Acute Toxicity of Beryllium Sulfate to Salamander Larvae (*Ambystoma* spp)

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The effects of exposing common guppies to beryllium sulfate in hard and soft water, respectively, were studied extensively in a number of static bioassays (SLONIM 1973), including two incorporating radioberyllium as a tracer (SLONIM and DAMM 1972). This beryllium salt was 100 times more toxic to guppies in soft water (20-25 mg/l as $CaCO_3$) than in hard water ($\geq$400 mg/l); the median tolerance limit at 96 hours of exposure was 20.1 mg/l Be^{2+} in hard water and 0.19 mg/l in soft water (SLONIM 1973). The precise relationship between the median tolerance limit for beryllium sulfate in guppies and water hardness was described recently (SLONIM and SLONIM 1973).

The acute toxicity of beryllium sulfate to another class of organisms, the amphibians, was investigated using the same bioassay technique as reported previously with the guppies. The larvae of two species of salamanders (*Ambystoma*), which are usually larger than guppies, were used in this study. Amphibians, long used in organ regeneration experimentation, have been used in the past in beryllium studies. NEEDHAM (1941) was the first to use beryllium, in the form of beryllium nitrate, as a chemical tool to study the mechanism involved in tail development and regeneration in tadpoles. In 1949, THORNTON started a series of experiments using beryllium nitrate to analyze certain aspects of regeneration of the limbs of two *Ambystoma* larvae, *A. opacum* and *A. punctatum* (presently called *A. maculatum*), the same species studied in this laboratory. The results of four static bioassays, two of which were conducted simultaneously using larvae of different size, respectively, are reported in this paper.

Experimental

The analytical methods and materials, preparation and characteristics of hard and soft water test solutions, and the bioassay conditions, containers and procedures used in this study were described in detail previously (SLONIM 1973). The beryllium (Be) used in the past and present was in the form of $BeSO_4 \bullet 4H_2O$. The hard water was ground water supernate with a hardness of at least 400 mg/l (as $CaCO_3$); the soft water was a 1:20 dilution of the hard water with distilled water resulting in a hardness of 20-25 mg/l. The static bioassays were conducted according to standard procedures (Amer. Public Health Assn. et al. 1965).

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The salamander larvae, the aquatic stage of this organism, were all collected in Western Kentucky during the spring season. Specimens for the first bioassay were A. opacum, the marbled salamander, and were collected at Dawson Springs, while those used in the rest of the bioassays were A. maculatum, the spotted salamander, and came from The Land Between The Lakes area. The identity of these larvae was ascertained in the surviving adults after completion of the bioassays. All four bioassays were conducted within three months of each other (Table 1). The salamander larvae were sorted and tested at a large (45 mm average length), medium (35 mm) and small (25 mm) developmental size or stage. Two of the bioassays (A and C of Table 1) consisted of larvae of the same size, and in two assays (C and D of Table 1), larvae of different size were compared at the same time. A total of 380 larvae was used in this study, with 300 exposed to various Be concentrations and 80 to hard and soft water only (controls).

TABLE 1

Bioassay and organism data.

Bio-assay	Date	Salamander Species	N*	Size of Organism Weight, mg	Length, mm
A	4/24-28/72	marbled	120	333(217-445)	35.9(31-41)
B	5/ 1- 5/72	spotted	100	41.2(18-74)	20.1(12-24)
C	7/10-14/72	spotted	80	298(226-391)	35.5(32-40)
D	7/10-14/72	spotted	80	508(401-643)	44.8(42-48)

*N = number of organisms tested.

The salamander larvae were brought into the laboratory and placed in plastic trays, which were compartmentalized to hold one organism per cell. They were fed white worms (Enchytrea) twice per week and maintained at a room temperature of 23.5±1.5°C. After adjustment to laboratory conditions for a period of at least two weeks, the organisms were bioassayed in one-gallon (3.8 l) widemouthed jars, each containing five larvae per two-liter test solution ($BeSO_4$ or diluent). Duplicate jars were used for each test solution so that 10 organisms were tested at each Be level and control (diluent only). These organisms were monitored continuously every two hours; any unusual changes in appearance or behavior, or the date and time of death (removal) were recorded. The median tolerance limit (TL50) was estimated by the graphic interpolation method using a semi-log plot of the percent survivors versus beryllium concentration. Later the bioassay data were combined after the variability of the organisms was found to be insignificant and were analyzed by the least squares method in a computer program.

Results and Discussion

The results of the four bioassays are presented in Table 2. There was no change in response to Be in hard water from 24 to 96 hours of exposure in each of the bioassays; the median tolerance limit averaged 25.6 mg/l at 24 hours to 24.85 mg/l at 96 hours. The response to 10 and 100 mg/l Be in hard water (100% and 0% survival, respectively) was identical in all four bioassays. The TL50 values in Bioassays C and D were lower (not significant) than in A and B because C and D incorporated an additional Be test concentration to obtain a better estimate of the TL50. In soft water, beryllium sulfate was more toxic to the salamander larvae as the exposure period was increased, a response similar to that observed for the fish previously. The latent toxic effect of this salt in soft water was evidenced also by the fact that in 3 out of 4 bioassays at least 80% of the larvae survived at 10 mg/l during the first 24 hours of exposure. The slight difference in TL50 values between Bioassays C and D versus A and B reflect also the additional Be test solution incorporated in C and D. In two of the bioassays (C and D), 10 mg/l Be was the highest level tested in soft water and at least 60% of the organisms survived it the first 72 hours of exposure; thus, the TL50 for both bioassays was greater than 10 mg/l during this period. In contrast, in the other two bioassays (A and B), survival at 10 mg/l at the 48 and 72-hr periods, respectively, did not exceed 20%. Therefore, the mean of all four bioassays was at least 12 mg/l at 24 hours and 7 mg/l at 48 hours and equal to 5.65 mg/l at 96 hours of exposure. When the mean 96-hr TL50 values estimated by this method are compared between hard and soft water, $BeSO_4$ is approximately 4.4 times more toxic in soft than in hard water.

TABLE 2

Median tolerance limits of beryllium sulfate (in mg/l Be) by graphic interpolation.

Bio-assay	Hard Water 24	48	96	Soft Water 24	48	96hr
A	31.5	31.5	31.5	23.7	4.21	3.15
B	31.5	31.5	31.5	6.83	4.21	3.15
C	18.2	18.2	18.2	>10*	>10*	8.02
D	21.2	18.2	18.2	>10	>10	8.32
Mean:	25.60	24.85	24.85	>12*	>7*	5.65

*See text for explanation.

If some of the data in Tables 1 and 2 are arranged as shown in Table 3, the effect of size and species of the test organisms on acute toxicity can be examined. The 96-hr TL50 values were not different significantly among the three sizes of spotted salamanders (Bioassays B-D), especially in regard to the two sizes that were evaluated under the exact same conditions at the same time (C and D). Since the 96-hr TL50 of the marbled salamander (A) was identical to that of one of the spotted salamander assays (B), no apparent difference existed between these two species of salamanders.

TABLE 3

TL50 as a function of size and species.

Bio-	Test Organism		96-hr TL50	
assay	Species	Size	Hard Water	Soft Water
A	marbled	medium	31.5	3.15
B	spotted	small	31.5	3.15
C	spotted	medium	18.2	8.02
D	spotted	large	18.2	8.31

In the absence of any significant difference between size and species of the salamander larvae used, the bioassay data from all four bioassays were combined to increase the sample size and hence the reliability of the data. When the response to one beryllium concentration was compared between hard and soft water Be test solutions, the effect of water hardness on toxicity was very pronounced, as shown in Table 4. In hard water, all 40 organisms survived 10 mg/l Be throughout the exposure, whereas in soft water there was a significant decline in survival with time, from 72.5% at 24 hours to 20% survival at 96 hours of exposure.

TABLE 4

Comparative survivability at 10 mg/l Be^{2+} in hard and soft water.

	Hard Water	Soft Water
24-hr:	40/40 (100%)	29/40 (72.5)
48-hr:	40/40 (100)	18/40 (45)
72-hr:	40/40 (100)	12/40 (30)
96-hr:	40/40 (100)	8/40 (20)

The combined bioassay data were also treated to a regression analysis by the least squares method as was done previously (SLONIM 1973). The results are shown in Table 5 and include for each exposure period the regression equation expressed in terms of the log of the Be concentration as a function of the percent survival, the correlation coefficient, and the TL50 with its 95% confidence limits. The computer-derived TL50 values matched closely the mean values obtained by graphic interpolation (Table 2). The 96-hr TL50 was 26.3 mg/l Be in hard water and 4.7 mg/l in soft water; thus, $BeSO_4$ was 5.6 times more toxic in soft water than in hard water. This one order of magnitude difference in toxicity (in contrast to two for guppies) applies only to the two species of salamander used in this study. It is interesting to note further that the TL50 for the salamander species was close to that for guppies in hard water but very different (about 25-fold) in soft water; thus, these organisms differ essentially in their sensitivity to beryllium ions in soft water, with the *Ambystoma* species being much more tolerant than guppies.

TABLE 5

Analysis of the combined bioassay data by the least squares method.

Exposure Period (hr)	Regression Equation*	r†	TL50 and 95 Percent Confidence Limits (mg/l)
	Hard Water		
24	Log C = 1.8762 - 0.008850P	0.9440	27.1(20.5-36.0)
48	Log C = 1.8408 - 0.008408P	0.9209	26.3(18.8-36.8)
96	Log C = 1.8408 - 0.008408P	0.9209	26.3(18.8-36.8)
	Soft Water		
24	Log C = 1.9968 - 0.01677P	0.8994	14.4(8.02-25.8)
48	Log C = 1.7352 - 0.01511P	0.8947	9.54(5.60-16.3)
96	Log C = 1.1527 - 0.009687P	0.8773	4.66(3.01-7.21)

*C = concentration of Be^{2+} in mg/l and P = percent survivors.

†r = correlation coefficient.

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