AN ABSTRACT OF THE THESIS OF

Joseph Brian Pryor for the degree of Honors Baccalaureate of Science in Biology presented on May 22, 2014. Title: Comparative toxicological assessment of PAMAM and thiophosphoryl dendrimers using embryonic zebrafish.

Abstract approved: ________________________________

Stacey Harper

Dendrimers are well-defined, polymeric nanomaterials currently being investigated for their biomedical applications. Initially, higher generation (size) dendrimers were of interest because of their drug carrying capacity. However, increased generation was associated with increased toxicity. The objective of the present study was to investigate the role of surface charge and generation in dendrimer toxicity using embryonic zebrafish as a model vertebrate. Due to the generational and charge effects observed at the cellular level, higher generation cationic dendrimers were hypothesized to be more toxic than lower generation anionic or neutral dendrimers with the same core composition. Polyamidoamine (PAMAM) dendrimers elicited significant morbidity and mortality as generation was decreased. No significant adverse effects were observed from the suite of thiophosphoryl dendrimers studied. Exposure to ≥50 ppm cationic PAMAM dendrimers G3-amine, G4-amine, G5-amine, and G6-amine caused 100% mortality by 24 hours post-fertilization. The toxicity observed within the suite of varying dendrimers provides evidence that surface charge may be the best indicator of dendrimer toxicity. Dendrimer class and generation are other potential contributors to the toxicity of dendrimers. Further studies are required to better understand the relative role each plays in driving the toxicity of dendrimers.

Keywords: nanomaterials; in vivo; toxicity; generation; surface chemistry; zebrafish

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Comparative toxicological assessment of PAMAM and thiophosphoryl dendrimers using embryonic zebrafish

by

Joseph Brian Pryor

A PROJECT

submitted to

Oregon State University

University of Honors College

in partial fulfillment of the requirements for the degree of

Honors Baccalaureate of Science in Biology (Honors Scholar)

Presented May 22, 2014

Commencement June 2014
Honors Baccalaureate of Science in Biology project of Joseph Brian Pryor presented on May 22, 2014.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

______________________________
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Acknowledgements

Oregon State University provided support for JP through the following programs: Undergraduate Research, Innovation Scholarship & Creativity; Honors Experience Scholarship; Honors Promising Finishing Scholarship; and Summer Undergraduate Research Experience. This project was also partially funded by National Institutes of Health grants ES017552-01A2, ES016896-01, P30 ES03850. The authors would like to thank Dr. Josephine Bonventre for manuscript editing.
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1. Introduction

The use of polymeric nanomaterials such as dendrimers in the development and delivery of therapeutic compounds has emerged as a promising field of biomedical engineering. Dendrimers have a well-defined branching architecture consisting of an initiator core, a radiating interior structural layer composed of repeating generations (G0-G10), and terminal functional groups attached to the outermost generation (Figure 1). Subsequent generations increase in diameter, the number of terminal functional groups, and the number of vacancies available to carry therapeutics. The low polydispersity index and the ability to precisely control their surface chemistry make dendrimers useful for medical imaging, enhancing the solubility and/or bioavailability of drugs, brain-target drug therapy, and specific targeting mechanism for anti-cancer therapeutics and gene therapy. The stability, precise size, and defined chemistry of dendrimers also make them ideal for the development of structure-property relationships.

Despite the myriad of potential biomedical applications of dendrimers, previous research exploring the toxicity and biocompatibility of dendritic therapeutics suggests some dendrimers may display inherent toxicity attributed to a combination of generation and charge. Although the mechanism explaining generational effects is still unclear, higher generation dendrimers have been observed to cause nanoscale holes in the lipid bilayer, resulting in more toxicity while other authors have found that increasing generation attenuates toxicity; especially for very high generation dendrimers, perhaps due to conformational changes. Regardless of dendrimer generation, some cationic dendrimers have been shown to interact with negatively charged cell membranes. An increasing number of studies in diverse cell lines have shown that exposure to cationic dendrimers results in cell membrane permeability, cellular lysis, and cytotoxicity. Recent studies in mice and zebrafish (Danio rerio) have also shown polyamidoamine (PAMAM) dendrimers with amine terminated groups affected blood-clot
formation when administered intravenously, while neutral and anionic dendrimers of the same generation demonstrated no observable vascular complications. The authors suggest that these effects are driven by the high affinity amine for platelets or vascular endothelium. The observed toxicity with dendrimers however, is likely due to a combination of generation and charge, as the net charge is dependent on the generation. In contrast with PAMAM dendrimers, the limited research on thiophosphoryl dendrimers has demonstrated minimal inherent toxicity in vitro. The majority of studies to date have focused on dendrimer toxicity in cell culture, with fewer studies investigating the toxicity in vivo. Dendrimer toxicity was explored using several PAMAM (Figure 2) and thiophosphoryl (Figure 3) dendrimers that varied in generation and charge in embryonic zebrafish (Danio rerio). Due to both the generational and cationic toxicity observed at the cellular level, higher generation cationic dendrimers were hypothesized to be more reactive and thus more toxic than lower generation anionic or neutral dendrimers with the same core composition. Zebrafish were selected as a model organism due to their similarities in anatomy and physiology, cell structure and signaling process to other vertebrates and their tissue clarity which allows for visual inspection of most systems during development. In addition, the rapid development of zebrafish from a few cells to fully functioning juveniles by 120 hours post-fertilization (hpf) allows for high-throughput experimental designs at relatively low cost. The nature of biomedical applications associated with dendrimers makes understanding how their biological interactions relate to structural features, such as generation and charge, an important step in designing safer and more efficient nanopharmaceuticals.
Figure 1: Generation 3 dendrimer architecture. Dendrimers consist of core, interior branching, and terminal functional groups.
Figure 2: Chemical structure of generation 1 PAMAM dendrimer
Figure 3: Chemical structure of generation 1.5 thiophosphoryl dendrimer
2. Materials and methods

2.1 Dendrimers

Thiophosphoryl-phenoazymethyl(methylhydrazono) 0.5 Generation (G) and 1.5G dendrimers were purchased from Fisher Scientific (Fair Lawn, New Jersey, United States). The 2.5G, 3.5G and 5G were purchased from Sigma Aldrich (Saint Louis, Missouri, United States). All PAMAM dendrimers were commercially purchased through Dendritic Nanotechnologies Inc. (Mount Pleasant, Michigan, United States). Dry dendrimers were stored as shipped at 25°C while PAMAM G5-succinamic acid was shipped and stored in solution at -20°C; all dendrimers were tested without further modification or purification. Detailed core composition, generation, surface chemistry, and surface charges of the dendrimers used in this study are listed in Table 1.
Table 1: Physical characteristics of PAMAM and thiophosphoryl dendrimers

<table>
<thead>
<tr>
<th>Name</th>
<th>Core Composition</th>
<th>Generation</th>
<th>Surface Group</th>
<th>Charge</th>
<th>Number of Surface Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAMAM G3-Amine</td>
<td>1,4-diaminobutane</td>
<td>3</td>
<td>Amine</td>
<td>+</td>
<td>32</td>
</tr>
<tr>
<td>PAMAM G4-Amine</td>
<td>1,4-diaminobutane</td>
<td>4</td>
<td>Amine</td>
<td>+</td>
<td>64</td>
</tr>
<tr>
<td>PAMAM G5-Amine</td>
<td>1,4-diaminobutane</td>
<td>5</td>
<td>Amine</td>
<td>+</td>
<td>128</td>
</tr>
<tr>
<td>PAMAM G6-Amine</td>
<td>1,4-diaminobutane</td>
<td>6</td>
<td>Amine</td>
<td>+</td>
<td>256</td>
</tr>
<tr>
<td>PAMAM G6-Succinamic Acid</td>
<td>1,4-diaminobutane</td>
<td>6</td>
<td>Succinamic Acid</td>
<td>-</td>
<td>256</td>
</tr>
<tr>
<td>PAMAM G5-Succinamic Acid</td>
<td>1,4-diaminobutane</td>
<td>5</td>
<td>Succinamic Acid</td>
<td>-</td>
<td>128</td>
</tr>
<tr>
<td>PAMAM G6-Amidoethanol</td>
<td>1,4-diaminobutane</td>
<td>6</td>
<td>Amidoethanol</td>
<td>0</td>
<td>256</td>
</tr>
<tr>
<td>Thio G0.5-Aldehyde</td>
<td>Thiophosphoryl</td>
<td>0.5</td>
<td>Aldehyde</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Thio G1.5-Aldehyde</td>
<td>Thiophosphoryl</td>
<td>1.5</td>
<td>Aldehyde</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Thio G2.5-Aldehyde</td>
<td>Thiophosphoryl</td>
<td>2.5</td>
<td>Aldehyde</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Thio G3.5-Aldehyde</td>
<td>Thiophosphoryl</td>
<td>3.5</td>
<td>Aldehyde</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Thio G5-DPT</td>
<td>Thiophosphoryl</td>
<td>5</td>
<td>Dichlorophosphinothiol</td>
<td>0</td>
<td>48</td>
</tr>
</tbody>
</table>
2.2 Dendrimer Preparation

Dendrimer stock solutions were prepared at 1000 mg/L in embryo media on the day of the exposure and vortexed for 1-2 minutes to ensure uniform dispersion. An hour prior to exposures, 5-fold serial dilutions were conducted with embryo media to make exposure concentrations from 0.016 to 250 mg/L. Concentrations above 250 mg/L were considered outside the realistic exposure dose. Dilutions were stored in an incubator at 26.9°C until the exposure.

2.3 Zebrafish Exposure

Zebrafish (Danio rerio) embryos were collected from group spawns of wild-type D5 fish housed at the Sinnhuber Aquatic Research Laboratory (Oregon State University, Corvallis Oregon) and staged such that the chorion surrounding the embryo could be removed enzymatically at 6 hpf. Dechorionation removes the barrier provided by the chorion in an effort to increase the bioavailability of the particle interacting with the embryo. A 0.6% Instant Ocean® salt solution was added to deionized water to make embryo media. Embryo media was prepared and adjusted with sodium bicarbonate until a pH of 7.0-7.4 and a conductivity of 480-600 μS/cm was obtained. Newly fertilized embryos were collected and staged to ensure the zebrafish were treated at the same developmental stage. Zebrafish were kept at 26.9°C on a 14hr/10hr light/dark cycle. At 8 hpf, a total of 24 embryos per treatment were exposed individually in 96 well plates to 5-fold serial dilutions of dendrimers (0.016, 0.08, 0.4, 2, 10, 50, 250 ppm) or embryo media alone (control). At 24 hpf, embryos were assessed for mortality, developmental progression, notochord malformation, and spontaneous movement. At 120 hpf, embryos were assessed for mortality, as well as physical and behavioral malformations such as: axis, brain, circulation, eye, caudal fin, pectoral fin, jaw, otic, pigment, pericardial edema, yolk sac edema, snout, swim bladder, trunk, somite and a touch response according to previously published methods.
Data for individual endpoints analyzed here are freely available on the open-source Nanomaterial-Biological Interactions Knowledgebase (nbi.oregonstate.edu).

2.4 Statistical Analysis

Experimental replicates were analyzed using one way analysis of variance (ANOVA) to ensure replicates were not statistically different from one another. ANOVA was also used to compare mortality between the PAMAM and thiophosphoryl dendrimer groups. The Fisher’s Exact test was used to determine if individual sublethal responses differed significantly from control. Spearmans’ rank order was used to correlate percent mortality with dosages. All statistical analysis was performed using Sigmaplot version 12.3 (San Jose, California, United States) with a significance threshold of p≤0.05.

3. Results

3.1 Generation (Size) Effects

Identical dendrimers of different generations were compared to explore the role of generation on toxicity. PAMAM and thiophosphoryl dendrimers contained a generational series of four dendrimers each. PAMAM dendrimers elicited significant morbidity and mortality as generation decreased. In order to compare the PAMAM generations, the median lethal concentration (LC$_{50}$) was estimated through linear interpolation (Figure 4). As shown in Table 2, the LC$_{50}$ of cationic PAMAM dendrimers increased from 2 ppm for G3 PAMAM-amine to 18 ppm for G6 PAMAM-amine dendrimers. Thiophosphoryl dendrimers induced no significant morbidity or mortality at any concentration tested (LC$_{50}$ > 250 ppm).
Table 2: *LC*$_{50}$ concentrations calculated through interpolation of mortality across 8 dosages in embryonic zebrafish. *LC*$_{50}$ values were only calculated for dendrimers in a generational suite.

<table>
<thead>
<tr>
<th>Dendrimer</th>
<th>LC$_{50}$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAMAM G3-Amine</td>
<td>2</td>
</tr>
<tr>
<td>PAMAM G4-Amine</td>
<td>6.4</td>
</tr>
<tr>
<td>PAMAM G5-Amine</td>
<td>4.5</td>
</tr>
<tr>
<td>PAMAM G6-Amine</td>
<td>18</td>
</tr>
<tr>
<td>Thio G0.5-Aldehyde</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Thio G1.5-Aldehyde</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Thio G2.5-Aldehyde</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Thio G3.5-Aldehyde</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>
Figure 4: Concentration-response curves for embryonic zebrafish exposed to suite of PAMAM (A) and thiophosphoryl (B) dendrimers varying in generation (size), charge, and class. Controls were performed at 0 ppm. * denotes statistical significance (p ≤ 0.05).
3.2 Surface Charge

The toxicity of PAMAM dendrimers was assessed at 24 and 120 hpf by evaluating concentration effects on embryonic zebrafish mortality, development and malformations. Neutral thiophosphoryl dendrimers exhibited no observable malformations at any dose throughout the exposure. Exposure to ≥50 ppm cationic PAMAM dendrimers G3-amine, G4-amine, G5-amine, and G6-amine caused 100% mortality by 24 hpf. Significant cardiac impacts (pericardial edema) were observed at 10 ppm for cationic PAMAM G4-amine and neutral PAMAM G6-amidoethanol (p<0.05). However, negatively charged succinamic acid G5 and G6 dendrimers did not elicit any significant adverse effects even at the highest concentration tested. No other assessed endpoints were significant for either type of dendrimer at any dose or generation.

G6 PAMAM dendrimers that differed in surface chemistry and charge were compared for total mortality at 250 ppm. Cationic PAMAM G6-amine at 250 ppm was more toxic than both neutral PAMAM G6-amidoethanol and anionic PAMAM G6-succinamic acid at the same concentration (Figure 5). At 250 ppm, 100% mortality was observed in the PAMAM G6-amine at 250 ppm, compared to less than 25% mortality in the neutral and anionic dendrimers.
Figure 5: Impact of surface chemistry and charge on zebrafish mortality following exposure to 250 ppm G6 PAMAM dendrimer.
3.3 PAMAM vs. Thiophosphoryl

PAMAM dendrimers as a class were found to be significantly more toxic than thiophosphoryl dendrimers (p<0.05) (Figure 4). In addition, all PAMAM except G5-succinamic acid dendrimer were found to have a statistically significant correlation between the percent mortality and dose (Supplementary Table 1). PAMAM G6-amidoethanol was found to be statistically more toxic than thiophosphoryl G0.5, G1.5, and G3.5 (p<0.05).

4. Discussion

Dendrimers demonstrate generation and charge-dependent toxicity in multiple cell lines;\textsuperscript{20-22} yet little work has been done to investigate effects \textit{in vivo}. The suite of dendrimers investigated in this study allowed for comparative toxicological analysis between generation, charge, and dendrimer class as possible factors driving toxicity in an \textit{in vivo} embryo model. Mortality and cardiac impacts (pericardial edema) were significantly increased by higher generation PAMAM dendrimers (Figure 4). Pericardial edema is not necessarily a predictor of any specific pathway being triggered but rather an early stage stressor.\textsuperscript{34} Pericardial edema in zebrafish has been associated with exposure to a wide variety of toxicants including PAHs,\textsuperscript{35} dioxins,\textsuperscript{36} and gasoline oxygenates.\textsuperscript{37} The PAMAM dendrimers had relatively high toxicity in comparison to the thiophosphoryl dendrimers, indicating higher risk for the combination of surface chemistry and generation studied here. Significant toxicity was only observed with the PAMAM dendrimers, while thiophosphoryl dendrimers induced very little morbidity or mortality at the concentrations tested (0.016-250 ppm).

The results observed for PAMAM and thiophosphoryl dendrimers do not follow the trends previously observed in cell culture studies.\textsuperscript{16,20-23} Although some \textit{in vitro} studies found an increase in dendrimer
generation to be associated with an increase in toxicity, the data presented in this study did not corroborate these findings in an in vivo model at generations tested. A potential explanation is that the largest dendrimers tested in this study were PAMAM G6 and thiophosphoryl G5. In another study, it was found that only PAMAM dendrimers at the G7 level induced 15-40nm holes in the lipid bilayer.\textsuperscript{38} The proposed mechanism was through the removal and formation of dendrimer filled lipid vesicles. Smaller generation PAMAM dendrimers; however, did not display similar membrane disruptive potential.

Within the suite of five thiophosphoryl dendrimers, increasing generation resulted in no observable increase in toxicity up to the highest dose tested (Table 2). While PAMAM dendrimers demonstrated a generational effect, an increase in generation correlated to a decrease in toxicity (Table 2) similar to previously published studies.\textsuperscript{15} This is perhaps due to a lower bioavailability to the whole organism compared to cells in culture or the stability of higher generation dendrimers in embryo media. The steric hindrance of higher generation dendrimers is thought to make them more likely to change conformation and thus become less stable.\textsuperscript{39,40} \textit{In vitro} studies ensure the nanoparticle interacts with the cell membrane of a specific cell type. In contrast, the waterborne exposures used in the embryonic zebrafish assay do not indicate a direct dose, rather an exposure concentration, and the impact of surface chemistry and generational size on bioavailability is still unknown.

Cellular uptake of nanoparticles is influenced by their size,\textsuperscript{41} shape, surface charge, and hydrophobicity.\textsuperscript{42} Differential cellular uptake between neutral and anionic surface chemistry is unclear.\textsuperscript{43} In similar studies, it was found that anionic dendrimers were more toxic than neutral particles,\textsuperscript{43} but no significant difference between the two were observed here. Despite these contradictions, cationic dendrimers were consistently more toxic than anionic or neutral particles in this study (Figure 5) and other studies.\textsuperscript{25,43,15} The positive charge associated with PAMAM dendrimers allows for interactions with
important biomolecules such as DNA, lipid membranes, and mitochondria. Considerable research has demonstrated that cationic nanoparticles induce cellular uptake, indicating cellular uptake may be the driving force behind the toxicity associated with cationic dendrimers. Manipulation of the terminal amine to another functional group or methylation of the terminal amine has been shown to decrease their toxicity, likely due to a shielding of the cationic charge on the surface. The toxicity observed among the suite of PAMAM dendrimers supports the hypothesis that surface charge drives the uptake and thus toxicity of dendrimers in vivo. Future studies should focus on quantifying uptake and understanding how changes to the inherent features of dendrimers affects their biodistribution within organisms.

Dendrimer class also plays a role in toxicity with PAMAM and thiophosphoryl dendrimers displaying significant differences in toxicity. Neutral PAMAM G6-amidoethanol exhibited significantly higher toxicity than three of the thiophosphoryl dendrimers, thereby, demonstrating the inherent toxicity of PAMAM dendrimers. PAMAM dendrimers have been shown to cause instability in lipid bilayers, mitochondria, and proteins. Visual evidence showed that PAMAM dendrimers were transported into cells through endocytosis. Furthermore, evidence suggests cationic PAMAM dendrimers induce lysosomal instability through alkalinization. The instability is likely due to a combination of both an increase in pH as well as a lipid bilayer instability through nanoscale hole formation. Smaller PAMAM dendrimers (G1-G5) have been shown to prefer water (plasma), whereas higher generation (G6 and G8) dendrimers were demonstrated to partition at the octanol/water interface. In combination with multiple studies demonstrating the lipid altering capacity of dendrimers, PAMAM dendrimers are anticipated to enter the plasma and result in cardiac malformations. In contrast, the surface groups of thiophosphoryl dendrimers are aldehydes, which have been shown to increase erythrocyte membrane stability and thermal durability, potentially leading to hepatic toxicity. PAMAM degradation is an area
with limited research that could potentially help elucidate one mechanism of toxicity. PAMAM
dendrimers contained multiple amide bonds, which are subject to degradation into subunits of
ethylenediamine (EDA). If metabolism of PAMAM plays any role in driving the toxicity of this class,\textsuperscript{55} then
an increase in generation would result in an EDA increase, suggesting generation is still an important
variable to consider in dendrimer toxicity. Future studies should investigate the toxicity of dendrons
verses parent dendrimers as well as studying transformations of dendrimer platforms associated with \textit{in}
vivo metabolism and excretion.

The clearly defined structure and amenability of dendrimers makes them ideal for looking at structure-
activity relationships. These relationships can then be used \textit{in lieu} of empirical data for predicting
nanomaterial hazards. \textit{Principle} characteristics that contribute to the inherent toxicity must be revealed
in order to minimize the exposure and potential adverse impacts on living organisms. One way to
elucidate these characteristics is through rapid, high-throughput assays. Rapid assays such as the one
used in this study allow for accurate reliable data that can test a wide range of materials. The rapid
assay allowed for a thorough quick assessment of a wide range of dendrimers and to the best of our
knowledge, this is the first \textit{in vivo} study to address such a broad range of dendrimers.

The mechanisms by which dendrimers interact with biological systems are still not fully understood;
however, it has been clearly demonstrated that minor alterations in surface chemistry can be used to
modify dendrimer toxicity.\textsuperscript{45} Due to the inability to isolate size (generation) from surface charge in this
study, generational toxicity is still under investigation. Surface charge was found to be the most
effective indicator of relative toxicity in this study; however, core composition also clearly plays a role.
Even though it is important to understand how surface chemistry modifications alter dendrimer toxicity,
understanding the inherent features of the various dendrimer platforms that drive toxicity provides
information on which we can base the safe biological and therapeutic development of future
dendrimers. The results presented here help to elucidate the important role of dendrimer charge and class for understanding and mitigating the toxicity of dendrimers for biomedical applications.
Table 3: Strength of correlation between the concentration and response

<table>
<thead>
<tr>
<th>Dendrimer</th>
<th>Spearmen’s P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAMAM G3-Amine</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAMAM G4-Amine</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>PAMAM G5-Amine</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>PAMAM G6-Amine</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAMAM G6-Succinamic Acid</td>
<td>0.0212</td>
</tr>
<tr>
<td>PAMAM G5-Succinamic Acid</td>
<td>0.839</td>
</tr>
<tr>
<td>PAMAM G6-Amidoethanol</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Thio G0.5-Aldehyde</td>
<td>0.29</td>
</tr>
<tr>
<td>Thio G1.5-Aldehyde</td>
<td>0.578</td>
</tr>
<tr>
<td>Thio G2.5-Aldehyde</td>
<td>0.794</td>
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<tr>
<td>Thio G3.5-Aldehyde</td>
<td>0.29</td>
</tr>
<tr>
<td>Thio G5-Aldehyde</td>
<td>0.794</td>
</tr>
</tbody>
</table>
Literature Cited


37. Bonventre JA, White LA, Cooper KR. Craniofacial abnormalities and altered wnt and mmp mRNA expression in zebrafish embryos exposed to gasoline oxygenates ETBE and TAME. *Aquatic Toxicology*. 2012;120–121(0):45-53.


