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BERYLLIUM UPTAKE BY THE COMMON GUPPY USING RADIOISOTOPE ^{7}Be.

II. BERYLLIUM CONCENTRATION IN FISH

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AEROSPACE MEDICAL RESEARCH LABORATORY
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WRIGHT-PATTERSON AIR FORCE BASE, OHIO

NOTICES

The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

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13. ABSTRACT

Two bioassays were conducted in which radioactive beryllium, Be-7, was added as a tracer to beryllium sulfate solutions in order to obtain some insight into beryllium toxicity in fish. Tracer mechanics and procedures for measuring beryllium uptake in guppies were determined in the previous phase. The bioassay data indicated that the amount of beryllium concentrated within the guppies (and fry) was influenced by the beryllium concentration in solution and by fish size (inversely), and to a less extent by exposure period, but not by fish age, buffering of the beryllium solution, or water hardness. Generally, beryllium uptake varied greatly among guppies exposed to the same environmental conditions. Examination of individual tissues and organs of six of the guppies revealed that most of the beryllium was concentrated in the viscera; beryllium uptake was highest (per total organ) in the gastrointestine and kidney of the 9 organs analyzed provisionally. These studies suggest that the lethality of beryllium in fish is not dependent upon the amount concentrated within the fish, but more likely on the effect of beryllium on a particular target organ, cellular or subcellular component. More work is required to determine the mechanism of beryllium toxicity in fish.

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Bioassay Data						
Fish; Guppies; Fry						
Beryllium Uptake						
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Mechanism of Toxicity						

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FOREWORD

The research covered in this report was accomplished during the spring and summer of 1970 by Dr. A. R. Slonim, Toxic Hazards Division, Aerospace Medical Research Laboratory, WPAFB, and Captain Frederick C. Damm,* Engineering and Experimentation Division, Nuclear Engineering Center of the Air Force Institute of Technology, WPAFB. This study was conducted in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630204, "Environmental Pollution Aspects of Propellants and Materials."

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This technical report has been reviewed and is approved.

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SECTION I

INTRODUCTION

The effects of beryllium sulfate on hard and soft water and on guppies have been studied in this laboratory for the past 2 years (ref 1, 2). Included in the program was a two-part study to monitor beryllium toxicity in fish by the use of radioactive beryllium, ^{7}Be. In the first part, preliminary to the biological aspects, tracer mechanics, preparations and procedures were determined (ref 3). The present report describes the results of the second part consisting of two tracer-applied bioassays in which the concentration (uptake) of beryllium by guppies from solutions was measured and evaluated under certain conditions (e.g., as a function of fish age, size, beryllium concentration, buffering, and exposure time).

SECTION II

EXPERIMENTAL

GENERAL PREPARATIONS AND PROCEDURES

The preparation of hard and soft water and beryllium sulfate solutions as well as the instrumentation and analyses used in conjunction with this study have been described in detail in an earlier report (ref 1). Some of the beryllium solutions were buffered by the addition of $NaHCO_3$, USP; the molar ratio of buffer to beryllium salt was 0.5 unless stated otherwise. The soluble beryllium salt used was $BeSO_4 \cdot 4H_2O$, and the isotope (^7Be) was in the form of $BeCl_2$ and contained radioactivity in the amount of 0.4 millicurie per milliliter. The beryllium concentration is expressed in terms of the ion, Be^{2+}, usually in milligrams per liter (mg/l).

The preparation of beryllium standard and water sample with the isotope as well as the measurement of radioactivity by means of a scintillation counter, which utilized a sodium iodide crystal as a gamma energy detector, have been described in the report covering the preliminary phase of this work (ref 3). The procedure for estimating beryllium uptake in the fish was outlined in the previous report also (ref 3).

MATERIALS AND METHODS FOR A STATIC BIOASSAY

The selection and preparation of materials for the static bioassay studies, which were conducted according to standard procedures (ref 4), are described briefly below.

Aquaria Materials

One 20-gallon and two 10-gallon aquaria were used to contain the fish to be tested in a bioassay. The 20-gallon aquarium was the "stock tank" into which the fish were originally placed; it contained water of moderate hardness, 160-245 mg/l (as $CaCO_3$). The 10-gallon aquaria were the "acclimatization tanks" containing hard water ($\geqslant$ 400 mg/l) and soft water

(20-25 mg/l), respectively, in which the fish were maintained for at least 7 days prior to the start of a bioassay. In each aquarium was placed a thermostatically-controlled heater, an aquarium thermometer, an artificial plant ring, which served also as a breeding area, and a filter containing charcoal and filter floss (figure 1). The mean aquaria temperature was 22.2 C ± 1.1 C.

Figure 1. Bioassay Employing a Beryllium Radioisotope as a Tracer of Beryllium Concentration. Two jars each containing 5 fish are used per test solution. Shown also are the aquaria used for maintaining the stock and test organisms (see text).

Fish

The fish selected for the beryllium bioassay was the common guppy, Lebistes reticulatus. This fish is a small, sturdy, warm-water fish which can tolerate changes in pH and temperature as well as adapt well to water of greatly different hardness better than other species.[1] In view of these characteristics plus the relatively small size of the bioassay work area, this fish appeared to be very suitable for bioassay studies. Young adult guppies were purchased from a local supplier. The fish were fed TetraMin® (a commonly available product of West Germany); this food was more digestible when ground-up in a blender.

Bioassay Preparation

Each weighed amount of $BeSO_4 \cdot 4H_2O$ was diluted to volume in a 2-liter volumetric flask with either hard or soft water. The contents of each flask were poured into a wide-mouthed, 1-gallon glass jar into which five fish were immediately placed from the "acclimatization tank." Five guppies per 2-liter volume meets the recommended limit of 2 grams of fish per liter liquid volume (ref 4). Two jars were used per beryllium concentration, so that 10 fish were tested at each level. The jars were arranged in two rows, with those containing identical solutions placed one behind the other and covered with a stainless steel screen (figure 1).

Radioisotope Application

The concentration of beryllium used in each of the two bioassays ranged from 0.01 to 100 mg/l Be^{2+}. A small quantity of isotope (e.g., 0.2 ml) was added to each 2-liter test solution. The amount of isotope was thus diluted 1:10,000 from its original level, so that each $BeSO_4$ solution, regardless of concentration, contained the same amount of radioactivity, namely 0.04 microcurie per millimeter.

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[1]Pickering, Q. H. (EPA Fish Toxicology Lab, Ohio): Personal communication.

Analyses and Monitoring

The analyses of the beryllium solutions (described in ref 1) were conducted before, during and immediately following a bioassay or after the death of the last survivor of a test solution. Monitoring of the response of the fish to the toxic metal was continuous around the clock, in cooperation with monitors working on other animal experiments. The loss of balance and other symptoms of toxicity were noted; the date and time of death, based on a 24-hour clock, were recorded. Upon death, each radioactive fish was blotted quickly with a paper towel to remove excess water, placed in a small plastic vial (with cover), and stored in a refrigerator prior to being weighed and measured for radioactivity. The data were first evaluated for the percent survivors at the 24-, 48-, and 96-hour periods to determine the median tolerance limit (TL_{50}) values. For the purposes of this study, the data from the two bioassays incorporating the ^{7}Be isotope were combined and evaluated in terms of the various parameters mentioned above (section I).

It should be noted that the bioassays were conducted according to standard procedure and not originally designed to evaluate the aforementioned parameters. Thus, the results described in the following section reflect an analysis of the data <u>after</u> completion of the bioassays. In cases where the numbers of fish compared under a given condition were not consistently large, the results should be considered as suggestive rather than conclusive.

SECTION III

RESULTS AND DISCUSSION

FISH AGE AND SIZE

Beryllium uptake by guppies from aqueous beryllium solutions was evaluated first as a function of fish age and size. The intake of beryllium was compared in guppies exposed to the same amount of aqueous $BeSO_4$ for the same period of time. Table I[2] shows that no correlation existed between beryllium uptake and the three different age groups of guppies (fry, young adult, and adult) that were exposed to 1.0 mg/l Be^{2+} in hard water for 4 days. This result agrees favorably with the observation that the toxicity of $BeSO_4$ solutions was the same for fry as it was for adult guppies (ref 2). The beryllium uptake of a larger population of guppies that died after about 1-hour exposure to a high level of beryllium (100 mg/l) was compared on a weight basis alone. The guppies were classed into three weight groups. The results, presented in table II, showed a decrease in mean Be uptake with increasing size of fish following exposure to $BeSO_4$ in hard water or soft water. No difference in uptake was noted between hard and soft water Be solutions by guppies of the same weight group. (The individual Be uptake values incorporated in this table are compared in appendix I.) In spite of the small numbers of fish examined at some of the weights, these data showed that increasing the mean size of guppies (at least twofold) resulted in a general decrease in mean beryllium uptake. This inverse relationship was supported further by the observation that of 23 guppies concentrating beryllium at over 100 micrograms per gram body weight, 20 were in the lowest weight group (A of table II), and three were in the low end of weight group B. Moreover, if the 9 fry that also concentrated beryllium at this high level were added to the list, then 29 out of 32 fry and adult guppies with over 100 μg/g uptake values were in the smallest size group.

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[2]Tables are located at end of report (pages 15-23).

CONCENTRATION IN ENVIRONMENT

Beryllium uptake as a function of the amount of Be in solution was evaluated in several ways from the large amount of available bioassay data. Generally, Be uptake was compared at different levels of beryllium and between hard and soft water solutions for the same exposure period. Because the guppy kills were widely distributed over time, the data in table III were confined to one hard water group and three soft water groups, each of the latter representing a different exposure period. The results showed that the mean Be uptake increased with increasing concentration of beryllium per exposure period in hard water and soft water; in the latter case, one exposure group (3.5 hours) showed little change due probably to the small number of fish evaluated. When beryllium solutions were buffered by the addition of sodium bicarbonate (ref 1), similar results occurred, as shown in table IV. The inconsistency observed in Be uptake in buffered soft water cannot be explained by these data at the present time. In general, a positive relationship between beryllium uptake and aqueous concentration was indicated in both the unbuffered and buffered bioassays.

Since the results of the concentration data (tables III and IV) were based on relatively small numbers of fish, Be uptake in 10 or more guppies at each environmental concentration level was determined. This was accomplished by combining all the fish per aqueous concentration independent of (a) fish weight, since the large majority of fish were in the low weight group, and (b) exposure time, since there were indications that the exposure period may not be critical (see below). Combining the data of tables III and IV with those of guppies that died at longer exposure periods, respectively, approximately doubled the sample size to 138 guppies. The results are shown in table V. The mean Be uptake increased with increasing concentration in hard water and soft water. A similar increase in uptake was observed with the buffered solutions, although the mean Be uptake was small for 9 guppies at 10 mg/l Be in buffered soft water (not tabulated), indicating some degree of inconsistency in response to this buffered solution. There was no consistent difference in Be uptake between unbuffered and buffered hard water solutions or between unbuffered and buffered soft water solutions, except for an unusually higher uptake value in the buffered than unbuffered soft water solutions. Notably, some fish were able to concentrate beryllium considerably more than others of the same body weight and under the same exposure conditions, whereas some of the expired fish showed relatively low amounts of the metal (cf, e.g., range values of tables II-IV). The foregoing results indicate that the amount of beryllium absorbed in the body is not an important determinant of beryllium lethality.

EXPOSURE TIME

Beryllium uptake as a function of exposure time was next examined in detail, including some data on chronic exposures. (Any period within the 4-day bioassay is classed here as acute exposure and that exceeding 1 week as chronic exposure.) In contrast to the previous situation, Be uptake was compared at varying exposure times with the Be concentration in solution kept constant. In table VI, beryllium uptake was analyzed at two levels of Be in hard water and at three levels in soft water. The results showed an increase in mean Be uptake with increasing exposure time at the 10 and 100 mg/l Be levels in both hard and soft water. At the 1.0 mg/l Be level in soft water, there was no consistent increase in uptake with increasing exposure time. Examination of some of the single uptake values (not tabulated) revealed some inconsistencies in hard water also; for example, at 10 mg Be/liter, Be uptake in one guppy that expired in 1 hour was 25 μg/g, whereas that of one guppy (in same weight group) at 81 hours was as low as 13 μg/g. Thus, although the tabulated data indicate that generally Be uptake increases with time, it was not totally consistent. This was borne out also when Be uptake versus exposure time was evaluated in the buffered solutions, as seen in table VII. The results showed that the trend was slightly reversed in soft water from the previous situation in that Be uptake increased with time at the 1.0 mg/l Be level, but not at the 10 and 100 mg/l Be levels. Most of the Be uptake values on the fish and fry used in the two bioassays are presented as a function of exposure time in appendix II. As shown in this scattergram, the Be uptakes of the

20 fry generally matched those of 165 adults (of varying size) in terms of exposure time and also environmental concentration; the uptake values were on the average higher in the fry than the adults, as expected from differences in fish size (discussed above).

The chronic exposure to beryllium study utilized fish that survived the first bioassay and were maintained and monitored along with the second bioassay. Only guppies exposed to low levels of Be would endure for periods up to 2 weeks (or slightly more); the tolerable levels for this were 0.01 mg/l Be in soft water and 1.0 mg/l Be in hard water. The results, presented in table VIII, showed that the amount of Be concentrated in all 4 fish at the lowest Be exposure level in soft water (0.01 mg/l) was consistently low for a long period of time, similar to the acute exposures (see appendix II). On the other hand, the response of 5 fish to beryllium in hard water from 8 to 17 days was inconsistent; however, much of the discrepancy in Be uptake may reflect in part the large weight differences noted among these 5 fish. The apparent inconsistent results of this very small chronic exposure study were similar to those found in the acute exposures. As mentioned previously, the widespread distribution of guppies over the entire bioassay period accounted for inadequate numbers of fish at some exposure times. Thus, although an inconsistent or low correlation between Be uptake and exposure period is suggested by these data, more fish must be analyzed at each exposure period to determine definitely the relationship between uptake and exposure time.

WATER HARDNESS

To evaluate any difference in Be uptake between hard and soft water solutions with the number of variables reduced as much as possible, uptake data were assembled on guppies exposed to the same beryllium level in solution for the same period of time (±0.5 hour). The results, presented in table IX, showed closely matching mean uptake values, but with a wide range, between hard and soft water solutions, being higher at the 100 mg/l than 10 mg/l Be levels. A similar response was seen between buffered hard and soft water solutions, but the difference between the two was greater in buffered than unbuffered solutions. Thus, in general there appears to be no significant difference in the average amount of beryllium concentrated in guppies that expire following exposure to the same level of beryllium for the same period of time in hard versus soft water. This means that the fish exposed to the same environmental conditions can concentrate Be within the body to the same extent from either hard or soft water; and this is independent of any toxic manifestations of the metal, since beryllium was shown to be more toxic in soft water than in hard water (ref 2, 5). The above observations tend to support an earlier statement that the amount of beryllium taken up by the body may not be an important factor in Be lethality; perhaps the ability to penetrate better to a vital organ from a soft water than hard water medium is what is toxicologically important. The mechanism by which beryllium becomes more toxic to fish in soft water than in hard water remains obscure.

CONCENTRATION IN TISSUES

In addition to the fish sacrificed earlier following the end of a bioassay (table I), six additional radioactive fish were sacrificed for microscopic dissection and evaluation in an attempt to determine the uptake of beryllium first by different parts of the body and then by certain organs. To determine if beryllium acts mainly through adsorption onto the skin and gills or is concentrated within the body, each of two fish was deskinned, and the skin and carcass placed in separate vials for radioactivity measurements. The data in table X show clearly that relatively little beryllium was adsorbed by the skin, but was almost totally in the carcass (averaging 97.5% for both fish); in the large fish examined (Fish B), the Be concentration in the carcass was about 22 μg/gram.

Two of the above 6 fish were dissected into three parts to determine which section of the body - the head, mid or visceral, or tail - concentrated the most beryllium. The two dissection lines were approximately along the gill line and just behind the visceral sac. Usually the tail section was the longest and the visceral section the heaviest of the fish; for example, the length of the head, visceral, and tail sections of one female guppy (Fish C, table XI) was 4.5, 7.5, and 18 mm, respectively. Table XI shows that the section with the greatest mass contained the highest concentration of beryllium, but that the amount relative to the head section varied greatly (e.g., 2- and 56-fold in the two fish shown).

The two remaining fish (of average-to-large size) were dissected and certain of their organs removed for analysis. Because some of the organs were extremely difficult to excise due to their minute size and unfamiliar location in the body, it was not possible to obtain similar organs from both fish. More intact organs were removed from the second fish than the first as experience was gained in the dissection process. Table XII presents the concentration of beryllium in those organs isolated intact from both fish; the results are expressed on an organ rather than weight basis. The organ that concentrated the most beryllium in both guppies was the gastrointestinal tract; this was significant even in the fish exposed to the lowest level of beryllium tested (Fish F). It is possible to combine the data of Fish E and Fish F to obtain some insight into organ affinity for beryllium. By setting the Be uptake of the gastrointestine at 100 for each fish, the relative uptake values for the other organs are obtained. Thus, the nine organs examined can be arranged into four groups in descending order of Be uptake as follows:

gastrointestine > kidney > ovary >>> gills, liver, brain, heart, eye, spleen

Be uptake of the six organs in the last group was too low to definitely serialize them, although they are listed in somewhat numerical order as calculated from the radiochemical data. Histopathologic examination of some of our exposed fish lends some support to the above observations. Except for the gastrointestine which was not well-preserved for examination and so was omitted, the kidney showed marked changes by beryllium over other organs and especially the unexposed controls.[3] In general, these preliminary organ uptake data should serve as a basis for further work in this area; e.g., much more organ uptake values along with organ weights are needed to establish a definite organ-beryllium relationship.

SECTION IV

CONCLUSIONS

Information assembled from two tracer-applied bioassays with a limited number of fish revealed that beryllium uptake in guppies correlated indirectly with fish size and directly with the concentration of beryllium in solution and, to a less extent, the exposure period. The ability to concentrate beryllium within the fish was not altered by the age of the fish, buffering of the beryllium solution, or water hardness. Generally, the amount of beryllium found in expired guppies under the same environmental conditions varied considerably. On the other hand, certain organs of the body (e.g., the gastrointestine and kidney) concentrated more beryllium than other organs. These studies indicate that the lethality of beryllium on fish is not dependent upon the amount concentrated within the fish, but more likely on the effect of beryllium on a particular target organ, cellular or subcellular component. More research is required to determine the mechanism of beryllium toxicity in fish.

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[3]Tucker, J. (EPA, Duluth, Minn.): Personal communication.

APPENDIX I

COMPARISON OF BERYLLIUM UPTAKE ACCORDING TO WEIGHT OF GUPPIES EXPOSED TO 100 mg/1 Be^{2+} IN HARD AND SOFT WATER FOR ONE (±0.5) HOUR

HARD WATER		SOFT WATER	
Fish weight, g	Be uptake, μg/g	Fish weight, g	Be uptake, μg/g
0.03 - 0.19		0.03 - 0.19	
0.038	33	0.030	173
0.050	144	0.047	56
0.051	77	0.053	19
0.053	43	0.079	170
0.075	38	0.084	41
0.078	81	0.091	12
0.088	44	0.093	44
0.089	24	0.094	77
0.105	55	0.107	51
0.126	12	0.114	38
0.151	23	0.116	47
0.082*	52*	0.155	41
		0.089*	64*
0.20 - 0.36		0.20 - 0.36	
0.211	45	0.205	36
0.211	22	0.223	31
0.218	31	0.226	30
0.31	37	0.254	25
0.238*	38*	0.300	12
		0.242*	27*
		0.37 - 0.53	
		0.383	9
		0.466	17
		0.510	17
		0.453*	14*

*Mean value

APPENDIX II

SCATTERGRAM OF CONCENTRATION OF BERYLLIUM PER GRAM OF FISH (AND FRY) VERSUS EXPOSURE TIME. DATA COMBINED FROM TWO BIOASSAYS IN WHICH RADIOISOTOPE ^7Be WAS USED AS A TRACER. Values are in μg Be/g fish

Be concn in water (mg/l)	Beryllium uptake (μg/g) per exposure time in hours: 0 - 2	2.5 - 4.5	5 - 9	10 - 16	18 - 24	25 - 43	45 - 63	65 - 93hr	Be uptake mean	No. of fish
			HARD	WATER						
0.01					0.2	0.1	0.3	0.1,0.1,0.2	0.16	6
1	11	27		24		3		14	16	5
Buf-1*		13,38	88	182	10			12,27	53	7
10	25	10,11,12,18	9,12,12,12,16, 16,20,28,29,69					13	19	16
Buf-10		20,22,28,36,37, 39,40,42,42	69						38	10
100	12,22,23,24,31, 33,37,38,43,44, 45,55,77,81,144	22,22,144						266,760	96	20
Buf-100		24,25,49,51,52, 65,65,76,85,182							67	10
			SOFT	WATER						
0.01	0.07		0.05,0.06,0.08	0.03	0.03	0.08,0.2		0.2,0.3	0.11	10
1		4,7,14	3,10	1,1,1,2,2,2,2, 6,7,7,7,8,9,12	1				5	20
Buf-1		31,38,188	36,164	34,218	181		10	155	106	10
10	3,6,11	5,6,16	6,12,12,15,22	6					10	12
Buf-10		4,8,13,17	5,6,7,10,15						9	9
100	9,12,12,17,17, 19,25,30,31,36, 38,41,41,44,47, 51,56,77,170,173								47	20
Buf-100	81,122,123,157, 177,194,197,240	51,161							150	10
			FRY IN	HARD WATER						
0.01							0.15	0.2,0.3,0.4	0.25	4
1								10,80,305	132	3
100		120,136,180,382							205	4
			FRY IN	SOFT WATER						
0.01		0.9						0.4	0.7	2
1			4,6	7					6	3
100	139	265,529,581							379	4

*Buf = Buffered solution of $BeSO_4$ (see text); -number represents concentration of Be^{2+} in mg/l.

TABLE I

BERYLLIUM UPTAKE AS A FUNCTION OF AGE AND SIZE OF GUPPIES SURVIVING 1.0 mg/l Be^{2+} IN HARD WATER FOR 96 HOURS*

Fish description		Microgram Be/gram fish
Adult (regular size)	- 0.176 g, 26.5 mm	11.9
	- 0.169 g, 30.0 mm	160
Young adult (small)	- 0.035 g, 19.5 mm	20.2
Fry	- 0.013 g	80
	- 0.019 g	305

*Guppies sacrificed at termination of bioassay

TABLE II

BERYLLIUM UPTAKE AS A FUNCTION OF THE WEIGHT OF GUPPIES EXPOSED TO 100 mg/l Be^{2+} IN HARD AND SOFT WATER FOR ONE (±0.5) HOUR

Weight Group*	Fish weight, g		No. of fish	Be uptake, μg/g	
	mean	range		mean	range
HARD WATER					
A	0.082	0.04-0.15	11	52.1	12-144
B	0.238	0.21-0.31	4	33.5	22-45
SOFT WATER					
A	0.089	0.03-0.16	12	64.1	12-173
B	0.242	0.21-0.30	5	26.6	12-36
C	0.453	0.38-0.51	3	14.2	8.7-17

* A = 0.03-0.19 g; B = 0.20-0.36 g; C = 0.37-0.53 g

TABLE III

BERYLLIUM UPTAKE BY FISH AS A FUNCTION OF BERYLLIUM CONCENTRATION IN HARD AND SOFT WATER

Be concn in water (mg/l)	Exposure time (hr)	No. of fish	Be uptake, μg/g mean	Be uptake, μg/g range
		HARD WATER		
10	3.5	4	12.6	10-18
100	3.5	3	62.9	22-144
		SOFT WATER		
10	1	3	6.7	3.4-11
100	1	20	47.3	8.7-173
1	3.5	3	8.6	4.4-14
10	3.5	3	9.0	4.6-16
0.01	8	3	0.06	0.05-0.08
1	8	2	6.3	3.1-10
10	8	5	13.6	6.4-22

TABLE IV

BERYLLIUM UPTAKE BY FISH AS A FUNCTION OF BERYLLIUM CONCENTRATION IN BUFFERED* HARD AND SOFT WATER

Be concn in water (mg/l)	Exposure time (hr)	No. of fish	Be uptake, μg/g	
			mean	range
		HARD WATER		
10	2.5	9	33.9	20-42
100	2.5	10	67.4	25-182
		SOFT WATER		
1	3.5	3	85.7	31-188
10	3.5	3	12.7	8-17
100	3.5	2	106	51-161

*Molar ratio of $NaHCO_3$ to $BeSO_4$ = 0.5

TABLE V

BERYLLIUM UPTAKE BY TEN OR MORE GUPPIES AS A FUNCTION OF CONCENTRATION IN VARIOUS SOLUTIONS AND INDEPENDENT OF FISH SIZE AND EXPOSURE TIME

Be concn in water (mg/l)	Exposure time (hr)	No. of fish	Be uptake, µg/g	
			mean	range
		HARD WATER		
10	1-81	16	19	10-69
100	0.3-75	20	96	12-760
		SOFT WATER		
0.01	2-89	10	0.11	0.03-0.34
1	3.5-18	20	5.3	0.9-14
10	1-10	12	10	3.4-22
100	0.3-1.5	20	47	8.7-173
		BUFFERED* HARD WATER		
10	2.5-5	10	38	20-69
100	5	10	67	24-182
		BUFFERED* SOFT WATER		
1	3.5-71	10	106	10-218
100	2.5-3.5	10	150	51-240

*See legend under Table IV

TABLE VI

BERYLLIUM UPTAKE BY FISH AS A FUNCTION OF EXPOSURE TIME. ACUTE EXPOSURE TO $BeSO_4$ IN HARD AND SOFT WATER

Be concn in water (mg/l)	Exposure time (hr)	No. of fish	Be uptake, µg/g mean	Be uptake, µg/g range
		HARD WATER		
10	3.5	4	12.6	11-18
10	5.5	9	21.6	8.8-69
100	0.25	2	37.6	31-45
100	1	13	48.6	12-144
100	3.5	3	62.9	22-144
100	75	2	513	226-760
		SOFT WATER		
1	3.5	3	8.6	4.4-14
1	8	2	6.3	3.1-10
1	10	5	6.0	1.4-8.8
1	12	2	10.0	7.6-12
1	14	6	2.6	1.2-6.9
10	1	3	6.7	3.4-11
10	3(±0.5)	3	9.0	4.6-16
10	8	5	13.6	6.4-22
100	0.25	9	26.2	12-51
100	1	11	64.5	8.7-173

TABLE VII

BERYLLIUM UPTAKE BY FISH AS A FUNCTION OF EXPOSURE TIME. ACUTE EXPOSURE TO $BeSO_4$ BUFFERED* IN HARD AND SOFT WATER

Be concn in water (mg/l)	Exposure time (hr)	No. of fish	Be uptake, μg/g	
			mean	range
		HARD WATER		
10	2.5	9	33.9	20-42
10	5.5	1	69.0	---
		SOFT WATER		
1	3.5	3	85.7	31-188
1	8	2	99.8	36-164
1	10	2	126	34-218
10	3(±0.5)	4	10.6	4.4-17
10	5.5	5	8.4	5.1-15
100	2	8	161	81-240
100	3	2	106	51-161

*See legend under Table IV

TABLE VIII

BERYLLIUM UPTAKE BY FISH AS A FUNCTION OF EXPOSURE TIME. CHRONIC EXPOSURE TO $BeSO_4$ IN HARD AND SOFT WATER

Environmental condition	Exposure time (days)	No. of fish	Be in fish (μg/g)
0.01 mg/l Be in soft water	11	3	negl,* 0.02, 0.03
	15	1	0.5
1.0 mg/l Be in hard water	8	1	5
	11	1	6
	12	1	157
	13	1	9
	17	1	274

*negl = negligible (<0.01 μg/g)

TABLE IX

COMPARISON OF BERYLLIUM UPTAKE BETWEEN HARD AND SOFT WATER SOLUTIONS BY GUPPIES UNDER THE SAME ENVIRONMENTAL CONDITIONS

Exposure condition		HARD WATER		SOFT WATER	
Be concn (mg/l)	Time (hr)	No.of fish	Be uptake (μg/g)	No.of fish	Be uptake (μg/g)
10	3(±0.5)	4	12.6(10-18)	3	9.0(4.6-16)
100	1(±0.5)	15	47.1(12-144)	20	47.3(8.7-173)
		BUFFERED* HARD WATER		BUFFERED* SOFT WATER	
10	3(±0.5)	9	33.9(20-42)	4	10.6(4.4-17)
100	2.5(±0.5)	10	67.4(24-182)	10	150 (51-240)

* See legend under Table IV

TABLE X

COMPARISON OF BERYLLIUM UPTAKE BETWEEN SKIN AND CARCASS OF TWO GUPPIES EXPOSED TO LOW LEVELS OF $BeSO_4$ SOLUTIONS FOR 96 HOURS

Fish	Environmental condition	Microgram Be Skin	Microgram Be Carcass
A	0.01 mg/l Be in soft water	0.002	0.055
B	1 mg/l Be in hard water	0.104	6.455*

*This relatively large fish showed beryllium to be concentrated mainly in the carcass (0.30g) at a level of 21.5 microgram per gram.

TABLE XI

COMPARISON OF BERYLLIUM UPTAKE BETWEEN DIFFERENT SECTIONS OF GUPPIES EXPOSED TO 1 mg/l Be^{2+} IN HARD WATER FOR 96 HOURS

Fish	Section*	Total Be, µg	Section Wt, g	µg Be/g section
	head	0.08	0.028	2.8
C	visceral	14.0	0.089	157.3
	tail	<0.01	0.041	negl
	head	0.12	0.046	2.6
D	visceral	0.37	0.083	4.5
	tail	0.04	0.046	0.9

*See text for explanation

TABLE XII

COMPARISON OF BERYLLIUM UPTAKE BY DIFFERENT ORGANS OF TWO GUPPIES EXPOSED TO LOW LEVELS OF $BeSO_4$ IN HARD WATER FOR 96 HOURS

Fish	Environmental condition	Organ	Microgram Be (per organ)
E	1 mg/l Be in hard water	heart	negl*
		gastrointestine	0.62
		kidney	0.10
		liver	negl
		gills	negl
		rest of carcass	2.1
F	0.01 mg/l Be in hard water	heart	negl*
		gastrointestine	0.10
		spleen	negl
		ovary	0.001
		eye	negl
		brain	negl
		liver	negl
		rest of carcass	0.002

*negl = negligible, which for Fish E is <0.10 µg Be^{2+} and for Fish F, exposed to one hundredth the Be level of Fish E, is <0.001 µg Be^{2+}

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