THE EFFECTS OF HIGH MINERAL CONTENT WATER ON THE FECUNDITY OF MICE

by

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TABLE OF CONTENTS

INTRODUCTION

An adequate supply of clean palatable water, readily available at any time the animal desires a drink is a prime prerequisite for optimum results in animal production. The most important factor affecting the palatability of clean water, free from harmful amounts of organic matter, is its mineral content. Water decreases in palatability when the total amount of mineral exceeds 500-1,000 ppm. Minerals usually present to some extent in drinking water are salts of zinc, copper, manganese, and iron. They are not cumulative poisons and become toxic at levels much higher than usual. Of the 30 or more mineral salts present in many surface and deep wells, by far the most abundant are the carbonates, bicarbonates, chlorides, and sulfates of sodium, potassium, magnesium, and calcium. Together they comprise from around 95-99% of the total content of most natural water.

Drinking water has often been incriminated in problems of health. This has been particularly true if the water has an unusual taste, odor, or appearance. Advanced knowledge and research has shown that the presence or absence of minute quantities of certain elements, may cause serious consequences, especially to developing fetuses. Because of this, there seemed to be ample reason to investigate the drinking water in a case being studied by the Iowa State University Veterinary Pathology Department and described herein.

In the spring of 1962, Mr. Milo Throlson, Sheyenne, North Dakota, purchased 14 sows which had just been bred, and brought them to his ranch.

Seven sows aborted approximately 3 days before farrowing and many of the full term piglets were deformed. That same year a herd of 75 Hereford-crossbred cows were wintered on this ranch. Twelve cows failed to conceive and 3 had calves which had short, yellow hair coats. Professional veterinary assistance was obtained and after ruling out infectious diseases it was concluded that the water was the caustive agent. A water sample was submitted to the North Dakota State Laboratory, Bismarck, North Dakota, for analysis. The well from which this water was obtained was drilled in 1961 and since that time 3 additional wells have been drilled in an attempt to secure suitable water.

This investigation was undertaken in an attempt to determine if feeding this water to mice would:

1. have any effect on fecundity and gestation,
2. possibly cause anomalies in off-spring,
3. influence the estrus cycle,
4. cause an increase or decrease in water consumption,
5. effect the rate of gain of the off-spring,
6. alter the blood picture, especially the hemoglobin content, packed cell volume, red blood cell count, or white blood cell count, and
7. produce gross or microscopic lesions.

LITERATURE REVIEW

Heller (1932 and 1933), Heller and Owens (1935), Anonymous (1942-1943) and Velu (1937) concur that swine, chickens, cattle, and sheep can survive and remain in good health on saline water up to 15,000 ppm of minerals such as bicarbonates, chlorides, and sulfates of sodium and calcium and up to 10,000 ppm of corresponding salts of potassium and magnesium. Limits of tolerances of alkaline waters containing sodium and calcium carbonate are around 5,000 ppm.

Anonymous (1961) states that due to lack of palatability, farm animals will not voluntarily drink a highly mineralized water (one containing more than 1,000 ppm of minerals such as the carbonates, bicarbonates, chlorides, and sulfates of sodium, potassium, magnesium and calcium.) When forced to drink it, they can gradually acquire a tolerance to the taste and physiological effects of water containing from 10,000 to 15,000 ppm of these minerals, (depending on the nature of the minerals), without detriment to health. Beyond these limits of tolerance the water is toxic and unfit for animal consumption.

It is estimated that more than 90% of the earth's water is unfit for consumption by land animals or use by land plants because of its excess salinity. Due to its solvent action on any form of matter with which it comes in contact, water free from traces of dissolved minerals does not occur anywhere in nature. Rain water, the purest form of natural water, contains trace amounts of gases from the atmosphere.

Arena (1963, pp. 213-216) states that a unique feature of iron

metabolism is that iron has no organ of excretion. The capacity to regulate the amount of iron in the body lies in the absorption mechanism. Presumably the mucosal cells regulate the rate of absorption from the gastrointestinal tract and seem to act as a barrier to the rapid entrance of iron into the circulation. In iron poisoning this mechanism fails to control the iron intake. Death in humans has resulted from ingestion of ferrous sulfate 40-1,600 mg/kg of body weight. Poisoning is manifested by lethargy, vomiting, fast and weak pulse, low blood pressure, and coma. These symptoms subside, the animal appears to be recovering, and then cyanosis, vasomotor collapse and pulmonary edema develop. Coma and death follow within 12-48 hours.

Garner (1967) cites that the excess iron upon absorption combines with apo-ferritin, and that it is the formation of an excess of ferritin which leads to the rapid development of shock and presumably liver injury, which is due to the direct action upon hepatic tissue. At post mortem, in naturally occurring cases, the only organ consistently affected is the liver, the characteristic finding being periportal necrosis.

Coup and Campbell (1964) found that cattle fed excessive quantities of iron given as iron in water used to irrigate pastures in which cows were taking 40-60 grams of iron a day would cause dark colored evil smelling frothy feces together with a decline in body weight, milk and fat yield, and general condition of the coat. However, iron in the natural state is in the ferrous form, and this experiment was carried out with iron in the ferric form. The possibility that iron in drinking water may

affect livestock cannot be entirely discounted. However, there is no experimental evidence on this possibility.

Grosskoph (1966) in his investigations found that high levels of bicarbonates had no effect on feed or water intake or any of the ruminal functions of sheep.

Ranjhan (1965) working with water with high calcium contents on 50 buffalo calves, found his results seemed to suggest that the presence of calcium in large amounts in water in their area might have an important bearing on calculi formation. Considering the views of Swingle (1953) it could be suggested that calcium content of water might play an important role in disturbance of crystalloid-colloid imbalance of urine and thus calculi formation.

Crawford (1968), Bostrom and Wester (1967) and Anonymous (1967) showed the death rate from cardiovascular disease dropped as the calcium content of the water rose, which is contrary to the normal assumption that the high level of calcium in blood vessels would necessarily show an increase in cardiovascular disease.

Anonymous (1959) cited some work done in which they found that the high salinity of water had no effect on feed consumption of rats, but did affect the water intake. The water intake was increased. High levels of $NaSO_4$ and $MgSO_4$ caused reduced growth rates. Diarrhea was also seen in some mice, but the symptoms were mild.

Pierce (1960) found that sheep getting NaCl and $NaSO_4$ drank more than those getting rain water in amounts up to 60% more. Those getting

$NaSO_4$ drank up to 80% more than those getting rain water. The sheep getting $NaSO_4$ showed higher SO_4 in their blood plasma than a control group, while NaCl had no effect.

Peterson (1952) in an analysis of more than 2000 replies to questionnaires sent to people drinking highly mineralized water in North Dakota indicated that water containing more than 750 ppm of sulfates is usually laxative and that water containing less than 600 ppm is normally safe. Magnesium sulfate is more purgative than sodium sulfate. Persons who have recently started to use the water can tolerate lower concentrations than those accustomed to it. The author considers that the maximum level of 250 ppm sulfate recommended by the United States Public Health Service could be raised to 500 ppm without producing any undesirable physiological effects on those consuming the water.

Chien et al. (1968) discovered that infants may be affected with tasteless drinking water which carries a laxative dose of sulfates, if they are given such water in their formulas. That the water may be the cause of the diarrhea is often unsuspected, for parents and older siblings may long since have grown "immune" to the laxative action of the water even though it may contain 400 to 1,000 mg/l of sulfate. Their work showed it unsafe for infants to consume water with greater than 400 mg/l of sulfates.

Larson and Bailey (1913) in an experiment in South Dakota fed cattle water with 7369 ppm total mineral and found they drank less water but voided more urine. After 2 years they were slaughtered and no ill effects

were seen. The salt was mainly $NaSO_4$. The feces became soft when the cattle first went on the experiment.

Anonymous (1942-1943) and Spafford (1941) in tests with white mice corroborated in large measure the previous findings of the Oklahoma Experimental Station that these animals can, in certain instances, survive on water of 1% or more mineralization with individual salts. Mixtures, particularly those of chlorides, nitrates, and sulfates proved harmful in smaller quantities than did many single salts.

Mulhearn (1957) relates that sulfides give an objectionable odor to water, but aeration will usually correct the condition. The sulfates of sodium and potassium give water a bitter taste.

Winks (1963) explained how sheep can tolerate high levels of sodium chloride and sodium sulfate by virtue of a renal adjustment which favors increased filtration and the elimination of the ingested salt. When first put on high levels of chlorides and sulfates, sheep did not drink the water well and when they did they often had a diarrhea. They soon became accustomed to this water and did not evidence diarrhea thereafter. When cattle were given water containing 400 grains per gallon of total soluble solids, 200 grains of which were in the form of sulfates and chlorides of magnesium and calcium, it was refused by them. They would die of thirst rather than drink such water.

Ballantyne (1957) doing some work in Alberta, Canada, found that water containing 1,300 grains per gallon of sodium and magnesium sulfates, caused death in cattle--it was a 2% solution. This was from a newly dug

well 9 feet deep. Other wells on the premises were satisfactory, but two wells had 252 and 520 grains per gallon of the above laxatives, and caused some problems. In case no. 2 a well unused for 3 years was used. Cattle appeared normal for 2 days and on the 3rd day were visibly affected, were down and unable to rise. The mucous membranes were cyanotic. Three died. The rest recovered when moved to different pasture and good water was given. The water had 1,146 grains per gallon of Glauber's and Epsom salts plus 116.6 grains of sodium chloride. In case no. 6 cattle developed diarrhea when drinking water with 415 grains per gallon of Glauber's salts. In another case listed as no. 8, calves drinking from a new well developed diarrhea, and two showed incoordination and convulsions. It contained 5380 ppm Glauber's salts. He listed the minimum toxic levels of NaCl-50 grains/gal; $NaSO_4$-100 grains/gal; $MgSO_4$-100 grains/gal; NaSO4 and MgSO4-100 grains/gal; and Iron-3ppm, higher levels causing digestive disturbances affecting the pylorus and duodenum.

Skold and Jensen (1961) took young rabbits, mice, and guinea pigs and allotted them to 2 groups. Over a period of 3 months, those in one group were supplied with water having a high mineral content; those in the other group with water containing a low concentration of mineral salts. The average daily gains of the animals in the 2 groups were used as the variates for comparison of the effects of the 2 kinds of water. The guinea pigs supplied with water having a low mineral content made significantly higher gains in body weight than did guinea pigs supplied with highly mineralized water. Rates of gains for rabbits and mice were not

significantly different. The water in this work contained the following:

Total solids	9725 ppm
$CaSO_4$	2328 ppm
$MgSO_4$	3947 ppm
$NaSO_4$	895 ppm
NaCl	236 ppm
Na_2CO_3	546 ppm

Standard levels of the following were listed as acceptable by the United States Public Health Service in its 1961 revision:

Arsenic	.01 mg/l	
Copper	1.0 mg/l	
Chloride	250.0 mg/l	
Iron	.3 mg/l	
Manganese	.05 mg/l	
Nitrate	45.0 mg/l	
Sulfate	250.0 mg/l	
Zn	5.0 mg/l	
Total dissolved solids	500.0 mg/l	(This has also been quoted as 1000 mg/l).

Snell (1956) made the following observations concerning the reproduction of the laboratory mouse: (1) copulation is accompanied by the formation of a vaginal plug, the presence of which is a sign mating has occurred. The plug is formed by a mixture of the secretions of the vesicular glands and the coagulating glands of the male, and in the mouse usually fills the vagina from the cervical canal to the vulva, from which it may even protrude. Plugs in the mouse usually persist for 18 to 24 hours, occasionally for as long as 48 hours, after which they are sufficiently loosened, probably as the result of leukocytic action, to fall out almost entirely. (2) Gestation in non sucking mice is from 19-20 days, and estrus occurs about 20 hours after parturition. (3) litter size shows an increase from the first to third pregnancy after which it

starts to decrease:

1st litter	average sixe 5.13	variation 0.08
2nd litter	average size 6.35	variation 0.09
3rd litter	average size 6.46	variation 0.09
4th litter	average size 6.21	variation 0.10
5th litter	average size 5.53	variation 0.11
6th litter	average size 4.62	variation 0.13
7th litter	average size 4.01	variation 0.14
8th litter	average size 3.50	variation 0.34
Total	5.56	0.04

(4) The sex ratio is usually 1:1. (5) Study of post natal development results in interesting observations; the young are born hairless, with the eyes and ears shut. The sex can be determined at birth. By 9 days the females show nipples. The ears open at 3 days and the coat is well developed at 2 weeks. By the time the young are 12-14 days of age the eyes open, external ears grow rapidly, first moult begins, large follicles develop in the ovaries and the young start to eat their first solid feed. The first fertile mating usually occurs at 7-10 weeks.

Allen (1922) made the following observations of the estrus cycle in the mouse: the cycle usually requires 4-6 days and is divided into 5 phases; diestrus--the generative organs are relatively quiescent and anemic, proestrus--the organs are hyperemic and there is growth and hypersecretion of the mucosal layer, estrus--the epithelium is both cornified and non cornified, metestrus 1-- leucocytes begin to penetrate the epithelium, metestrus 2-- the epithelium shows degeneration and leucocytes are numerous.

There are 2 variations of the estrus cycle. First, instead of a rapid recurrence of the second cycle, a long diestrus interval may inter-

vene resulting in an apparently normal cycle of 8 or more days duration. At times in this cycle there may be slight indications of estrus in the smear at the midinterval suggesting possibly that the failure of a second expected heat period is only partial. Second, there may be a continuation of estrus usually evident for 1 day or less, for 2, 3, or more days. Due chiefly to these 2 types there is a great variation in the estrus cycle. Also certain strains have various cycles.

Grüneberg (1939) found that the fecundity of cross-bred mice was greater than Pure Line or Inbred strains.

Browman (1937) reports that too frequent or incorrect vaginal examinations may induce prolonged vaginal cornification.

Crew and Mirskain (1930) noted that fruitful mating is exceedingly rare in the suckling mother, but if the litter is removed immediately after birth the mating associated with estrus phase which normally follows within 24 hours after parturition is, with few exceptions, followed by a pregnancy of normal duration of 19-20 days. Pregnancy and lactation are two phases of equal duration, each separated by an estrus phase.

Crew and Mirskain (1930) also noted that the second postpartum estrus occurs spontaneously between days 21 and 26 following parturition. By permitting the young to suckle the mother a delay in the normal process of implantation and embryonic development is seen. By removing the litter at 7 and 14 days, estrus usually occurred at the 2nd and 4th day. Suckling is the agent primarily responsible for the maintenance of the lactation interval, and when it is removed, no matter at what stage of the interval, the interval terminates and the estrus phase replaces the reproductive phase.

MATERIALS AND METHODS

Water Source

Water for this study was obtained from the Milo Throlson ranch (well No. 2) Sheyenne, North Dakota, and from the water supply of Iowa State University, Ames, Iowa. Twenty gallons of water from each source were held in plastic containers under refrigeration. Bacteriological and chemical analyses were performed on representative samples of water from each source.

A prefiltration and postfiltration analyses were done on the North Dakota water by the Federal Water Pollution Control Laboratories, United States Department of Interior to determine the trace elements and other minerals. A prefiltration sample of North Dakota was also analyzed by the Chemical Engineering Department, Iowa State University for minerals other than trace elements.

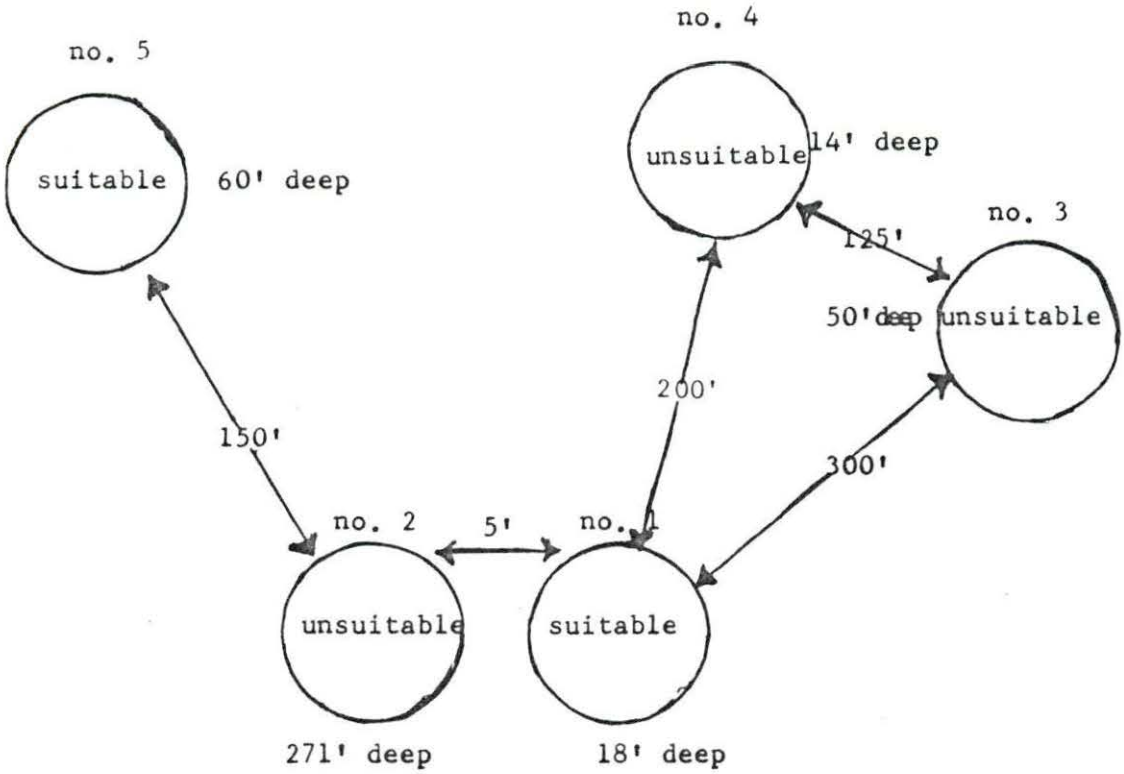
A diagram of relative locations, depth, and drilling dates of the wells on the Throlson ranch is presented in Figure 1. The wells are labeled suitable or unsuitable according to owner's evaluation.

Experimental Animals

Source

Mice for this study were obtained from the Iowa State University Genetics Laboratory. Seven K strain albino males and 20 wa-2v strain black females were used as first generation breeding stock. These were divided into 2 groups of 10 each. One male was put with 5 females. One

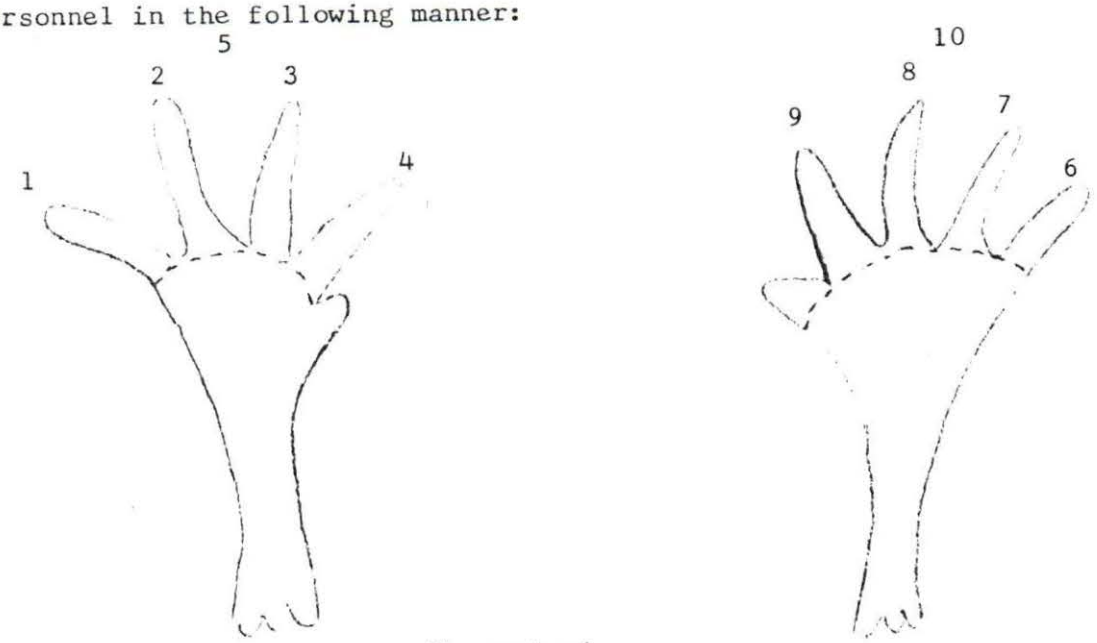
Figure 1. Location and description of wells on Throlson Ranch



no. 1 was original well on farm at time of purchase
no. 2 drilled in 1961 -- 271 feet deep
no. 3 drilled in 1963 -- 50 feet deep
no. 4 drilled in 1965 -- 14 deep deep
no. 5 drilled in 1969 -- 60 feet deep

group of 10 was put on the water from North Dakota and one group of 10 was put on the water from Ames.

The mice were toe-marked for identification by the Genetic Laboratory Personnel in the following manner:



Ventral view

Front feet ---- digits
Hind feet ----- tens

Vaginal Smears

This part of the study was designed to determine if the estrus cycle of mice might be altered by the ingestion of North Dakota water. Daily vaginal smears were made from three mice through 4 estrus cycles after which they were fed the test water and observed for 3 cycles.

Making a Pipette

A small pipette was made by heating a Pasteur pipette over a flame and drawing it out to a fine tip. The tip was rounded by heating so

that there would be no sharp edges to injure the vaginal mucosa. A small rubber bulb was attached to the pipette.

A solution used for vaginal lavage was prepared by adding 20 drops of methylene blue to 50 ml of normal saline solution. The vaginal washings were stained with methylene blue thus making cellular identification much easier.

A weak alcohol solution was drawn into the pipette between each sampling to clean it. The pipette was washed daily with hot water and soap and stored in 70% ethyl alcohol.

Vaginal smear

In order to do a vaginal lavage the mice were grasped by the loose skin behind the head with the thumb and forefinger of the left hand. The hind parts of the mice were controlled by holding the tail with the other fingers. The pipette was held in the right hand and about 2 drops of solution was drawn into it. The tip was gently inserted about 1 mm into the vagina and the solution injected into the vagina. The bulb was released to draw the solution back into the pipette. The vaginal lavage was discharged onto a clean glass slide and air dried. Smears were examined under a microscope to determine the stage of estrus.

Care of Mice

The mice were housed in standard mouse boxes. The bedding in the boxes was changed once a week.

All mice were fed commerical mouse cubes supplemented with whole yellow corn every 2nd day. Cheese and a green leafy vegetable were fed

once a week. An adequate supply of North Dakota water or Iowa State University water was available at all times to respective groups.

Experimental Design

The 20 females and 7 males previously described were divided in 2 groups. One group of 10 females with 3 males were fed North Dakota water. The other group of 10 females and 4 males consumed Iowa State University water.

Each of the above groups was subdivided in 2 more groups consisting of 5 femals and 1 male. Each of these subgroups was placed in a mouse box for first generation breeding stock. As soon as copulation was evidenced or pregnancy was noted, the females were removed and housed 2 to each mouse box.

One litter consisting of 11 offspring from the first generation breeding stock that had been fed the North Dakota water and another litter with same number of offspring that had consumed the Iowa State University water were kept until they had gone through 1 breeding period. The other litters were examined at birth for any abnormalities and killed.

To determine if the North Dakota or Iowa State University water had had any effect on estrus cycle, blood picture, and second gestation period, the original 20 females were rebred to males of the respective experimental groups. Ten days after the birth of their 2nd litter these females were killed.

All adult mice were killed by stunning and exsanguination by severing the brachial artery. Blood was collected in a tube containing EDTA for a complete blood count.

The necropsy was performed by laying the mouse on its back and removing the ventral body wall from the thorax to the pelvis. Exposed viscera were examined for gross changes.

Tissues for histopathological study were fixed in 10% formalin. The following tissues were collected: gonads, urinary bladder, uterus, kidneys, adrenal glands, liver, colon, and a femur. Tissues were embedded paraffin, sectioned at 8 u, mounted on glass slides and stained with Harris hematoxylin and eosin.

RESULTS

Data from the analysis of water are presented in Tables 1, 2, 3, and 4. Trace elements in both North Dakota and Iowa State University water were within acceptable levels with the exception of iron in suspension in the North Dakota water.

Table 1. Iowa State University water analysis for trace elements dissolved and suspended

	ug/l.(PPB)		ug/l.(PPB)
1. Zinc	4.	13. Nickel	5.
2. Cadmium	5.	14. Cobalt	5.
3. Arsenic	24.	15. Lead	10.
4. Boron	2100.	16. Chromium hexavalent	2.
5. Phosphorus	5000.	17. Vanadium	10.
6. Iron	26.	18. Barium	340.
7. Molybdenum	10.	19. Strontium	100.
8. Manganese	30.		
9. Aluminum	2000.		
10. Beryllium	10.		
11. Copper	240.		
12. Silver	.5		

Table 2. North Dakota water analysis for trace elements in solution

		ug/l.(PPB)			ug/l.(PPB)
1.	Zinc	150.	13.	Nickel	5.
2.	Cadmium	5.	14.	Cobalt	5.
3.	Arsenic	24.	15.	Lead	10.
4.	Boron	1150.	16.	Chronmium hexavalent	2.
5.	Phosphorus	5000.	17.	Vanadium	10.
6.	Iron	10.	18.	Barium	100.
7.	Molybdenum	10.	19.	Strontium	100.
8.	Manganese	180.			
9.	Aluminum	2000.			
10.	Beryllium	10.			
11.	Copper	8.			
12.	Silver	.5			

Table 3. North Dakota water analysis for trace elements dissolved and suspended

		ug/l.(PPB)			ug/l.(PPB)
1.	Zinc	156	13.	Nickel	40.
2.	Cadmium	20	14.	Cobalt	40.
3.	Arsenic	50	15.	Lead	40.
4.	Boron	10	16.	Chronmium	20.
5.	Phosphrus	100	17.	Vanadium	40.
6.	Iron	4800.	18.	Barium	2.
7.	Molybdenum	40.	19.	Strontium,	2.
8.	Manganese	39.			
9.	Aluminum	68.			
10.	Beryllium	.20			
11.	Copper	20.			
12.	Silver	2.0			

Table 4. North Dakota analysis for other than trace elements

Biochemical oxygen demand		10.5 mg/l as oxygen
Chemical oxygen demand	unsettled	93.8 mg/l as oxygen
	settled	36.1 mg/l as oxygen
Nitrate		0.22 mg/l as N
Nitrite		trace
Ammonia		2.06 mg/l as N
Iron (total)		337.3 mg/l as Fe
Sulfate		2449.8 mg/l as SO_4
Hardness (Ca, Mg, Ba, Sr)		1605.7 mg/l as $CaCO_3$
Selenium		less than 10ug/l as Se
Total solids	unsettled	5613 mg/l
Volatile total	unsettled	787 mg/l
Fixed total	unsettled	4827 mg/l
Total phosphorous (hydrolyzable and ortho)		3.64 mg/l as PO_4
Ortho-phosphate		0.14 mg/l as PO_4
Sodium		390 mg/l as Na
Potassium		24 mg/l as K
Sulfide		roughly 0.10 mg/l as S

Water analysis data for components other than trace elements are presented in table 4. Iron levels were approximately 1,100 times higher than acceptable levels. Sulfate levels and total solids respectively were 10 times acceptable levels.

A comparison of the consumption of water from the ranch in North Dakota and the water from the Iowa State University was made. Two similiar groups of mice were obtained from the Diagnostic Laboratory, Ames, Iowa, and the water consumption was measured daily with a graduated beaker.

Table 5. Comparison of water consumption

Date	Consumption of University water	Consumption of North Dakota water
2/3/70	40 ml	50 ml
2/4/70	40 ml	50 ml
2/5/70	35 ml	60 ml
2/6/70	60 ml	60 ml
2/7/70	35 ml	50 ml
2/8/70	40 ml	50 ml
2/9/70	40 ml	65 ml
2/10/70	45 ml	55 ml
2/11/70	40 ml	45 ml
2/12/70	45 ml	55 ml
2/13/70	55 ml	45 ml
2/14/70	55 ml	45 ml
2/15/70	30 ml	20 ml
2/16/70	30 ml	40 ml
2/17/70	30 ml	50 ml
2/18/70	50 ml	30 ml
2/19/70	10 ml	50 ml
2/20/70	35 ml	50 ml
2/21/70	35 ml	40 ml
2/22/70	40 ml	50 ml
2/23/70	40 ml	50 ml
2/24/70	35 ml	40 ml
Total	865 ml	1030 ml

Gross Pathology

No visible gross lesions were observed on necropsy.

Histopathology

Detectable histopathological changes were limited to the intestinal mucosa and renal glomeruli. The intestinal villi of the mice drinking Nortk Dakota water were more distinct and prominent, but appeared to be less cellular than in the mice consuming Iowa State University water. This observation may be a reflection of a difference in cellular hydration due to the high mineral intake. Renal changes were limited to a dilation of Bowman's space in the glomeruli of mice drinking North Dakota water.

DISCUSSION

From an analysis of the North Dakota water it was determined there was 10 times the accepted levels of sulfates which is 250.0 mg/l. Solids were 5-10 times the accepted levels which are usually listed as 500.0 mg/l, but occasionally is given as 1000.0 mg/l. Iron was extremely high being about 1100 times the accepted level of .3 mg/l. It should be pointed out that the iron level in the water consumed by the mice was actually lower than that since the iron would not all stay in suspension.

The primary objective of this investigation was to determine if there would be an adverse effect on the fecundity of mice from the feeding of high mineral content water. A comparison of the litter size of the first gestation indicated only an average of .1 more young in the mice fed Iowa State University water than those fed North Dakota water.

In the second gestation there was an average difference of 2.3 more young on the Iowa State University water than those on the North Dakota water. In the 2 litters, each with 11 offspring, from the first gestation which had been kept through one breeding period, there was an average of 1.1 more young among the group fed Iowa State University water than those fed North Dakota water (tables 6, 7, and 9). In the first gestation breeding there was 1 stillborn mouse in each group. In the second gestation there was 1 stillborn and 1 cyclop born in the group fed North Dakota water and no stillborn or anomalies to those fed Iowa State University water. The 2 above litters which had been kept from the first gestation did not produce any stillborn or anomalies.

Daily vaginal smears on all the females were not taken to determine

Table 6. A comparison of the litter size from the first gestation of the two groups[a]

Mice on University water			Mice on North Dakota water		
Mouse no.	Litter size	Remarks	Mouse no.	Litter size	Remarks
4	11	Saved for breeding	3	11	Saved for breeding
5	11	None	2	10	None
6	8	None	1	4	None
3	4	None	5	4	None
9	9	None	12	13	None
7	9	None	4	11	None
1	4	None	8	4	None
11	9	1 born dead	6	7	1 born dead
12	8	None	32	8	None
2	0[b]	None	9	8	None
	Total 73			Total 80	
	Av. 8.1			Av. 8	

[a] A comparison of the two groups through the first gestation shows no appreciable difference in their fecundity.

[b] Non-breeder.

Table 7. A comparison of the litter size from the second gestation of the two groups

Mice on University water			Mice on North Dakota water		
Mouse no.	Litter size	Remarks	Mouse no.	Litter size	Remarks
4	7	None	2	10	None
5	9	None	8	3	1 born dead
9	10	None	1	9	None
3	8	None	3	8	None
6	5	None	4	2	1 abnormal
12	0	None	6	5	None
2	0	None	32	3	None
7	0	None	9	4	None
11	0	None	5	0	None
1	0	None	12	0	None
	Total 39			Total 44	
	Av. 7.8			Av. 5.5	

Since there were 5 non-breeders in the group on the Iowa State University water, and 2 non-breeders in the group on the North Dakota water, the author did not feel there was an appreciable difference in the two groups.

Table 8. Comparison of the total weights of the two litters (11 in each) at 45 days of age

University water		North Dakota water	
5 males, 6 females	217 grams	4 males, 7 females	223 grams

Table 9. Comparison of the litter size of the two groups

University water			North Dakota water		
Mouse no.	Litter size	Remarks	Mouse no.	Litter size	Remarks
1	10	None	1	5	None
2	8	None	2	9	None
3	12	None	3	10	None
4	8	None	4	10	None
5	8	None	5	8	None
6	8	None	6	7	None
			7	6	None
	Total 54			Total 55	
	Av. 9			Av. 7.9	

A comparison of these two groups, both in weight at 45 days, and litter size, did not show any appreciable differences.

estrus cycle status because the work of Browman (1937) showed that too frequent or incorrect vaginal examinations may induce prolonged vaginal cornification. Therefore vaginal smears were made from only 3 female mice being fed North Dakota water. Normal vaginal smears are illustrated in Figures. Estrus cycles were not visibly altered by feeding of high mineral content water.

The consumption of the North Dakota water was about 16% higher than the consumption of the Iowa State University water. The mice on the North Dakota water developed a diarrhea which subsided after a few days. It appeared that the increased salinity of the North Dakota water accounted for the additional consumption and probably caused the diarrhea. Apparently the animals adjusted to the high salinity with no unfavorable effects. This agrees with the works of Anonymous (1959), Peterson (1952), Pierce (1960), Chein et al. (1968), and Winks (1963).

A comparison of the growth rate, sexual maturity, and total weight of the 2 litters consisting of 11 off-spring in each which were kept from the first gestation, failed to show any appreciable variations as shown in Tables 8 and 9.

The hematologic results of these 2 litters are presented in Tables 10 and 11 and a comparison in Table 12 suggests very little difference between the 2 groups. From tables 13 and 14 which are summarized in table 15, it is noted that hematologic values in the breeding females fed the North Dakota water were slightly higher than those of the other group, but the differences were relatively small. The hematologic values of breeding males are listed in tables 16 and 17 and the comparison may be

Figure 2. Vaginal smear in early proestrus stage of estrouse cycle. Note decreasing numbers of leucocytes and increasing numbers of epithelial cells. Wright's Stain x 100

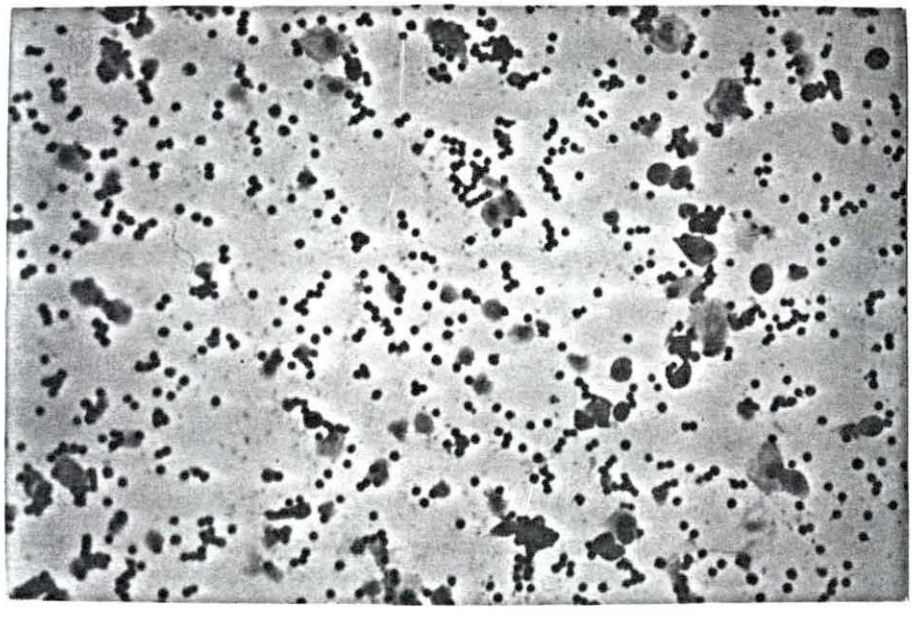


Figure 3. Vaginal smear in estrous stage of estrous cycle. Consists primarily of epithelial cells with some becoming cornified. Wrights Stain x 100

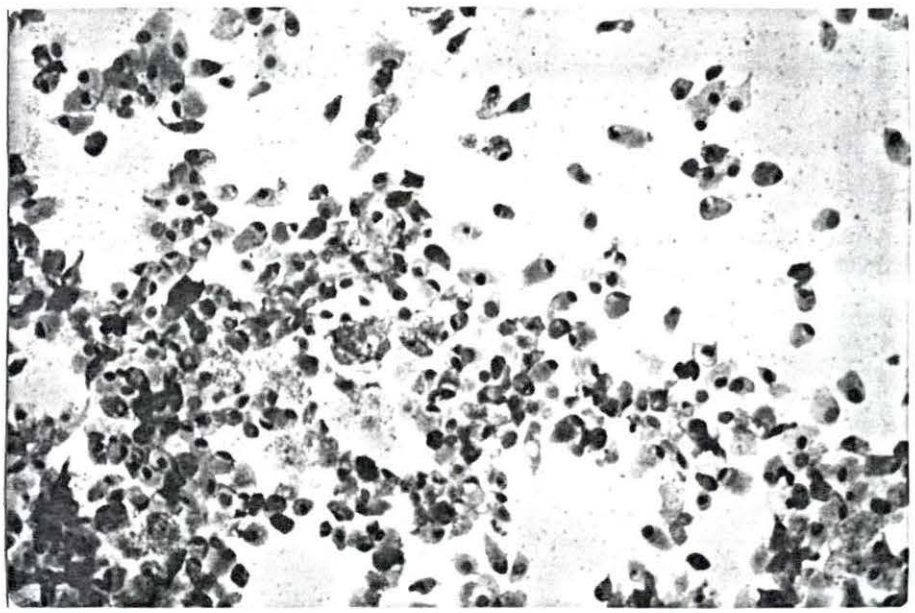


Figure 4. Vaginal smear in metestrus I stage of estrus cycle. Mostly cornified epithelial cells with a few leucocytes. Wright's Stain x 100

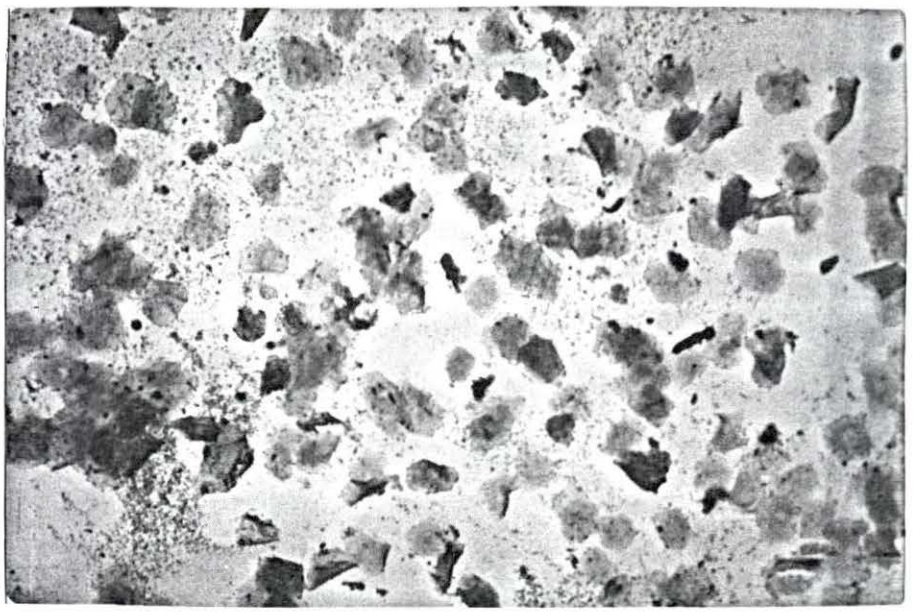


Figure 5. Vaginal smear in metestrus II stage of estrus cycle. Note the great increase in number of leucocytes and corresponding decrease of epithelial cells. Wright's Stain x 100

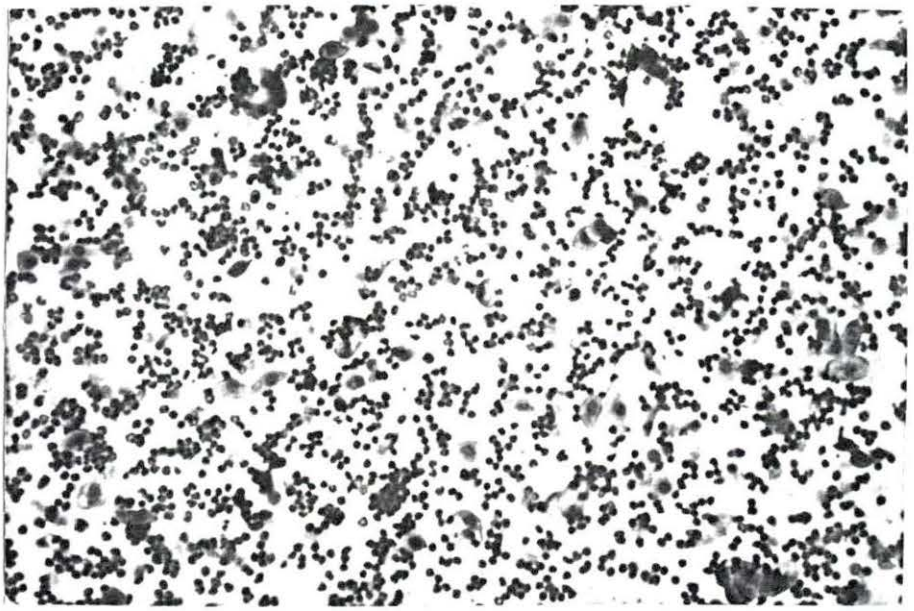


Figure 6. Vaginal smear in diestrus stage of estrus cycle. Note leucocytes decreasing in numbers and mild increase in epithelial cells. Mucos is present. Wright's Stain x 100

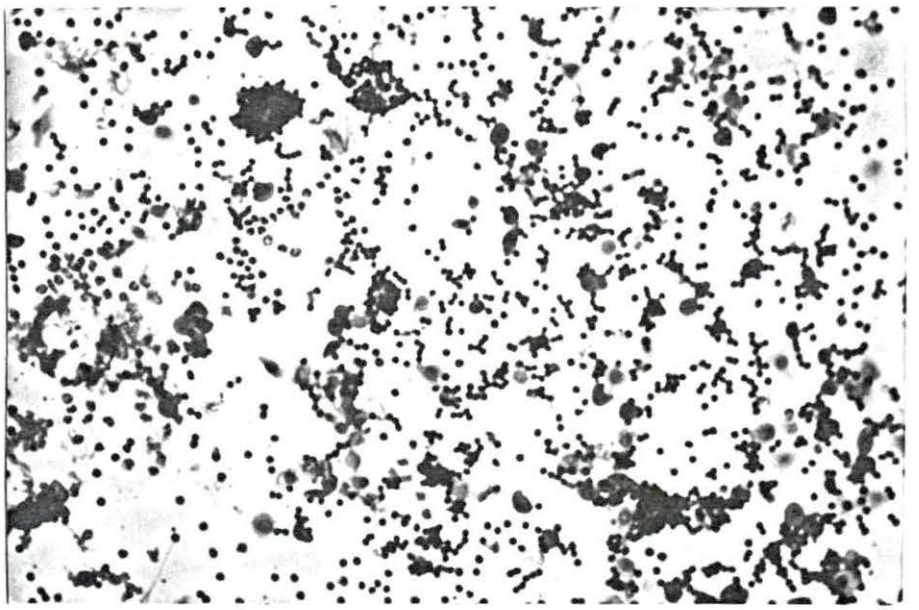


Table 10. The hematology of the litter of 11 offspring on University water

Mouse no.	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
Females				
1	Clotted sample			
2	11.8	30.0	7,000,000	12,300
3	Clotted sample			
4	Clotted sample			
5	12.1	30.0	6,040,000	4,200
6	9.8	27.0	5,100,000	5,000
Males				
1	13.8	39.0	7,440,000	4,100
2	12.8	33.0	6,960,000	6,300
3	13.0	35.0	7,270,000	8,900
4	13.0	36.0	7,380,000	8,900
5	Clotted sample			
Av.	12.3	32.9	6,741,000	7,100

Table 11. The hematology of the litter of 11 offspring on North Dakota water

Mouse no.	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
Females				
1	13.8	38.5	6,960,000	10,300
2	11.4	30.0	6,140,000	4,000
3	11.3	32.0	6,230,000	5,600
4	8.7	23.0	4,930,000	4,000
5	12.1	35.0	6,530,000	4,600
6	13.6	39.0	6,950,000	4,400
7	14.4	45.0	8,750,000	5,400
Males				
1	11.4	-	5,740,000	4,400
2	Clotted sample			
3	15.6	48.0	8,160,000	5,500
4	15.6	46.0	8,660,000	4,500
	12.8	37.4	6,905,000	5,270

Table 12. Comparison of hematology of the two litters

	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
North Dakota water				
Av.	12.8	37.4	6,905,000	5,500
University water				
Av.	12.3	32.9	6,741,000	7,100

From these results, it may be concluded that there is no significant difference between the two groups.

Table 13. Hematological results of the females which were given North Dakota water

Mouse no.	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
2	10.7	32	6,030,000	11,900
8	13.2	46	8,020,000	5,500
1	14.8	52	9,150,000	9,500
3	13.2	41	7,050,000	5,000
4	13.6	41	7,970,000	13,400
6	10.7	33	6,570,000	5,950
32	11.1	33	7,000,000	5,925
9	9.8	29	5,830,000	7,650
5	12.8	35	7,930,000	8,750
12	14.0	39	7,840,000	5,200
Av.	12.4	38	7,339,000	7.877

Table 14. Hematological results of the original females on the University water

Mouse no.	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
4	9.8	23.5	5,750,000	3,300
5	9.0	24	4,870,000	11,600
9	12.8	38	6,590,000	8,700
3	10.7	33	7,480,000	8,700
6	10.4	32	8,650,000	5,520
12	12.8	40	6,770,000	2,375
2	12.1	36	7,920,000	5,250
7	10.7	30	6,630,000	7,480
11	11.8	35	7,770,000	10,400
1	14.4	42	8,270,000	3,800
Av.	11.4	33.3	7,070,000	6,712

Table 15. Hematological comparison of the two groups of original females

	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
North Dakota water				
Av.	12.4	38.1	7,339,000	7,877
University water				
Av.	11.4	33.3	7,070,000	6,712

Table 16. Hematological results of the original males which were used in this study

Mouse no.	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
North Dakota water				
1	12.5	35	7,770,000	2,925
2	14.0	41	8,300,000	6,620
3	Clotted			
University water				
1	11.8	33	8,040,000	8,550
2	13.2	36	9,740,000	3,700
3	14.4	40	8,120,000	2,900
4	12.8	38	7,650,000	5,690

Table 17. A comparison of the hematology of the two groups in Table 16

	Hemoglobin g/100ml	Packed cell volume %	Red blood cells /mm^3	White blood cells /mm^3
North Dakota water				
Av.	13.25	38	8,035,000	4,772
University water				
Av.	13.05	37	8,375,000	5,210

noted in table 17. The differences between the 2 groups are too small to be of significance.

There was an absence of detectable gross lesions attributable to the feeding of high mineral content water. Microscopic changes were found in the intestinal mucosa and renal glomeruli. The intestinal villi of the mice drinking North Dakota water were more distinct and prominent, but appeared to be less cellular than in the mice consuming Iowa State University water. This observation may be a reflection of a difference in cellular hydration due to the high mineral content. Renal changes were limited to dilatation of Bowman's space in the glomeruli of mice drinking North Dakota water. This suggests that mice are able to adapt to high mineral content water with no visible ill effects.

SUMMARY AND CONCLUSION

1. The feeding of high mineral content water to breeding mice over a 5 month period and through two gestations did not produce any appreciable effect on their fecundity.

2. One anomaly - a cyclops - was observed. It was from a female being fed North Dakota water. Since Mr. Throlson had reported "awful" looking piglets, it might suggest that the North Dakota water may produce some anomalies.

3. No change could be detected in the estrus cycle of mice fed the North Dakota water. Failure to cycle regularly was not uncommon in either group. Long diestral periods were observed in both groups.

4. Mice fed North Dakota water consumed more water than those fed Iowa State University water probably caused by the high salinity of the North Dakota water. They developed a diarrhea soon after being fed the water, but this ceased after a few days. It could be concluded that mice can adjust to water with a high mineral content.

5. The rate of gain of the off-spring fed the North Dakota water was greater than those on Iowa State University water. Apparently they were more hydrated than the mice consuming Iowa State University water.

6. Only slight variations were found in hematologic values. In all groups the hemoglobin content and the packed cell volume were slightly higher in the mice fed North Dakota water than those drinking Iowa State University water which may be related to the high iron content of the water. The red blood cell count was higher in 2 out of 3 groups fed North Dakota water. The number of white cells was lower in 2 out of 3

groups drinking the North Dakota water.

7. No gross lesions and only limited histopathological changes were observed that were attributable to the North Dakota water. This would indicate that mice have the capacity to adapt to high mineral content water.

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