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***Project title*:**
Environmental Safety and Health (ESH) Impacts of Emerging Nanoparticles and Byproducts from Semiconductor Manufacturing - Toxicity Assessment and Prediction

Deliverable:
Report of tests to screen the toxicity of Phase I-nanoparticles with identified contaminants

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Background:

Numerous reports published in recent years indicate a growing concern for the potential toxicity of engineered nanomaterials (Balbus et al. 2007; Nel et al. 2006; Handy & Shaw, 2007). Toxicity research is a high priority for the semiconductor industry due to the fact that some nanoparticles (*e.g.* chemo-mechanical planarization (CMP) slurry particles) are currently used in semiconductor manufacturing, and various new nano-sized materials (nanowires, carbon

nanotubes, immersion lithography nanoparticles) are being considered for upcoming manufacturing processes. Predicting the potential toxicity of emerging nanoparticles (NPs) will require hypothesis-driven research that elucidates how physicochemical parameters influence toxic effects on biological systems. Of particular concern are NPs of less than 0.1 μm that would escape normal mechanisms of cellular defense (Gwinn & Vallyathan, 2006; Stern & McNeil, 2008). The intrinsic capacity of NPs to penetrate biological tissue may in itself not be the primary cause of toxicity; rather surface properties of NPs may accentuate (or minimize) toxicity. These include high specific surface area, reactive surfaces, and adsorptive surfaces for other toxic chemicals. Contaminants can also accumulate in NPs via nano-capillary condensation (Kelvin effect) in the particle pores. NPs have very high surface curvatures, engendering high surface tensions and energies that might have unique effects on living cells. Reactive radical species can have prolonged lifetimes when sorbed onto NPs. There is a growing consensus that reactive oxygen species (ROS, composed primarily of hydroxyl radicals, hydrogen peroxide and superoxide) are a major contributing factor of NP toxicity (Gwinn & Vallyathan, 2006; Limbach et al., 2007). ROS are normally produced in and around living tissues; however, overproduction can lead to cell toxicity and loss of cell and tissue function.

The goal of this project is to characterize the potential toxicity of current and future NPs and NP-byproducts of SC manufacturing. The information will be used to develop mechanistic hypotheses that will be applied to developing rapid toxicity assessment protocols applicable in the industrial workplace, as well as to predicting the ESH impacts of NPs based on physicochemical properties. Our hypothesis is that the size and size distribution of NPs intrinsically makes them more adsorptive to external chemicals, and these surface molecules can contribute to the observed toxic effects of NPs on cells.

Objective and key findings:

The goal of the task was to determine if contaminants interact with nanoparticles to cause a synergistic increase in NP toxicity. Nanoparticles of aluminum oxide (Al_2O_3) and hafnium oxide (HfO_2) were exposed to inorganic arsenic (As) and the toxicity was tested with yeast cells and human lung epithelial cells.

Aluminum oxide NPs were effective in adsorbing inorganic arsenic. Compared to literature data with micron sized activated alumina, nano-sized aluminum oxide was approximately 5-fold more effective in adsorbing arsenic. Also nano-sized hafnium oxide adsorbed arsenic to a limited extent. Nanoparticles with adsorbed arsenic were found to be nontoxic to oxygen uptake activity of yeast cell even at relatively high concentrations, 20 mg

As(III) L^{-1} adsorbed onto 800 mg L^{-1} Al_2O_3 or HfO_2. On the other hand, with a real time electrode impedance assay (xCELLigence) using lung epithelial cells, the aluminum oxide NPs were found to cause a partial inhibition which was either not enhanced or only slightly enhanced with adsorbed As(III) 0.1 mg L^{-1} on 250 mg L^{-1} Al_2O_3 depending on the cell culture medium. In conclusion, arsenic was effectively adsorbed by NPs; however, no evidence was obtained for a noteworthy synergistic toxicity caused by the adsorbed arsenic.

Technical Results and Data:

Nano γ-aluminum oxide (Al_2O_3 of 50 nm diameter) and hafnium oxide (HfO_2 of 100 nm diameter) was used to study the adsorption of pentavalent and trivalent arsenic (As(V) and As(III), respectively). The goal was to determine if arsenic adsorbed onto NPs would display a synergistic toxicity to cells beyond the sum of the toxicity of arsenic and Al_2O_3 alone. The toxicity testing was done with As(III) since it is considered to be more toxic than As(V) (Sierra-Alvarez *et al*. 2004). Two toxicity testing systems were utilized. Firstly, the O_2-uptake assay was utilized with baker's yeast cells (*Saccharomyces cerevisiae*). In this assay, the consumption of O_2 in the headspace is monitored as a measure of cell activity. Secondly, a human epithelial lung cell line (16HBE14o-) was used in conjunction with a label-free, real time cell cytotoxicity array test based on monitoring impedance of an electrode at the base of each well of the array (xCELLigence system from Roche) (Hondroulis *et al*. 2010). The impedance is a measure of the attachment of 16HBE14o- cells on the electrode which is indicative of cell growth and healthy epithelial function.

Adsorption isotherms of As(V) and As(III) were performed with Al_2O_3 as shown in Figures 1 and 2. Both As(V) and As(III) were adsorbed very well by nano-Al_2O_3 at pH 5 and 8, but neither were adsorbed very effectively at pH 12. The maximum measured adsorption at pH 8 was 11 and 15 mg As g^{-1} Al_2O_3 with an equilibrium concentration (Ce) of 8 and 12 mg As L^{-1} for As(III) and As(V), respectively. All isotherms showed a good fit with either Langmuir or Freundlich sorption equations as shown in the Figures. The adsorption of both arsenic species by nano-Al_2O_3 was superior to micron sized activated alumina. The adsorption capacity of nano-sized alumina was approximately 5-fold higher compared to micron sized activated alumina (Sun *et al.,* 2010). HfO_2 also adsorbed As(V) and As(III) as shown in the isotherm of Figure 3. However at pH tested of 5, the maximum adsorption capacity was approximately 3 mg As g^{-1} HfO_2.

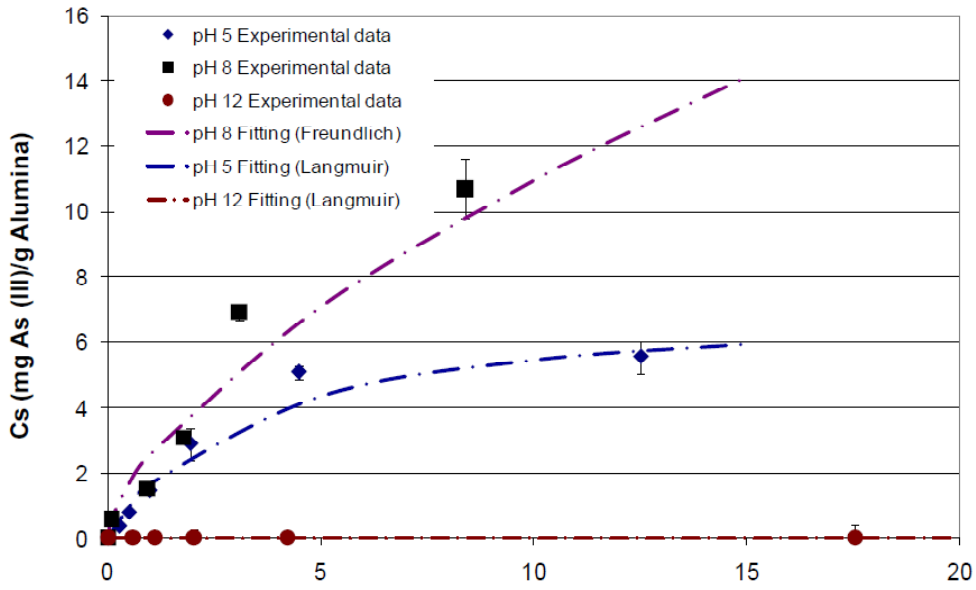

Fig. 1. Adsorption isotherm of As(III) on Al_2O_3 nanoparticles at pH 5, 8 and 12. The graph shows the adsorbed mass of As per unit Al_2O_3 (Cs) as a function of the aqueous As equilibrium concentration (Ce).

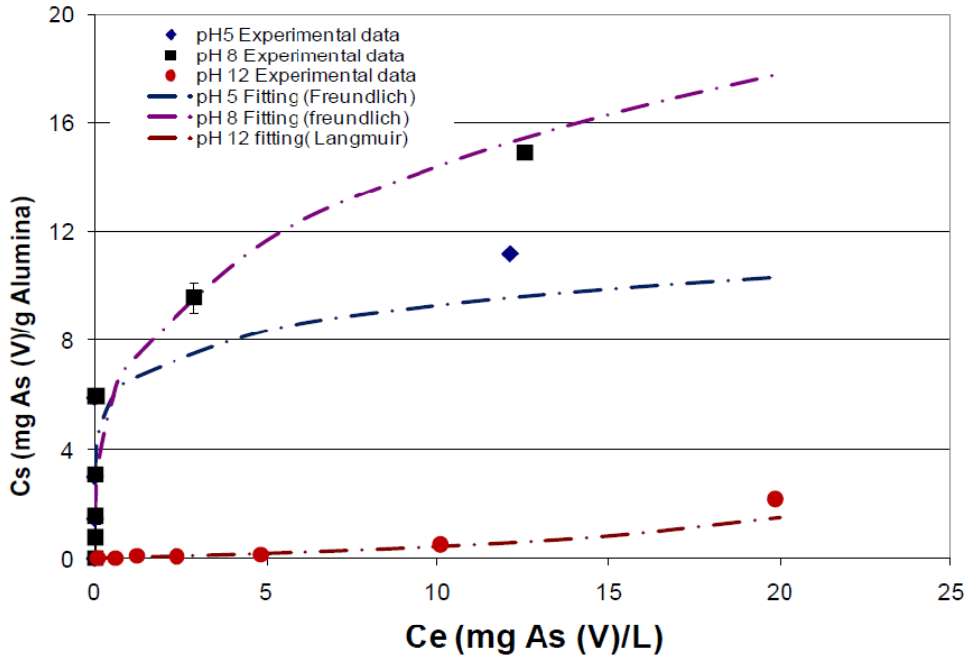

Fig. 2. Adsorption isotherm of As(V) on Al_2O_3 nanoparticles at pH 5, 8 and 12. The graph shows the adsorbed mass of As per unit Al_2O_3 (Cs) as a function of the aqueous As equilibrium concentration (Ce).

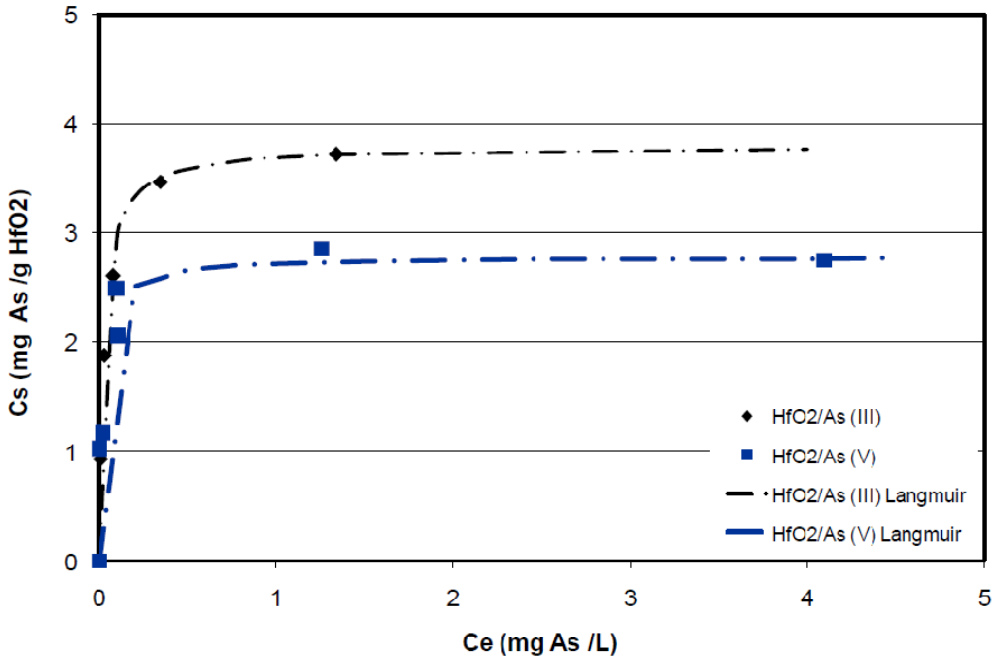

Fig. 3. Adsorption isotherm of As(III) and As(V) on HfO_2 NPs at pH 5. Graph shows the adsorbed mass of As per unit HfO_2 (C_s) as a function of the aqueous As equilibrium concentration (C_e).

In order to test the synergistic toxicity of As(III) and nanoparticles adsorbing As(III), it was first necessary to determine the toxicity of As(III) to yeast cells in the O_2-uptake test. Figure 4 shows the results of the O_2 consumption versus time during the assay of yeast cells exposed to varying As(III) concentrations. The graph shows partial inhibition of O_2-uptake at 30 mg L^{-1} As(III); whereas concentrations of 40 to 60 mg L^{-1} As(III) caused complete inhibition of O_2 uptake. The slope of the O_2 consumption time course graphs was used as an indicator of activity. The normalized activity as a function of As concentration is plotted in Figure 5, showing a 50% inhibiting concentration of 33 mg L^{-1} As(III). A similar test was also conducted for Al_2O_3 and HfO_2 nanoparticles at concentrations ranging from 0 to , mg L^{-1}. The results indicated absolutely no detectable toxicity even at the highest concentration tested of 1000 mg L^{-1} Al_2O_3 or HfO_2. In order to assess the synergistic toxicity, the combined treatment of 20 mg L^{-1} As(III) adsorbed onto 800 mg L^{-1} Al_2O_3 or HfO_2 was tested as shown in Figures 6 and 7. In both tests all treatments were non-toxic as evidence by the same rate of O_2 uptake in the control (with no additives, or single additives) as well as the full treatment with the combined additives. Thus in conclusion in the yeast assay there was no evidence of any synergistic toxicity to yeast cells caused by As(III) adsorbed by NPs.

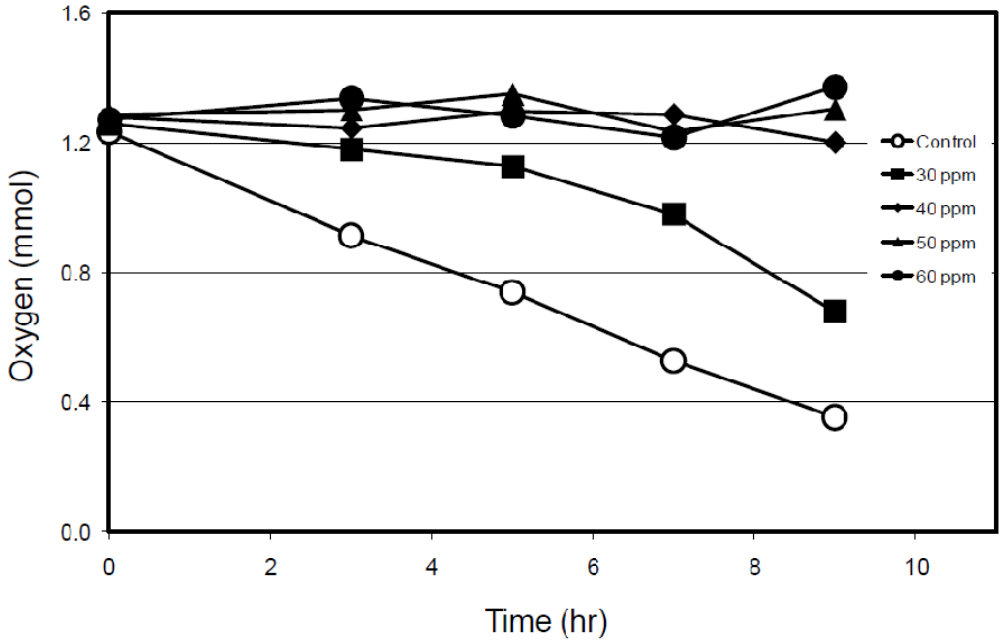

Fig. 4. O_2 uptake versus time in the yeast assay exposed to As(III) concentrations ranging from 0 to 60 mg L^{-1}.

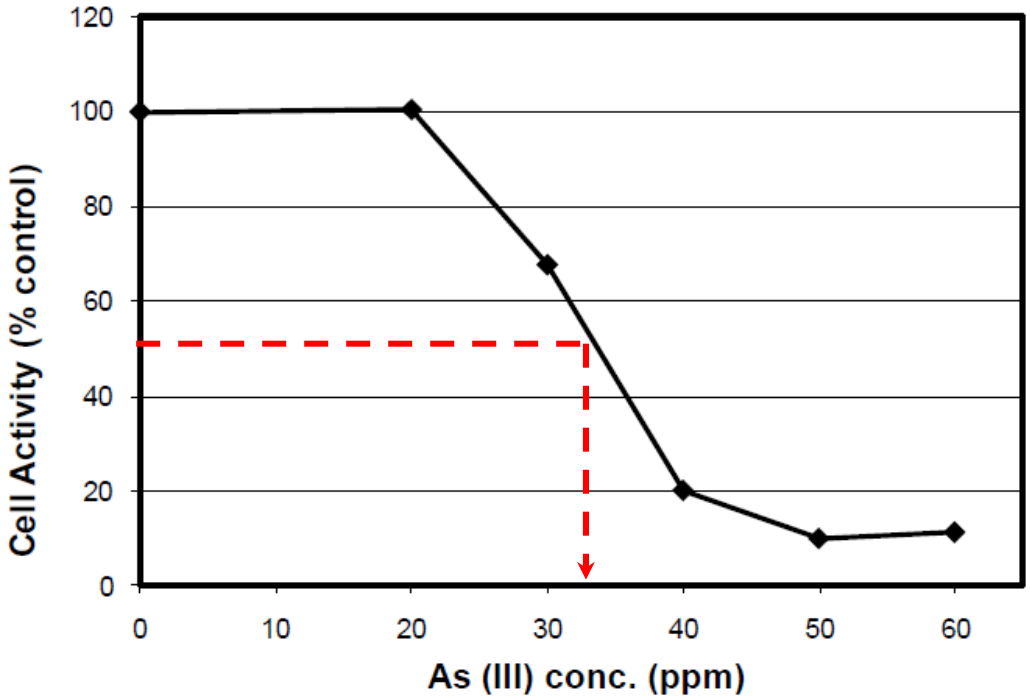

Fig. 5. Normalized O_2 uptake activity as a function of As(III) concentration. The 50% inhibiting concentration is 33 mg L^{-1} As(III).

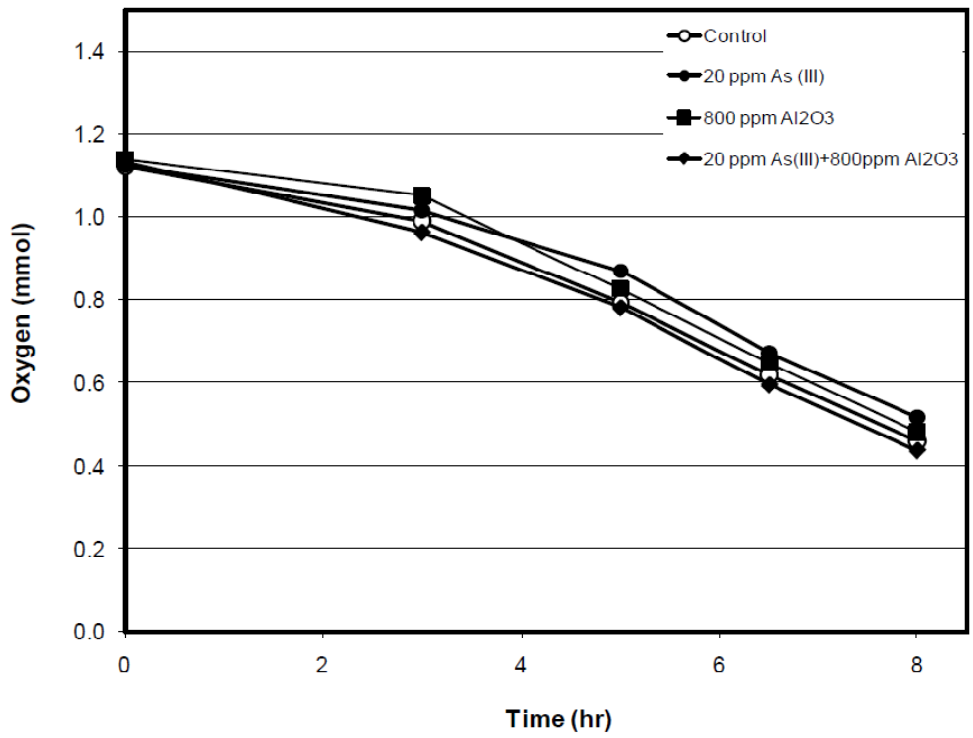

Fig. 6. O_2 uptake versus time in the yeast assay exposed to a combined treatment containing 20 mg L^{-1} As(III) 800 mg L^{-1} Al_2O_3 NPs as well as controls with just As(III) or just Al_2O_3.

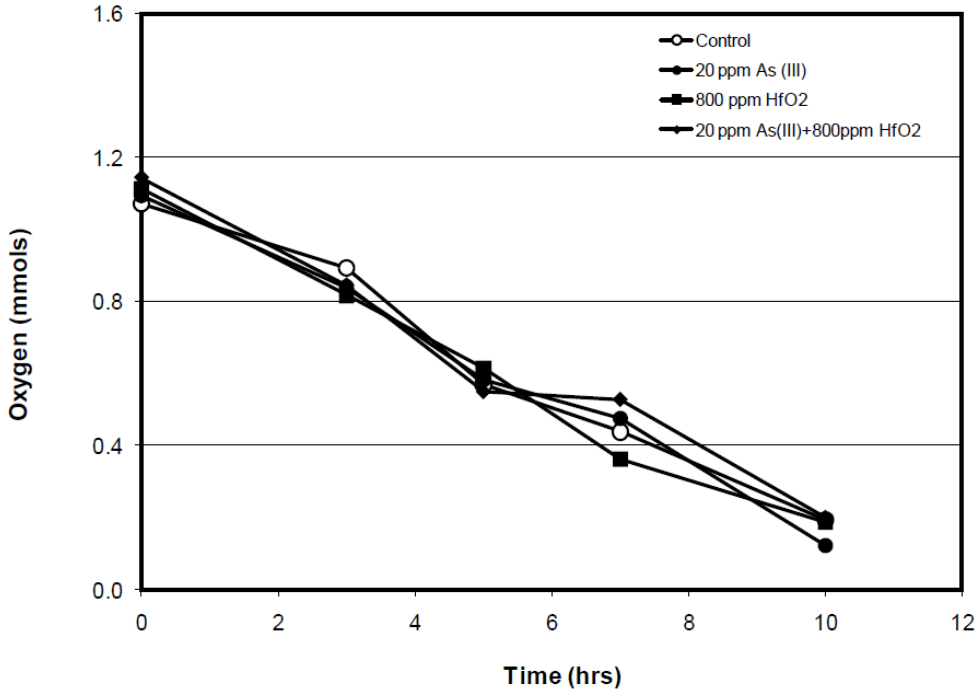

Fig. 7. O_2 uptake versus time in the yeast assay exposed to a combined treatment containing 20 mg L^{-1} As(III) 800 mg L^{-1} HfO_2 NPs as well as controls with just As(III) or just HfO_2.

Additional toxicity tests were conducted with 16HBE14o- cells using the xCELLigence system. In this test, the sub-toxic concentration of arsenite chosen was 100 µg L^{-1} combined with 250 mg L^{-1} of Al_2O_3. The results show a small inhibitory impact of the combined As and Al_2O_3 treatment in minerals essential medium (MEM) (Figure 8a). This impact was greater than the response by As(III) alone and greater than the small response by either 250 or 500 mg L^{-1} of Al_2O_3 alone. However, in the experiment with a different medium, Hank's Buffered Salt Solution (HBSS), the combined treatment was not different than the Al_2O_3 alone treatments. In fact in HBSS the most noteworthy impact is that Al_2O_3 alone causes partial inhibition of 16HBE14o-. Although, initially Al_2O_3 nanoparticles increase impedance, the effect only 4 h after addition is a more rapid drop off in impedance compared to the control with no additions.

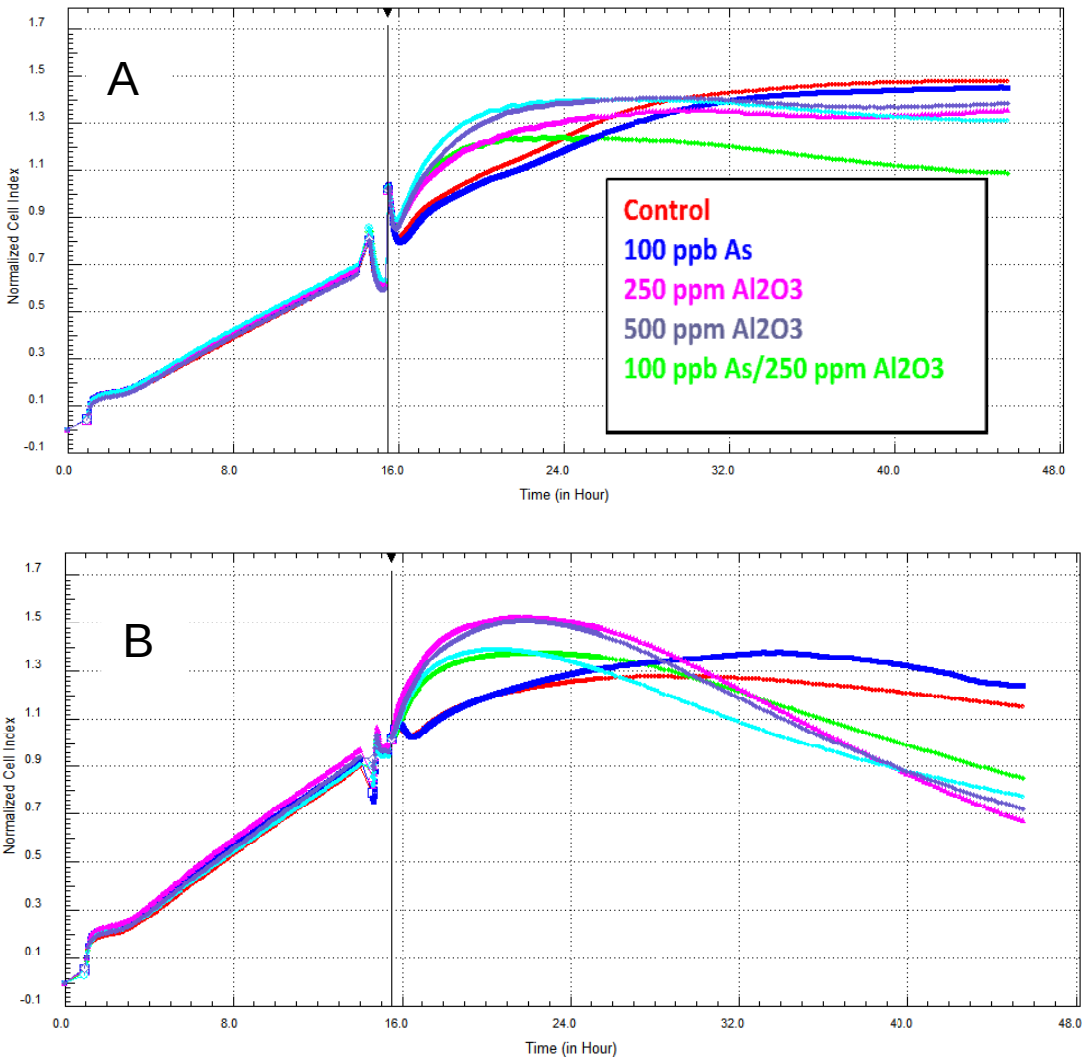

Fig. 8. Normalized Impedance output from the xCELLigence test using 16HBE14o- cells spiked with treatments at 16 h. Panel **A** shows results in minerals essential medium (MEM). Panel **B** shows results in Hank's Buffered Salt Solution (HBSS) medium.

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