GEOTHERMAL ARSENIC IMPACTS ON STREAM ANIMALS

M. H. Timperley
Environmental Scientist, Environmental Research Group,
Institute of Environmental Health & Forensic Sciences, Auckland, N.Z.

Linda Hill
Research Scientist, New Zealand Pharmaceuticals Ltd, Palmerston North, N.Z.

SUMMARY -Freshwater snails, *potamopyrgus antipodarum*, exposed to a mean water concentration of arsenic of 0.088 mg/l in a stream receiving geothermal fluid, did not contain more arsenic than snails exposed to water concentrations of arsenic less than 0.001 mg/l. Irrespective of the exposure, arsenic in the snails was concentrated onto the outer surface of their shells. An abiotic mechanism is postulated. The reason for the low arsenic concentrations in the snail soft body parts was shown to be the low concentrations in the main component of the snail diet, chain diatoms. Snails fed with filamentous green algae containing high concentrations of arsenic, accumulated arsenic to high levels in their soft body parts.

1. INTRODUCTION

Arsenic is a constituent of most geothermal fluids (Ellis and Mahon, 1977) and the discharge of these fluids can result in contamination of receiving environments. In New Zealand, the discharge of separated water from the Wairakei geothermal power station over the past 30 years, has caused arsenic concentrations in the Waikato River immediately below the Wairakei geothermal power station to rise above drinking water standards (Aggett and Aspell, 1980). In some of the hydroelectric reservoirs on the Waikato River, the sediments and aquatic plants contain arsenic concentrations of up to 1000 mg/kg (Aggett and Aspell, 1980). The practice of discharging separated water to natural waters is being replaced by reinjection but intermittent discharges from holding ponds is likely to remain common.

Prior to 1986, the water quality criterion most often applied for controlling arsenic discharges to waters was that promulgated for potable drinking water. This criterion is 0.05 mg/l of total dissolved arsenic (World Health Organisation, 1971). The use of this criterion for a relatively long period had perhaps lead to its acceptance as an appropriate criterion for protecting aquatic life, but in 1986, the U. S. Environmental Protection Agency (1986) published two new criteria specifically derived for this purpose. These criteria were a 1 hour average to protect from acute effects and a 4 day average to protect from chronic effects. The arsenic concentrations were 0.360 mg/l and 0.190 mg/l respectively.

The four-fold increase from the commonly used, pre-1986 value of 0.05 mg/l to the new 4 day criterion of 0.190 mg/l caused some scepticism, not only among those who did not understand the basis for the pre-1986 criterion, but also among some scientists who, from their general experience with metals in waters, felt that the new criterion was just too high.

In 1986 proposals for developing the Mokai geothermal field situated just north of Taupo on the Central Volcanic Plateau, required environmental impact assessments. During the course of work for these assessments, a small population of the freshwater snail *Potamopyrgus antipodarum* (Gray, 1843) was discovered living in a stream which received a natural input of geothermal water. The stream water was warm and contained about 0.1 mg/l total dissolved arsenic. A brief study of these snails indicated that they may be stunted in their growth because of exposure to either heat or arsenic. The discovery of this population living in a concentration of arsenic about 50% of the new 4 day criterion offered the opportunity to validate the criterion from field observations.

The objectives of this study were to firstly examine the breeding and growth dynamics of the snail population to identify any abnormalities and to secondly distinguish and quantify the impact on the snails of elevated temperature and arsenic.

2. METHODS

2.1 Site description

The Waipapa stream, which receives geothermal arsenic and heat, flows from the Mokai Geothermal field, north into Lake Whakamaru on the Waikato River (Fig 1.). The inflow of geothermal fluid occurs about 4 km from the confluence with the Waikato River. A population of snails in the outlet stream of Lake Ngapouri which has a low concentration of arsenic and a natural temperature regime, was used as a control for the study. Lake Ngapouri is located about 40 km north of Taupo just west of the road to Rotorua. Snails from a stream near the coast, west of Auckland were used for laboratory experiments.

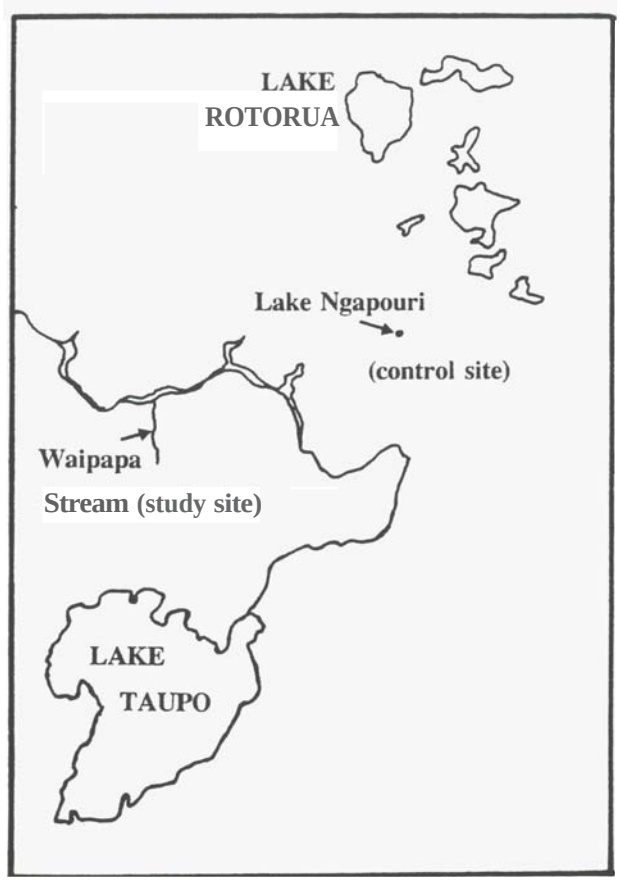

Figure 1- Location map showing the study and control sites.

2.2 Population dynamics

Snails were collected at monthly intervals from both the study and control sites. Pebbles from the stream bed and vegetation from the stream banks were gently but completely brushed into a nylon net (mesh 0.5 mm) until it wasjudged that sufficient snails had been collected. These samples were sorted in the laboratory, the snails were rinsed in distilled water and then dried at 60°C. Each snail was weighed to determine the size distribution of the snails in each sample. The temperature of the stream water at the time of sampling was recorded.

2.3 Arsenic analysis

The analysis of inorganic and organic forms of arsenic was based on methods described by Aggett and Aspell (1976) and Anderson *et al.*, (1986) using arsine generation with sodium borohydride and a heated quartz tube on an atomic absorption spectrophotometer. For the determination of As(III) only, the sample was maintained at a pH of 6.0 with a citrate buffer during reduction with sodium borohydride to arsine. For the determination of total inorganic arsenic, As(V) was reduced to As(III) with potassium iodide in hydrochloric acid before analysis. The As(III) was then reduced to arsine with sodium borohydride in 5M hydrochloric acid for analysis. Total dissolved arsenic; As(III), As(V) and organic arsenic (here represented by dimethylarsinic acid, DMAA) were determined by first reducing As(V) to As(III) as described above and then reducing As(III) and DMAA in mercaptoacetic acid to arsine with sodium borohydride. As(III) was determined directly and As(V) and DMAA by difference.

2.4 Sample preparation, digestion and analysis

Snails for total arsenic analyses, were dried at 60°C then ground in an agate mortar and pestle to a uniform powder. This powder (0.05 g) was dissolved overnight in concentrated nitric acid (5 ml) in teflon beakers. The mixture was then heated gently for 2 hours. Further nitric acid (1 ml) was added and the mixture evaporated to about 0.5 ml. Hydrochloric acid (0.050 ml) was then added with gentle warming and the digestate was made up to 5 g for analysis. Recoveries of As(III), As(V) and dimethylarsinic acid by this digestion procedure were in the range of 91 to 100%.

Snail shells and bodies were separated manually under a dissecting microscope. The components were dried and digested as described above.

The black external coating on the snail shells was dissolved by standing the shells in cold oxalic acid (0.05M) for 1 hour. This extract was analysed for total dissolved arsenic and for iron by flame atomic absorption spectrophotometry. For examination by infrared spectroscopy the coating was dissolved in dilute sodium hydroxide (0.05M) and then recovered from solution by precipitation with dilute hydrochloric acid. The precipitated matter was dried and the spectrum obtained in potassium bromide.

Sediments were dried at room temperature and separated by sieving into the following fractions: 0.50-0.20 mm, 0.20-0.12 mm, 0.12 mm-0.058 mm and < 0.058 mm. The dried, sieved sediment (0.5 g) was allowed to stand overnight in nitric acid (5 ml, concentrated) then heated to boiling for 2 hours. Further nitric acid (5 ml, concentrated) and perchloric acid (1 ml, concentrated) were added and the mixture was evaporated until the appearance of dense white fumes. This latter treatment was repeated, then the digestate was made up to 10 ml for analysis of total dissolved arsenic.

Aquatic macrophytes and algae were washed in distilled water then dried overnight (90°C). The dry weed (0.02 to 0.05 g) was digested by the procedure described above for snails.

2.5 Bioassays

Snails of similar size, collected from the Auckland west coast site, were placed in a glass aquarium which had been previously acid-cleaned. The substrate in the aquarium consisted of pebbles collected from the same site. Both water and sediment from this site had been shown to have very low concentrations of arsenic. The aquarium was inoculated with stream water containing predominantly *spirogyra,* a periphytic green algae, to provide food for the snails. At the start of the experiment, the concentration of

arsenic in the aquarium water was raised to approximately 0.1 mg/l. The aquarium water was analysed daily and the concentration adjusted if necessary to maintain the concentration at this level. At the end of the experiment the snails were removed and some were analysed by the methods described above for arsenic while others were dissected to examine stomach contents.

Perspex sheets were used as artificial substrates in both the aquarium and in the stream at the study site to gather periphytic growth for analysis of the snail diet. These sheets were suspended in the aquarium in such a manner that the snails could not reach them. In the stream, the substrate were suspended inside a plastic bucket with holes around the base. The growth on the substrate was removed with a flat plastic blade, dried and analysed for arsenic. A small quantity was examined fresh to identify the material present.

The stomach contents of snails from aquarium and the study site stream were examined to identify the predominant organisms in their diet.

3. RESULTS

Table 1. summarises the range of water temperatures over the duration of the study and the concentrations of arsenic in the stream waters and sediments.

Table 1. The range of stream water temperatures and the arsenic concentrations in waters (mg/l) and sediments (mg/kg) at the study and control sites.

	Study site	Control
Temperature	20 to 25°C	9 to 20°C
As in water	0.088(0.016)[13]*	<0.001[4]
As in sediment**		
0.50-0.20 mm	12	6
0.20-0.12 mm	12	28
0.12-0.058 mm	11	18
<0.058 mm	12	24

* mean(standard dev.)[n]

** at each site the result for each fraction is the mean of two samples.

The temperature at the study site remained within a relatively narrow, warm range whereas at the control site temperatures spanned the range normally observed in lakes of the Central Volcanic Zone. The results show that despite the fact that water arsenic concentrations at the control site were less than the detection limit of 0.001 mg/l, the sediments at the control site contained as much or more arsenic than the sediments at the study site.

The changes in the size classes of the snails at the two sites are presented in Fig 2. The size structure of the snail population at the control site changed in the expected manner with an increase in the proportion of juveniles during spring and the growth of these during summer and winter. At the study site, there was a more constant proportion of juveniles throughout the year and the steady decline in the proportion of juveniles through to the end of November seen at the control site was not so marked at the study site. The pattern at the study site is typical of a tropical environment with juveniles occurring in high numbers during all seasons. The maximum size of the adult snails at the study site was similar to that at the control site contrary to the tentative conclusion made from the 1986 observations that the snails at the study site were not reaching their full adult size.

Snails from the study site and the control site were analysed for total arsenic and the results are presented in Table 2.

Table 2. Total arsenic concentrations (mg/kg dry weight) in snails from the study and control sites.

	Study site	Control
Number of samples	10	9
Mean As concentration	50	54
standard deviation	17	12

Despite the low water concentrations of arsenic at the control site, the total concentration of arsenic in the snails was almost identical to that at the study site. These results for the snails correspond closely with those for the sediments which also had similar concentrations at both sites.

Previous studies have shown that many aquatic organisms metabolise inorganic arsenic to organic forms. Since this appeared to be a mechanism these snails might use to detoxify the relatively large amounts of arsenic they contain, the snails were analysed for total arsenic and total inorganic arsenic to give a measure of organic arsenic. The results of these analysis are shown in Table 3.

Table 3. The concentrations (mg/kg) of total arsenic (inorganic plus organic) and total inorganic arsenic in whole snails from the study and control sites.

	Replicate			
	1	2	3	4
Inorganic arsenic	15	11	7	16
Total arsenic	17	10	7	16
% inorganic arsenic	88	110	100	100

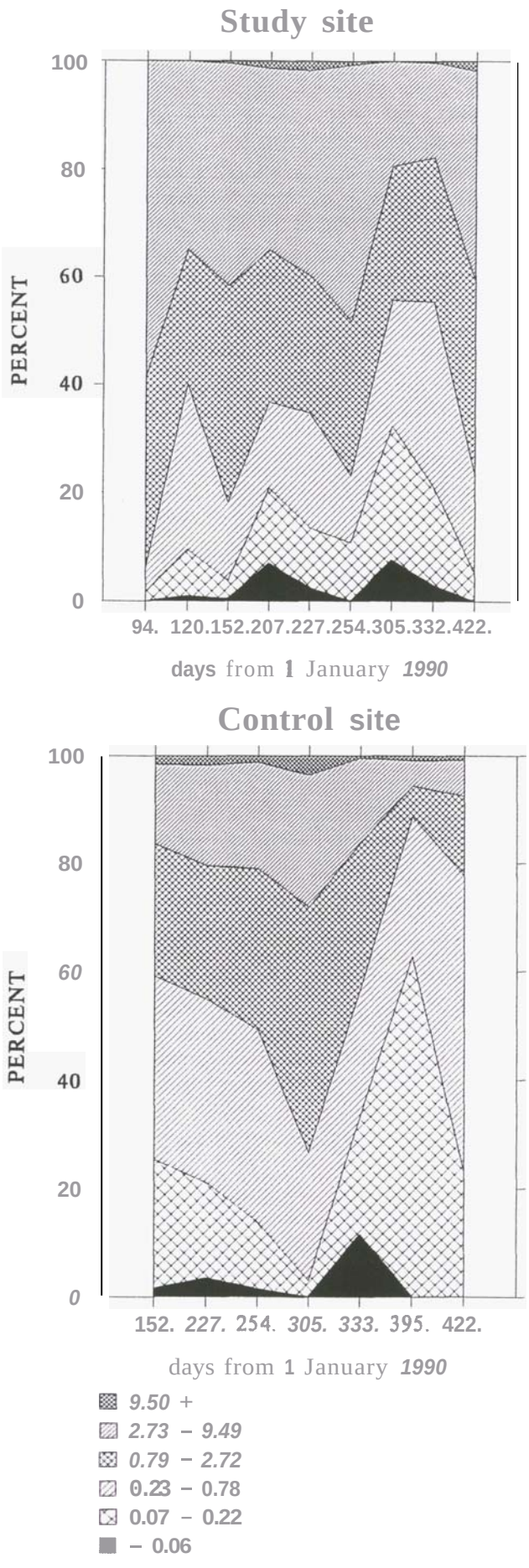

Figure 2- Size distributions of snails, presented as six weight (mg) classes, at the study and control sites.

These results show that, within the precision of the measurements, all of the arsenic in the snails is in inorganic form.

It was considered unlikely that the snails could live with the measured amounts of inorganic arsenic in their soft body organs (unless they had evolved some protective metabolism) and the most obvious alternative was that the arsenic was associated with the shells where it could be stored without harm to the snail. To test this hypothesis, snails from both sites were dissected into body and shell and analyses of these separate components are given in Table 4.

Table 4. Total arsenic concentrations (mg/kg dry weight) in body and shell components of snails from the study and control sites. For both sites the first result is for one sample dissected after drying and for the study site the second result is for one sample dissected while fresh (and then dried before analysis).

Component	Study site	Control
As in body	7, 2	5
As in shell	19, 24	17

Clearly, the major part of the arsenic in the snails from both sites is associated with the shell. To elucidate the location of the arsenic, i.e. deposited in the shell material or on the surfaces of the shell, whole snails were treated with dilute oxalic acid which had been shown to dissolve the black surface coating of the shell without visible attack on the shell mass. The oxalic acid extracts were analysed for arsenic and also iron because of the known affinity of precipitated iron oxides for arsenic. The results are shown in Table 5.

Table 5. Arsenic and iron concentrations in dilute oxalic acid extracts of snail shells. The extract concentrations are expressed in terms of the dry snail weight (mg/kg). The results for two samples from each site are given.

		Study site	Control
Arsenic			
	oxalic acid extract	32, 24	70, 42
	residual*	3, 3	3, 2
Iron			
	oxalic acid extract	800, 800	10270, 7170
Arsenic: iron			
	oxalic acid extract	1:25, 1:33	1:150, 1:170

* whole snail after oxalic acid extraction

The major part of the arsenic in the snails together with the black shell coating and substantial amounts of iron were dissolved into the oxalic acid solution. At each site the arsenic:iron ratios in the extracts were very similar for the two samples examined although there was more iron and arsenic on the snail shells from the control site. Although the results provide circumstantial evidence that both arsenic and iron are contained in the black shell coating, the possibility that the iron and arsenic are not associated cannot be rejected. For example, iron might have been extracted from the snail body rather than from the shell.

Iron oxides on the surface of sediment particles readily adsorb arsenic from solution and the possible association of iron and arsenic on the exterior of the snail shells gives some support for the idea that the accumulation of arsenic on the shells had occured by a similar, abiotic mechanism. However, there was also the possibility that the arsenic on the shells had been excreted with the black coating as the snails grew.

The first step towards distinguishing between these two possibilities was to determine the nature of the black coating. The coating was removed in dilute sodium hydroxide, precipitated with acid and dried. The IR spectrum of the precipitate had all the features of protein and the nearest match was with a protein extracted from corn. It also matched very closely with animal proteins including keratin and wool. The presence in the coating of protein, a substance with a known ability to bind metals, does not preclude any of the possible mechanisms for arsenic accumulation onto the shell; abiotic adsorption onto the protein of both iron and arsenic from the water, or excretion of one metal and adsorption of the other, or excretion of both metals.

Further attempts were made to clarify which of the possible explanations for the presence of arsenic on the snail was correct. Proof of excretion would identify a deliberate metabolic process for arsenic de-toxification. Bioassay experiment were conducted using snails from the Auckland west coast which had had no significant exposure to arsenic. Snails and sediment (< 0.5 mm fraction) from this site had less than 1 mg/kg and 1 mg/kg of arsenic respectively.

Over a period of 4 weeks the mean arsenic concentration in the aquarium water was 0.083 mg/l. At the end of this period, the snails were removed and divided into three samples. These samples were analysed for total arsenic in the whole snails, total arsenic in shells and bodies, and total arsenic in oxalic extracts. The results are given in Table 6.

Table 6. Arsenic concentrations (mg/kg dry weight) in snails from the bioassay experiment.

	Total arsenic concentration
Whole snails	22
Components	
shell	15
body	29
total	44
Extracted	
oxalic acid extract	9
residue*	8
total	17

* whole snail after oxalic acid extraction

The whole snail total arsenic concentrations showed considerable variation among the three samples but despite this, the results showed that at least 50% of the whole body arsenic was present in the soft body with the remainder on the shell surface. This finding was in contrast to the results for snails from the study and control sites where the soft bodies contained only small amounts of arsenic.

It had been observed that at the study site, periphytic green algae was quite sparse in contrast to the bioassay aquarium where it dominated periphytic growth. This possibility that this difference was related to the snail diet was investigated by using artificial substrate to collect samples of periphytic growth. The stomach contents of the snails were also examined.

The growth on the artificial substrate from the study site was dominated by *melosira spp*, a chain diatom and this was also the main component of snail stomach contents from this site. The predominantly diatom mass recovered from these substrate contained 76 mg/kg (dry weight) of total arsenic.

The periphytic growth in the aquariums was predominantly *spirogyra,* a filamentous green algae and this was the main dietary component for the aquarium snails. The total arsenic concentration in the material recovered from the ayuarium substrate was 350 mg/kg (dry weight).

4. DISCUSSION

The variations in snail size at the study and control sites showed that although breeding occurred more or less continuously throughout the year at the study site in contrast to the typical seasonal cycle at the control site, the adult size of the snails at the study site was no smaller than at the control site. It is known that these snails are found in thermal waters only at temperatures below 28°C and laboratory studies have found that snail activity is first curtailed at this temperature when temperatures are progressively raised (Wintebourn, 1969). Our observations that the temperatures observed at the study site have no

deleterious effect on the snails, is consistent with Winterbourn's observations.

At both sites, almost all of the arsenic in the whole snails was located on the surface of the shell, probably on the exterior in association with the protein covering and iron. The presence of iron indicates that the mechanism of accumulation of arsenic onto the shell could be identical to that which controls the deposition of arsenic onto sediment, i.e adsorption of arsenic onto iron hydroxides and oxides. The similar sediment arsenic concentrations at the two sites supports this postulate. The snails in the bioassay experiments also accumulated arsenic onto their shell surfaces (about half of their total body burden of arsenic) but because this occurred over only a four week period, it is strong evidence against the excretion of arsenic by the snail into the shell protein covering during growth.

Despite the difference in water concentrations of arsenic at the study and the control sites (about 3 orders of magnitude), snails at both sites contained very little arsenic in their soft body parts. This was in contrast to the snails in the bioassay study. The major difference between the study site and the bioassay aquarium, was that snails at the study site were eating diatoms with a low arsenic concentration whereas the aquarium snails were eating green algae with a much higher arsenic concentration.

5. CONCLUSIONS

Elevated water temperatures at the study site do not adversely influence the breeding, growth and maximum adult size of the snails. The only obvious effect is to promote continuous breeding.

Water arsenic concentrations of about 0.1 mg/l do not cause body burdens of arsenic in snails any greater than are caused by water concentrations less than 0.001 mg/l. However, irrespective of the water concentration, arsenic is accumulated onto the external surfaces of snail shells in association with the protein covering and iron. The results imply that this accumulation is abiotic but conclusive evidence has not yet been obtained.

A comparison of snail diets at the study site and in bioassay aquariums shows that the reason for the low concentrations of arsenic in the soft body parts of snails from the study site is the low arsenic concentration in the principal component of their diet, chain diatoms. The green algae which snails consuined in tlie bioassay experiments, accumulated arsenic to much higher concentrations than found in the diatoms from the study site, and this resulted in higher arsenic levels in the soft body parts of the aquarium snails. If field populations of snails grazed on filamentous green algae exposed to high concentrations of arsenic, then the soft body burdens of arsenic could reach high levels.

Acute toxicity was not observed in the bioassay experiments and there could be two reasons for this. Either the body burdens did not reach toxic levels or the snails metabolised the arsenic to non-toxic organic forms.

6. ACKNOWLEDGEMENTS

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