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# Ecotoxicity assessment of ionic As(III), As(V), In(III) and Ga(III) species potentially released from novel III-V semiconductor materials

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ABSTRACT

III-V materials such as indium arsenide (InAs) and gallium arsenide (GaAs) are increasingly used in electronic and photovoltaic devices. The extensive application of these materials may lead to release of III-V ionic species during semiconductor manufacturing or disposal of decommissioned devices into the environment. Although arsenic is recognized as an important contaminant due to its high toxicity, there is a lack of information about the toxic effects of indium and gallium ions. In this study, acute toxicity of As(III), As(V), In(III) and Ga(III) species was evaluated using two microbial assays testing for methanogenic activity and $O_2$ uptake, as well as two bioassays targeting aquatic organisms, including the marine bacterium *Aliivibrio fischeri* (bioluminescence inhibition) and the crustacean *Daphnia magna* (mortality). The most noteworthy finding was that the toxicity is mostly impacted by the element tested. Secondarily, the toxicity of these species also depended on the bioassay target. In(III) and Ga(III) were not or only mildly toxic in the experiments. *D. magna* was the most sensitive organism for In(III) and Ga(III) with 50% lethal concentrations of 0.5 and 3.4 mM, respectively. On the other hand, As(III) and As(V) caused clear inhibitory effects, particularly in the methanogenic toxicity bioassay. The 50% inhibitory concentrations of both arsenic species towards methanogens were about 0.02 mM, which is lower than the regulated maximum allowable daily effluent discharge concentration (2.09 mg/L or 0.03 mM) for facilities manufacturing electronic components in the US. Overall, the results indicate that the ecotoxicity of In (III) and Ga(III) is much lower than that of the As species tested. This finding is important in filling the knowledge gap regarding the ecotoxicology of In and Ga.

## 1. Introduction

III-V materials such as indium arsenide (InAs), gallium arsenide (GaAs) and indium gallium arsenide (InGaAs) are novel materials with high electron mobility, low power requirement and favorable optoelectronic properties (Dick et al., 2010; Vurgaftman et al., 2001; Yamaguchi et al., 2008). Because of these attractive properties, III-V materials are increasingly used in semiconductor manufacturing as light emitting diodes (LEDs), laser diodes, liquid crystal displays (LCDs), biosensors and microcircuits (Dayeh et al., 2009; Joyce et al., 2011). III-V materials are also finding extensive application in thin-film photovoltaic devices, thin film photovoltaic solar modules and other photovoltaic devices (Miles et al., 2005). It has been reported that the demand for Ga and In has increased significantly during the past 30 years (Lovik et al., 2015; White and Hemond, 2012). The world primary production of Ga in 2014, estimated at 440 metric tons, was four times higher than in 2010 (US Geological Survey, 2015). This rapid growth has been mainly attributed to the higher content of GaAs in different electronic devices, especially smartphones, and increasing use of GaAs-based LEDs (Butcher and Brown, 2014). Global indium consumption in 2014 was about 1500 metric tons. Increased indium consumption was reportedly driven by increased demand for LCD televisions in developing countries and for smartphones and tablets in developed countries (US Geological Survey, 2015).

The extensive application of III-V materials may contribute to the release of hazardous materials through corrosion of decommissioned electronic and photovoltaic devices. Manufacturing activities could also potentially contribute to the environmental release of III-V materials. For example, chemical mechanical planarization (CMP) of thin films containing III-V materials, such as GaAs and GaInAs, generates waste effluents that may contain particulate and ionic III-V species. Waste streams containing high levels of arsenic and other hazardous materials require treatment prior to discharge to surface water in order to meet environmental regulations. In the United States, the arsenic discharge

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limits for effluents produced by facilities manufacturing electronic components are 2.09 mg/L as one-day maximum and 0.83 mg/L as 30-day average concentration (USEPA, 2015). However, there are no current regulatory limits for the discharge of indium (In) and gallium (Ga). Chen and coworkers measured the concentration of As, Ga and In in the groundwater at sites in and around an industrial park (with 350 companies manufacturing integrated circuits) and found that the semiconductor industry in the region had impacted the groundwater (Chen, 2006), with average levels of As, Ga and In detected of 34.2, 19.3 and 9.3 μg/L, respectively. These values were significantly higher than those in clean groundwater offsite.

Arsenic and arsenic compounds are well-known for their high toxicity and carcinogenicity. There is a close relation between As toxicity and speciation, and inorganic As species are usually more toxic than many organic As forms to living organisms (Sharma and Sohn, 2009; Shaw et al., 2007; Sierra-Alvarez et al., 2004). The toxicity of trivalent arsenic (As(III) or arsenite) is related to its high affinity for sulfhydryl groups of biomolecules (Aposhian and Aposhian, 2006). The formation of these bonds leads to inhibition of crucial enzymatic functions within the cells (Sharma and Sohn, 2009). On the other hand, the microbial toxicity of arsenate (As(V)) is often the result of its potential for phosphate replacement. Consequently, As(V) inhibits enzymes that use phosphate and uncouples ATP formation, which ultimately results in depletion of cell energy (Rosen and Tamas, 2010). Compared to inorganic arsenic species, there is a lack of available information regarding the toxicity of In(III) and Ga(III). In and Ga toxicity to humans is most evident from occupational exposure. Inhalation of In and Ga compounds such as indium tin oxide (ITO), indium phosphide (InP) and GaAs is linked to serious and sometimes fatal effects (Chen, 2007; Hines et al., 2013; Hoet et al., 2012; Nakano et al., 2014; Omae et al., 2011). So far only a very limited number of studies have evaluated the ecotoxicity of In(III) and Ga(III) (Jiang et al., 2015; Olivares et al., 2016; Zurita et al., 2007).

The objective of this study was to investigate the ecotoxicity of As (III), As(V), In(III) and Ga(III) species. To this end, two toxicity bioassays were performed, in which inhibition of bioluminescence activity in the marine bacterium, *Aliivibrio fischeri* (formerly known as *Vibrio fischeri*) and lethal effects on *Daphnia magna*, a multicellular aquatic microcrustacean, were tested. The Microtox test and acute toxicity bioassays with daphnids are used by municipalities and industries worldwide as standard methods to test the toxicity of chemicals and effluent streams. Both methods are accepted by organizations such as the U.S. Environmental Protection Agency (USEPA), the Organization for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO) (Parvez et al., 2006; Rubinos et al., 2014; Ventura et al., 2012; Zhu et al., 2010, 2009). In addition, two microbial assays, testing the impact of the various ionic species towards the methanogenic activity of an anaerobic mixed culture and on $O_2$ uptake by aerobic bacteria in wastewater treatment sludge. Methanogens and aerobic heterotrophic bacteria are microorganisms commonly found in natural environments that play important roles in matter and energy cycles (Madigan et al., 2014). These microorganisms are also widely used in wastewater treatment systems and, therefore, they are used as indicators in ecotoxicity studies (de Garcia et al., 2014; Ramos-Ruiz et al., 2016).

## 2. Materials and methods

### 2.1. Chemicals

Gallium(III) chloride ($GaCl_3$, CAS# 13450-90-3, > 99.99%), sodium meta-arsenite ($NaAsO_2$, CAS# 7784-46-5, ≥99%), sodium arsenate dibasic heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$, CAS# 10048-95-0, ≥98%) and citric acid ($C_6H_8O_7$, CAS# 77–92-9) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Indium(III) chloride tetrahydrate ($InCl_3{\cdot}4H_2O$, CAS# 22519-64-8, > 99.99%) was obtained from Strem Chemicals (Newburyport, MA, USA).

In(III) and Ga(III) have very low aqueous solubility in the neutral pH range (Wood and Samson, 2006). In order to avoid precipitation in the circumneutral bioassay media, the stock solutions of In and Ga were supplied with citrate at a molar ratio 1:3.75, metal: citrate. Citrate is a ubiquitous compound abundant in the natural environment and a common metabolite of living cells (Gamez et al., 2009). Citrate is also commonly used as a chelating agent in metal processing industries including semiconductor manufacturing (Thomas et al., 2000). Therefore, the use of citrate in this study is relevant to In(III) and Ga(III) exposure scenarios.

### 2.2. Microtox acute toxicity bioassay

Microtox is an *in vitro*, metabolic inhibition test system that uses a strain of a naturally bioluminescent marine bacterium named *Aliivibrio fischeri* that produces light as byproduct of cellular respiration. The toxicity of test chemicals can be recognized by the loss of luminescence level that results from cellular activity inhibition.

In the study, all samples were tested using a Microtox M500 analyzer following the Microtox acute toxicity test protocol (U.S. EPA, 2003). Microtox reagent (*A. fischeri*), reconstitution solution (ultra-pure water), osmotic adjusting solution (22% NaCl), and diluent (2% NaCl) were obtained from Fisher Scientific. Stock solutions, 6 mM As(III) and As(V) as well as 20 mM Ga(III) and In(III), were prepared prior to the experiments. The pH of each stock solution was adjusted to about 7.0 through addition of either diluted HCl or NaOH. In order to maintain the osmotic pressure of the samples, the stock solution was mixed with osmotic adjusting solution (10:1, v/v). Then nine dilutions were prepared by a 2-fold serial dilution using Microtox diluent. The concentration ranges used in this assay were: 0.01–2.7 mM for As(III) and As(V); and 0.04–9.10 mM for In(III) and Ga(III). Luminescent levels were tested after 0, 15 and 30 min of exposure. All tests were performed in duplicate; blank controls and samples with citrate alone were run in parallel. Percent microbial activity was calculated as described in the Supplementary Information (SI) Section.

### 2.3. Methanogenic toxicity bioassay

The methanogenic sludge was obtained from an anaerobic bioreactor treating brewery wastewater (Mahou, Guadalajara, Spain) and contained 7.92% of volatile suspended solids (VSS) per unit wet weight. Batch experiments were conducted in duplicate using glass serum flasks (160 mL) supplemented with inoculum (1.5*g* VSS/L), and acetate-containing basal medium (25 mL) (described in the SI Section) (Liang et al., 2013). The flasks were sealed with butyl rubber stoppers and aluminum crimp seals. Next, the headspace was flushed with a mixture of $N_2$ and $CO_2$ (80:20, v/v) to create anaerobic conditions. All flasks were pre-incubated overnight to ensure that the methanogens were adapted to experimental conditions. The following day, As (0–0.2 mM of As(III) or As(V)), In(III) (0–1.08 mM) or Ga(III) (0–2.50 mM) were added. Controls lacking toxicant addition and controls with citrate alone were run in parallel. Subsequently, the flasks were incubated in an orbital shaker (115 rpm) at 30 ± 2 °C. Methane ($CH_4$) production was determined periodically until 80% or more of the substrate in toxicant-free controls was depleted. The normalized microbial activity (NMA) was calculated as shown below:

$$NMA(\%) = \frac{Maximum \;\; Specific \; Activity \; of \; Experimental \; Group}{Maximum \;\; Specific \;\; Activity \; of \;\; Control} \times 100 \quad (1)$$

where the maximum specific activity was calculated from the slope of cumulative $CH_4$ production.