100	100
	5	7.00	745			100	100	100
	15	6.32	770			80	80	70
	30	5.90	910			30	30	20
	60	5.05	1175			0	0	0
275	0*	7.35	530	150	7.7	100	100	100
	1	7.30	550			100	100	100
	5	6.74	575			100	100	100
	10	6.25	587			100	90	90
	20	5.94	670			0	0	0
150	0*	6.82	305	66	7.7	100	100	100
	2.5	6.65	321			100	100	100
	5	6.40	340			80	80	70
	7.5	6.06	361			40	40	30
	10	5.91	380			0	0	0
22	0*	6.55	59.0	16	7.7	100	100	100
	0.1	6.53	59.5			90	90	80
	0.25	6.52	60.0			100	60	20
	0.5	6.47	61.7			90	30	10
	2	6.38	62.7			80	20	0

*Control (dilution water only).

TABLE 3. Relationship between the median tolerance limit (TL_{50}) of beryllium sulfate to guppies and water hardness per exposure period.

Exposure Period (hr)	Regression Equation*	r†
24	log t = 1.1965 log h - 1.7716	0.9999
48	log t = 1.4729 log h - 2.4478	0.9980
96	log t = 1.7060 log h - 3.0441	0.9955

*t = TL_{50} in mg/l Be and h = hardness of water in mg/l.

†r = correlation coefficient.

TL_{50} - Hardness Relationship

When the TL_{50} values in each exposure period were plotted versus water hardness, a regression was apparent. A regression line calculated by the least squares method was determined for each exposure period; the regression equation and correlation coefficient were computed also and are presented in Table 3. As the data indicate, the correlation coefficient of the log of the TL_{50} was very high for each exposure period; this was true also for the 24-hour regression line, which contained one less value than the 48 and 96-hour periods due to the lack of a specific TL_{50} value in soft water (see Table 2). The regression function between the TL_{50} and water hardness for the 96-hour exposure to $BeSO_4$ is illustrated in Figure 2; the dotted lines represent the 95% confidence limits.

TABLE 4. Test of the TL_{50} - water hardness relationship in two bioassays.

Water Hardness (mg/l)	Exposure Period (hr)	Median Tolerance Limit, mg/l Be^{2+}	
		Calculated*	Observed†
192	48	8.23 (5.92-11.43)	6.3
	96	7.10 (4.00-12.60)	6.3
72	48	1.94 (1.35-2.90)	2.8
	96	1.33 (0.71-2.61)	2.5

*Obtained from equations given in Table 3; 95% confidence limits are in parentheses.

†Determined by graphic interpolation of bioassay data.

Two bioassays using guppies from the same stock as those used above were run one week after the start of the above bioassays. The TL_{50} values at 48 and 96 hours of exposure obtained by the graphic interpolation method for each bioassay were compared with values calculated from the regression equations of Table 3. This evaluation is presented in Table 4. The observed TL_{50} was in agreement with the calculated value within the 95% confidence range at both exposure periods in both bioassays. The observed value could be checked also graphically with the computed regression line (e.g., Fig. 2). The relationship between the TL_{50} for $BeSO_4$ and water hardness can be described in logarithmic or exponential form. For the 96-hour exposure period, this relationship can be expressed as follows:

$$\log t = 1.7060 \log h - 3.0441 \qquad (1)$$

where $t = TL_{50}$ in mg/l Be and

h = hardness of water in mg/l

or exponentially: $$t = 0.0009034\, h^{1.7060} \qquad (2)$$

The equations for the 24 and 48-hour periods (Table 3) are applicable in the same manner. Thus, for any given level of water hardness, the TL_{50} (per exposure period) can be estimated for beryllium sulfate in guppies.

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