J Vector Borne Dis 48, September 2011, pp. 174–176

Environmental endocrine disruptors and their effects on *Biomphalaria glabrata* oviposition

Md. Touhid Iqbal & Shadman Sinha

Department of Biology, State University of New York, Geneseo, NY, USA

Key words Adaptive plasticity; *B. glabrata*; endocrine disruptors, oviposition, schistosomiasis

Schistosomiasis is a parasitic disease caused by several trematode species belonging to the genus of parasitic flatworms known as schistosomes. It is currently the most widespread of the water borne diseases and carries a number of socioeconomic burdens. Like all infectious diseases it greatly reduces quality of life of infected persons.

There are a number of drug treatments available to cure those infected with schistosomiasis[1]. However, large community-based drug treatments have been applied with very little success[2] since even a small number of individuals left untreated can pass schistosome eggs back into the water, maintaining high rates of reinfections[2].

Thus, the current focus for schistosomiasis control is through prevention methods. *Biomphalaria glabrata* is the species of snails that acts as vectors for *S. mansoni,* which is the most prominent of the schistosome species responsible for human schistosomiasis. Infection rates are found to be directly related to the population size of snails in endemic areas[2]. The majority of humans at risk of infection live in impoverished regions where most waterways are polluted and not well-maintained.

Previous studies show that the oviposition behavior of snails is sensitive to the surrounding environment; *B. glabrata* is sensitive to varying water quality[3] and their oviposition has also shown to be sensitive to concentrations of heavy metals in the surrounding environment[4].

More importantly Bisophenol A (BPA) has previously been shown to promote endocrine disruption and increase oviposition through superfeminization in a different species, the Ramshorn snail *Marisa cornuarietis*[5]. A link between plastics and increased oviposition of *B. glabrata* would raise incentives to clean waterways in the third world, offering a method for indirectly controlling the spread of schistosomiasis.

This study aimed to investigate if common pollutants found in waterways could have an effect on snail reproduction. Specifically, the link between known endocrine disruptors, such as plastics pollutants, and oviposition behavior of *B. glabrata* was of interest.

In all 36, healthy snails from the NMRI *B. glabrata* snail stock were chosen to be divided among six set ups. Pyrex loaf pans (12.7 × 23 cm, 1.5 L) were used to conduct the trials. Six snails were placed in six loaf pans with 800 ml of mineral balanced spring water at set pH. For one week snails were fed a diet of fresh lettuce, tetramin flakes, and dog kibble every 2 days. Oyster shells were also included as a source of calcium. Throughout the experiment a 12 h photoperiod and a room temperature of 27°C was maintained. At the end of 7th day trials were assigned. Water levels were always kept at 800 ml by addition of spring water; water was not replaced filtered at an attempt to simulate contaminated water in schistosomiasis endemic areas. Two trials were assigned as controls; the remaining four were subjected to varying amounts of sources of endocrine disruptors. Two trials were conducted in the presence of sandwich bags; one trial contained two shredded bags, while the other contained one intact bag. The remaining two trials were in the presence of a lid from a can of white tuna and tuna can tops with one-fourth components of a shattered blank compatible disc. Sandwich bag plastics are a known source of phthalates, while tuna cans and CD-Rs are a source of Bisophenol A. Both compounds have been known to show antiandrogenic as well as estrogenic effects.

Phthalate treament 1: This treatment consisted of a single shredded sandwich bag placed along the bottom of the loaf pan.

Phthalate treatment 2: Phthalates are esters mainly used as plasticizers and as PVC softeners. This treatment consisted of two sandwich bags (16.5 × 14 cm) shredded into equal parts, and placed at both ends of the loaf pans.

Bisophenol A treatment 1: This treatment consisted of a single tuna can lid which was heavily sanded down.

Bisophenol A treatment 2: Tuna cans, specifically, the can lids, and compact discs are common items made with BPA. This treatment consisted of a single tuna can lid which was sanded down heavily. A blank compact disc was also shattered, and exactly one-quarter of its components by mass was added to the loaf pan.

Snail groups were subjected to treatments for days, and then placed in circular glass dishes (10.5 × 4 cm deep) with 300 ml fresh spring water for one week. During this time they were fed lettuce, tetramin fish flakes, and dog chow daily along with regular removal of fecal waste and addition of fresh water. These conditions induced oviposition and the number of egg masses laid was recorded. Snail groups were once again subjected, respectively, to the same experimental trials for another 10 day period, following an identical seven day period where oviposition was induced and data were gathered.

Data used for comparison are the totals of both weeks of oviposition since it is unlikely that considerable effects of endocrine disruption will be seen after 10 days. Values for control treatments were averaged for a baseline egg mass count. The egg mass counts in different treatments were: control–20 (C1–7, C2–13); phthalate treatment 1–20, phthalate treatment 2–11, BPA1–23 and BPA2–15. The data indicate that oviposition was more prolific in the treatments phthalate treatment 2 and bisophenol treatment 1 (BPA1). The values for phthalate treatment 1 and bisophenol treatment 2 (BPA2) however are similar to the value for control 2. Oviposition was also more prolific during the second oviposition week for all treatments (Table 1).

Increased oviposition with increasing sources of xenoestrogen source is a logical trend given the estrogenic effects of these compounds in living organisms. In the Phthalate 2 and BPA 2 treatments it appears that the concentration of the respective compounds was negligible in altering oviposition. This supports the concept that a minimal threshold of xenoestrogen is needed for sensitivity and alteration of natural endocrine control of reproduction. Superfeminization, characterized by massive oocyte stimulation, has been observed in freshwater prosobranchia *Marisa cornuarietis* in response to BPA[5]. Furthermore, Marisa exhibited no signs of acute toxicity, indicating that the observed effects were strongly due to endocrine disruption. Likewise, each of the 36 original snails showed no signs of poor health or reduced activity throughout the experiment. The results of this experiment, in conjunction with other studies, demonstrate that estrogenic chemicals that cause reproductive effects in vertebrates and other aquatic animals also have effects in the fresh water pulmonate *B. glabrata*[6,7].

Results show that BPA2 treatment led to more egg masses with the comparable phthalate treatment 2; this indicates that BPA produces stronger endocrine disrupting effects in *B. glabrata* than phthalates at similarly reduced concentrations. The results also indicate that as exposure duration to possible sources of endocrine disruptors increase, oviposition increases. This is supported by the slow acting effects of hormones, especially steroidal compounds regulating gene expression, producing the hormonal alterations observed weeks after exposure.

Pulmonates produce many small egg masses during the spring-summer breeding season, and are considered seasonal breeders in the wild[8]. Literature regarding the effects of external conditions (pH, temperature, humidity, food and water quantity/quality) shows that environmental factors can regulate the endocrine system and reproduction[4]. This was observed with the behavior of the snails in which no egg laying was observed while exposed to BPA and phlthalate treatments, but oviposition was induced in response to clean water. A phenomenon termed adaptive plasticity allows snail populations to maximize productivity under normal environmental conditions, to compensate for harsh periods, without sacrificing their ability to react dynamically in a changing environment[9].

In schistosomiasis endemic countries, industrial waste water and sewage containing various heavy metals, toxins, and endocrine disrupting compounds, are dumped into rivers. Under these conditions, environmental factors signal the down regulation of reproductive hormones, and oviposition is limited. Relative increase in water quality and decrease in contaminants may elsewhere induce oviposition. Snail populations that have been subjected to xenoestrogens from contaminated effluent may then exhibit increased oviposition once factors such as heavy metal contamination, pH, and dissolved oxygen concentrations have been normalized.

The results of this study provide preliminary evidence that vertebrate endocrine disruptors, BPA and phthalates, also affect the freshwater pulmonate *B. glabrata*, notably through increased oviposition. This supports the notion that the basic signaling mechanisms are conserved throughout the animal kingdom. Given this, it is reasonable to conclude that the endocrine function of *B. glabrata* can be altered by numerous other compounds shown to affect vertebrates as well. Since *B. glabrata* is an intermediate snail host of *Schistosoma mansoni,* population control of

Table 1. Egg mass count during induced oviposition weeks

Treatment	Control 1	Control 2	Phthalate 1	Phthalate 2	BPA 1	BPA 2
1st ovipostion week	3	5	5	5	9	6
2nd oviposition week	4	8	15	6	14	9

these pulmonates is a prominent concern. These findings further raise the incentive to develop new methods to test for endocrine disruption, which depends on further research on molecular and signaling attributes of the pulmonate endocrine system. Strict control of *Biomphalaria* population will reduce schistosomiasis infections as well as improving the overall environmental condition for all organisms in the area. Although these data are from a single experiment not yet replicated, the strength of the response observed is worthy of note.

ACKNOWLEDGEMENT

Snails were provided by the Schistosomiasis Resource Centre, under the National Institutes of Allergy and Infectious Diseases Contract No. HHSN2722010000091.

REFERENCES

1. Louis-Albert TT, Shaw DJ, Polla L, Cioli D, Vercruysse J. Efficacy of praziquantel against *Schistosoma haematobium* infection in children. *Am J Trop Med Hyg* 2004; *71*(6): 778–82.
2. King CH, Sturrock RF, Kariuki HC, Hamburger J. Transmission control for schistosomiasis–why it matters now? *Trends Parasitol* 2006; *22*(12): 575–82.
3. Boyle JP, Timothy P. The effect of water quality on oviposition in *Biomphalaria glabrata* (Say 1818) (*Planorbidae*), and a description of the egg-laying process. *J Molluscan Studies* 2000; *66:* 83–93.
4. Ansaldo M, Nahabedian DE, Fonzo CD, Wider EA. Effect of cadmium, lead and arsenic on the oviposition, hatching and embryonic survival of *Biomphalaria glabrata*. *Sci Total Environ* 2009; *407:* 1923–8.
5. Oehlmann J, Schulte-Oehlmann U, Bachmann J, Oetken M, Lutz I, Kloas W, Ternes TA. Bisphenol A induces superfeminization in the Ramshorn snail *Marisa cornuarietis* (Gastropoda: *Prosobranchia*) at environmentally relevant concentrations. *Environ Health Perspect* 2005; *114*(S-1): 127–33.
6. Ulmer MJ. Notes on rearing snails in the laboratory. In: MacInnis AJ, Voge M, editors. *Experiments and techniques in parasitology.* San Francisco: WH Freeman 1970; p. 143–4.
7. Oehlmann J, Schulte-Oehlmann U. Endocrine disruption in invertebrates. *Pure Appl Chem* 2003; *75:* 2207–18.
8. Loreau M, Baluku B. Population dynamics of the freshwater snail *Biomphalaria pfeifferi* in eastern Zaire. *J Molluscan Stud* 1987; *53*(1): 249–65.
9. Jobling S, Casey D, Rodgers-Gray T, Oehlmann J, Schulte-Oehlmann U, Pawlowski S, Baunbeck T, Turner AP, Tyler CR. Comparative responses of mollucs and fish to environmental estrogen and an estrogenic effluent. *Aquatic Toxicol* 2004; *66:* 207–22.

Correspondence to: M.D. Touhid Iqbal, Department of Biology, State University of New York (SUNY), Geneseo, New York-14454, USA.
E-mail: mti@geneseo.edu; iqbal569@hotmail.com

Received: 26 April 2011 *Accepted in revised form:* 22 June 2011