

## The effects of organotin on female gastropods†

Cassander P. Titley-O'Neal,<sup>\*a</sup> Kelly R. Munkittrick<sup>b</sup> and Bruce A. MacDonald<sup>c</sup>

Received 7th January 2011, Accepted 21st June 2011

DOI: 10.1039/c1em10011d

Female masculinisation (FM) in gastropods (imposex, intersex and ovo-testis) has been documented in > 260 species globally. Biomonitoring studies use FM to indirectly assess the presence of tributyltin (TBT), a once popular biocide in antifouling paint, which has since been regulated. Laboratory studies confirm that FM in gastropods can be induced not only by TBT, but other tin (Sn) compounds, Sn “cocktails”, steroids, and other contaminants such as Aroclor 1260. Even though FM in gastropods in the field has been attributed to TBT from boating activity, there is evidence that imposex occurred prior to the advent use of TBT in the 1960s, and for some species imposex occurs naturally. There are roughly 42 species that do not elicit a FM response even if they are exposed to TBT under laboratory conditions, collected sympatrically with other species displaying FM, and/or have TBT in their tissues. A geographic analysis of field studies in four regions indicates that the bulk of studies were conducted in Europe > Asia and Oceania > North America > Latin America. More data are needed regarding FM in Africa, the Middle East, and Latin America since data regarding the masculinisation of female gastropods in these areas are lacking. The most studied gastropod species used in TBT biomonitoring studies is the dogwhelk, *Nucella lapillus*. This review summarizes the progression of the literature from 1962 to 2009 outlining how the terminology, science, and theories have evolved over time.

### 1 Introduction

Invertebrates are used globally in environmental monitoring programs to measure pollutant levels and the effects of these pollutants on organisms. Some standardized environmental

monitoring programs include the global Mussel Watch programme,<sup>1</sup> the Environmental Effects Monitoring (EEM) programs for metal mining<sup>2</sup> and pulp and paper mills<sup>3</sup> in Canada, and the Joint Assessment and Monitoring Programme (JAMP) in Europe.<sup>4</sup> Molluscs are used globally in ecotoxicology studies because they are common, highly visible, ecologically and commercially globally important, researchers have the ability to work with all life history stages, and the adults have a sedentary or sessile life history.<sup>5</sup>

Tributyltin (TBT), a once popular biocide in antifouling paint, is a known endocrine disruptor in two classes of molluscs, Bivalvia (bivalves) and Gastropoda (gastropods). In 1979 the first sign that TBT was having an adverse effect on non-target organisms came from France's Arcachon Bay.<sup>6</sup> TBT was linked as the causative agent to abnormal shell thickening, also known as “chambering” or “balling”, in the commercial Pacific

<sup>a</sup>Department of Biology, University of New Brunswick, P. O. Box 5050, Saint John, New Brunswick, Canada E2L 4L5. E-mail: c.titleyoneal@yahoo.com; Fax: +1-506-648-5811; Tel: +1-506-648-5620

<sup>b</sup>Canadian Rivers Institute, Department of Biology, University of New Brunswick, P. O. Box 5050, Saint John, New Brunswick, Canada E2L 4L5. E-mail: krm@unbsj.ca; Fax: +1-506-648-5811; Tel: +1-506-648-5825

<sup>c</sup>Department of Biology, University of New Brunswick, P. O. Box 5050, Saint John, New Brunswick, Canada E2L 4L5. E-mail: bmacdon@unbsj.ca; Fax: +1-506-648-5811; Tel: +1-506-648-5620

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1em10011d

#### Environmental impact

Imposex and masculinisation of female gastropods has been widely examined and this paper reviews more than 550 papers since 1962, and expands the global list of known affected species to > 260. There are no standardized approaches to assessment and studies have used more than 20 different indices. Previous studies have not focused on species that are not affected but this review documents >30 species that do not show a masculinisation response and summarizes evidence that imposex can occur naturally in some species, and occurred prior to the advent use of TBT in the 1960s. In this paper we also prioritize issues, species, and geographical areas that need further research, and make recommendations for reducing the diversity of approaches used.

oyster, *Crassostrea gigas*.<sup>7</sup> TBT also acts as an endocrine disruptor in female prosobranch gonochoristic gastropods,<sup>8</sup> and three types of masculinisation have been identified thus far. Two types of female masculinisation in gastropods are noticeable and well-known pseudohermaphroditism<sup>9,10</sup> or imposex,<sup>11</sup> and intersex<sup>12</sup> but the third, ovo-testis, is inconspicuous and has only been described by histological analysis.<sup>13–18</sup> Although TBT was first linked to chambering in *C. gigas*, the responses of female gastropods to butyltins have been regarded as the most complete example of endocrine disruption caused by an environmental contaminant.<sup>19</sup> Since there are three different types of masculinisation that are known to occur in female gastropods in response to organotin exposure, we will use the term female masculinisation (FM) when describing all three, and the individual name of each type where appropriate. Quantitatively, the literature on the effects of TBT on female gastropods in field studies (n = 416) has been dominated by imposex (n = 343) > intersex (n = 39) > ovo-testis (n = 34).

In imposex-affected females, the entire female genital system is conserved, but superimposed by male organs, penis and/or vas deferens as seen in dogwhelks, *Nucella lapillus*.<sup>20</sup> On the other hand, in intersex-affected females, such as the periwinkle, *Littorina littorea*, the pallial organs are modified towards male

morphological structures in the early stages that will eventually supplant the corresponding male formation, *i.e.* prostate gland, in higher stages.<sup>20</sup> In the last type of FM, ovo-testis, female gastropods were classified as masculine because oogenesis was suppressed and/or had spermatogenesis and/or seminiferous tube-like structures. This phenomenon was first observed in *N. lapillus* exposed to TBT under laboratory conditions from hatchlings to maturity for roughly two years.<sup>13</sup> Some of the primary species which ovo-testis has been detected in include: the sharp dwarf winkle, *Ocenebrina aciculata*;<sup>14</sup> the reticulated nassa, *Nassarius reticulatus*;<sup>21</sup> three species of abalone: *Haliotis madaka*,<sup>15–17</sup> *H. gigantea*,<sup>22,23</sup> and *H. roei*;<sup>17</sup> and in the ivory shell, *Babylonia japonica*.<sup>24</sup>

The following review constitutes a thorough analysis of the literature (n = 587) on the effects of organotins on female gastropods spanning a 47-year period (1962 to 2009) that extends across four geographic regions consisting of Europe, Asia and Oceania, North America and Latin America. It includes an updated list of 268 species in 33 families of gonochoristic gastropods affected by FM; an original unaffected list of 42 species in 20 families; a synopsis of conventional (n = 10) and alternative (n = 11) indices used to measure incidence and severity; a summary of individual compounds and “cocktails” (n = 31) known to induce FM in gastropods; and an outline of the progression of theories as they developed used to explain the induction of FM in gastropods. In addition, controversial evidence from museum samples collected prior to the 1960s when there was an increased use of TBT as a biocide in antifouling paint, and the occurrence of “natural” imposex in some species demonstrates that imposex does occur in some species without exposure to tin (Sn) compounds.

## 2 Literature review from 1962 to 2009 – global trends

### 2.1 Literature distribution

A review of the literature related to FM (imposex, intersex, and ovo-testis) in gastropods published from 1962 to 2009 was



Cassander  
P. Titley-O'Neal

*Cassander Titley-O'Neal is a recent PhD graduate of the University of New Brunswick. Her research interests include understanding the transport, fate, and impact(s) of anthropogenic pollutants on the environment and the organisms living in it.*



Kelly R. Munkittrick

*Kelly Munkittrick is the Canada Research Chair in Ecosystem Health Assessment in the Canadian Rivers Institute at the University of New Brunswick. His research program is related to assessing the environmental impacts of industrial and agricultural activities, and on developing methods for environmental effects monitoring and for the cumulative effects assessment of multiple stressors on aquatic environments. He was recently appointed the Scientific Director of the*

*Canadian Water Network, where he leads the development of an innovation network focused on providing clean, safe water across Canada and internationally.*



Bruce A. MacDonald

*Bruce MacDonald is a Professor of Biology and Associate Dean of Graduate Studies at the University of New Brunswick in Saint John. His research program involves the physiological ecology of suspension-feeding marine invertebrates in particular any mechanisms that facilitate particulate capture and improve the flow of energy to growth and reproduction. He is currently involved in projects to evaluate the efficiency of several marine species to serve as biofilters or energy recyclers to reduce the environmental impacts of salmon farming.*