Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Differential Effect of Micron- *versus* Nanoscale III-V Particulates and Ionic Species on the Zebrafish Gut

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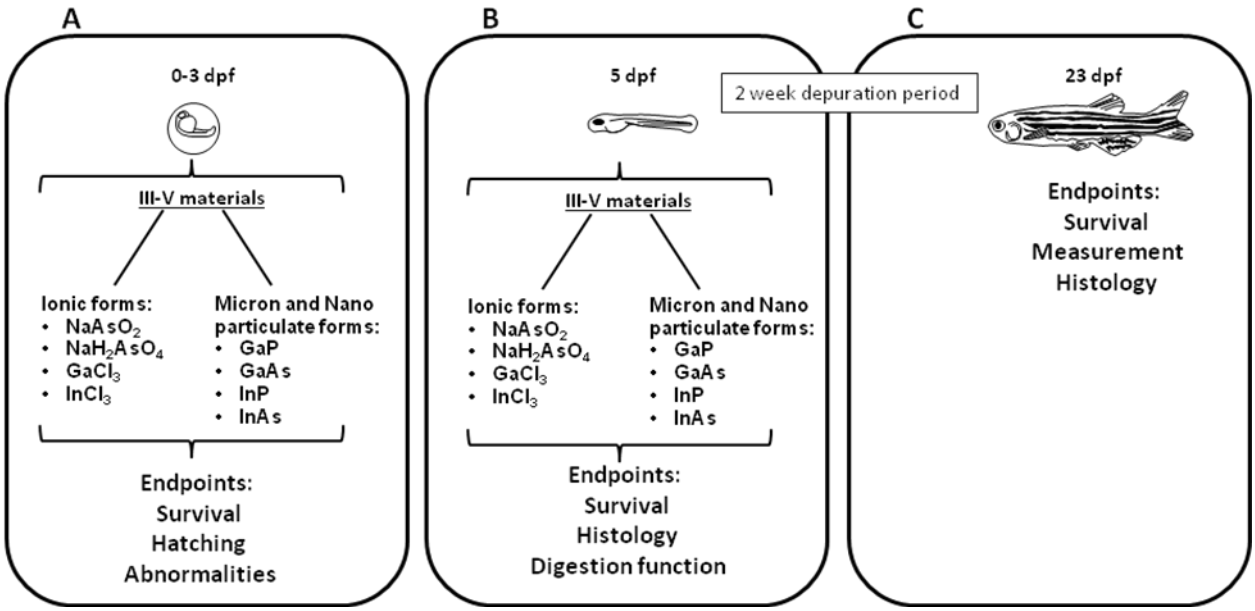
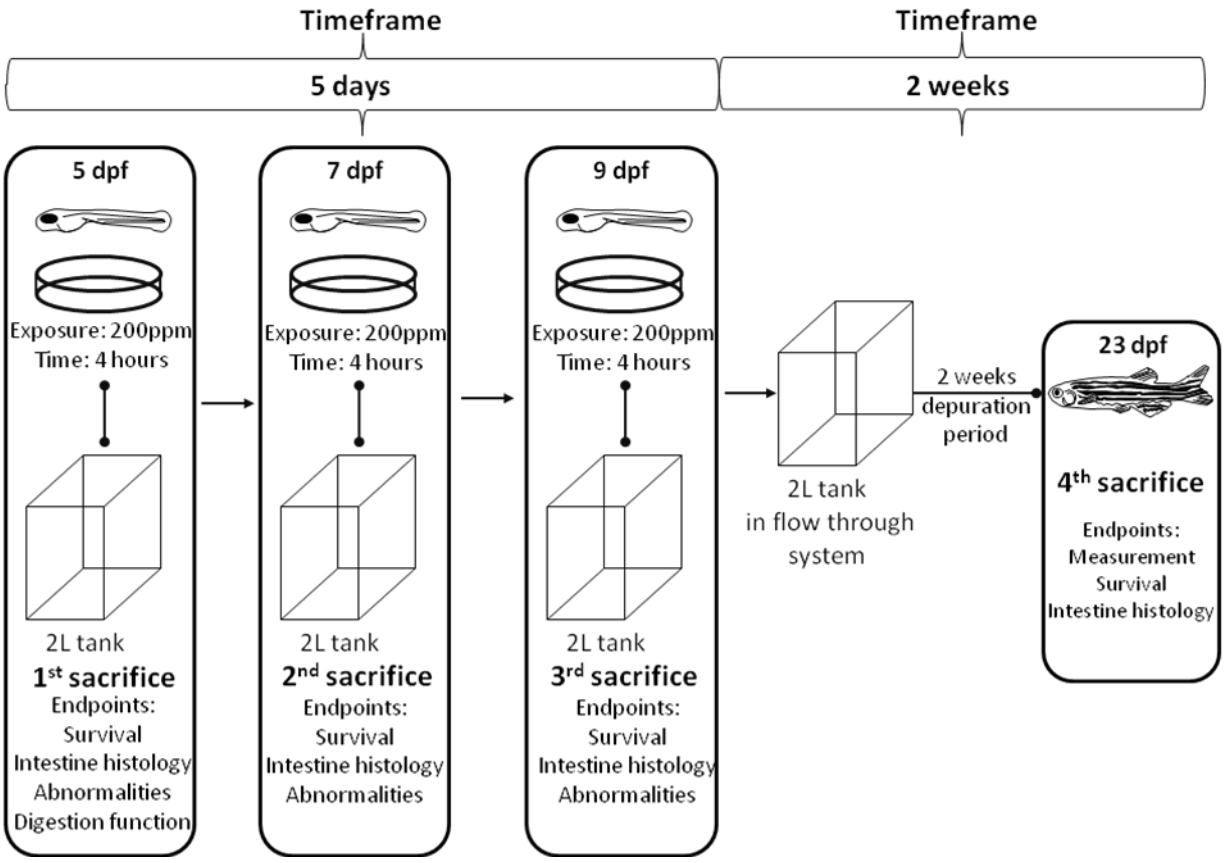
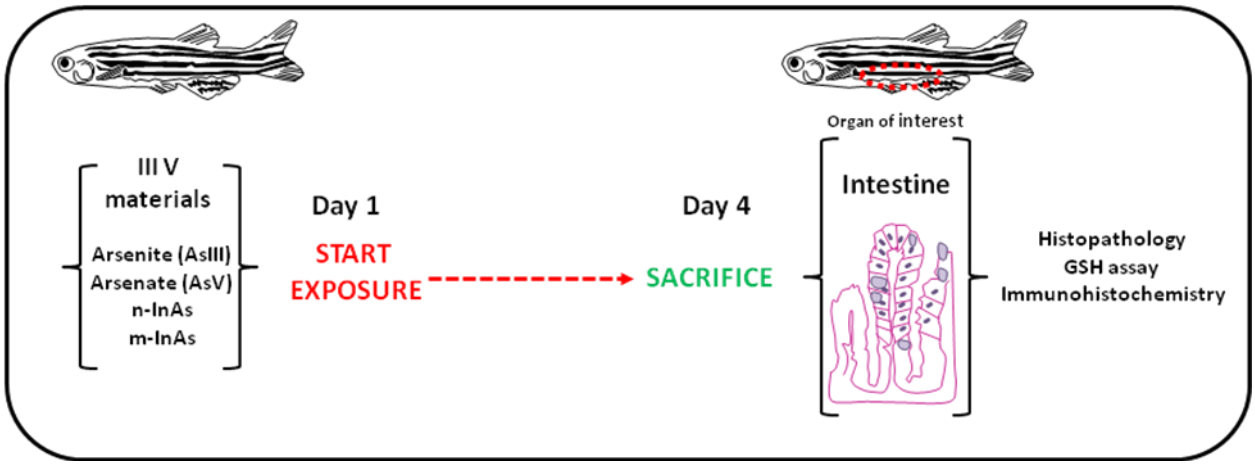


Schematic S1. Study plan for elucidating the effect of III-V materials on biological outcomes during zebrafish development. (A) High throughput screening of the toxicological potential of nano- and micronscale GaP, GaAs, InP, and InAs particulates, as well as representative ionic forms ($NaAsO_2$, NaH_2AsO_4, $GaCl_3$, $InCl_3$) in zebrafish embryos up to 3 dpf. The measured endpoints were survival, hatching interference and morphological abnormalities. (B) Pulse-exposure of zebrafish larvae to III-V materials to assess the effects of oral ingestion of the same materials (a schematic for the pulse-exposure protocol is shown in Schematic 2). The endpoints measured at 5, 7 and 9 dpf included intestinal histology, survival rate and use of an enzymatic assay for digestive function (only at 5dpf). (C) After a 2 week depuration period from the same batch of larvae included in (B), the overall health status was assessed by survival rate, body length and intestinal histology.



Schematic S2. Experimental plan for larval screening, using pulse exposures. Zebrafish larvae at 5 dpf were incubated with III-V material suspensions in Petri dishes. Thirty larvae were exposed on each occasion to III-V particulates and ionic forms at 200 ppm for 4 h. The larvae were then carefully and thoroughly washed before returning to standard aquarium tanks for regular feeding and water circulation. The same batch of larvae was used for a secondary and a tertiary exposure at 7 and 9 dpf. The endpoints included survival and intestinal histology. After the tertiary exposure and 14 days depuration, the larvae were assessed on 23 dpf for survival, growth, body length and histological analysis of the intestines.



Schematic S3. Schematic to show the experimental layout for adult exposure. There were 4 treatment groups (n-InAs, m-InAs, Arsenite (AsIII), Arsenate (AsV) and one control *n*=6. Zebrafish were exposed on Day 1 with the treatment groups at different concentrations. On Day 4 the fish were sacrificed and the organ of interest: the intestine was isolated.

Schematic S4. Schematic to show the III-V materials toxicity mechanism.

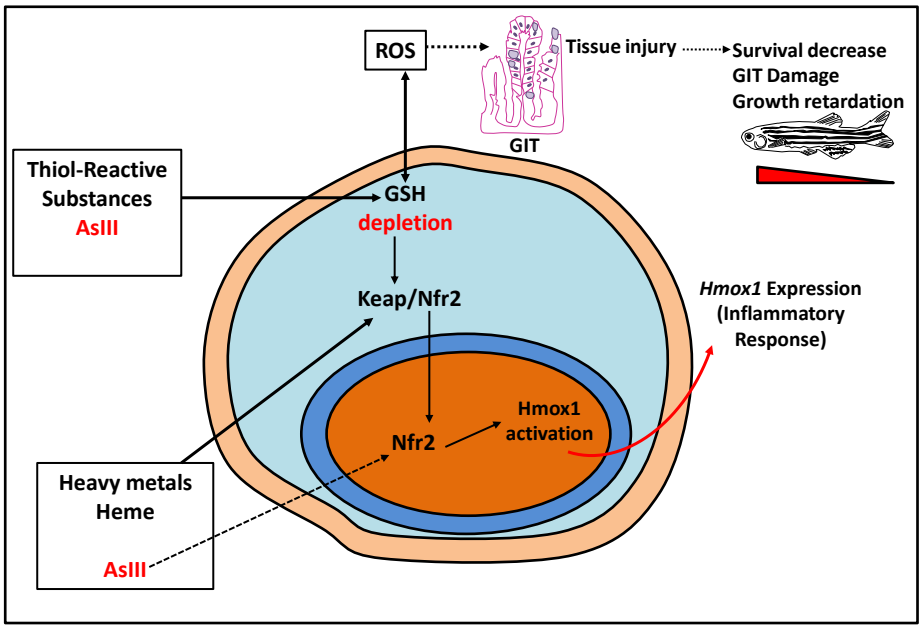


Table S1. Physicochemical characterization of the particulate III-V material library*

SRM Properties	Technique	Unit	Micron-size Particles				Nano-size Particles			
			m-GaP	**m-GaAs**	**m-InP**	**m-InAs**	**n-GaP**	**n-GaAs**	**n-InP**	**n-InAs**
Primary Size	TEM	nm	100-3000	200-700	200-1100	100-650	~10	<10	~10	20-200
Phase & Structure	XRD		Cubic GaP	Cubic GaAs	Cubic InP	Cubic InAs	Cubic GaP	Cubic GaAs	Cubic InP	Cubic InAs
Shape/ Morphology	TEM		Irregular	Irregular	Irregular	Irregular	Sphere	Irregular	Sphere	Irregular
Size in DI H_2O	HT-DLS	nm	442.3 ± 29.6	342.9 ± 5.2	246.3 ± 6.6	301.7 ± 7.2	450.2 ± 12.4	503.3 ± 13.2	290.1 ± 26.9	186.1 ± 2.5
Zeta Potential in DI H_2O	ZetaPALS	mV	-11.0 ± 2.1	-24.2 ±1.5	6.6 ± 0.6	-11.9 ±1.1	-8.0 ±1.3	-13.3 ± 1.2	-10.0 ± 1.0	-3.3 ± 1.2
Endotoxin[a]	Limulus Amebocyte Lysate	EU/mL	< 0.153	< 0.324	< 0.098	< 0.246	< 0.215	< 0.136	< 0.233	< 0.273

[a]The endotoxin levels of all the particles were less than the detection threshold (<2.5 EU/mL) in the Limulus Amebocyte Lysate assay kit (Lonza, Walkersville, MD).

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	HM 4hrs			SIF 120hrs		
NPs	**dH(nm)**	**PDI**	**ʒ potential (mV)**	**dH(nm)**	**PDI**	**ʒ potential (mV)**
nm-InAs	1145.9±28.8	0.358±0.022	-11.20±3.37	1746.71±119.3	0.362±0.022	-21.49±2.72
µm-InAs	1618.7±61.9	0.363±0.068	-23.01±1.17	1255.0±136.1	0.243±0.014	-28.70±1.60
nm-GaAs	1800.5±446.8	0.376±0.040	-11.32±2.59	1349.5±18.5	0.236±0.019	-18.08±1.75
µm-GaAs	4747.8±1428.7	0.374±0.034	-16.71±0.16	3003.6±557.1	0.389±0.024	-27.41±2.64

Table S2 : Characterisation of III-V materials (InAs/GaAs particulates) in Holtfreter's Medium at *t*=4hours and simulated intestinal fluid at *t*=120 hours.

	As (III)	**As (V)**
	%	
µm -GaAs	95.1	4.9
nm-GaAs	100.0	0.0
µm -InAs	89.1	10.9
nm-InAs	80.7	19.3

Table S3. Statistical analysis to show the percentage release of AsIII and AsV in HM from µm GaAs, nm-GaAs, µm InAs, nm-InAs at 200 ppm over 4 hours. Using disposable cartridges[1] for fractioning we estimated the percentage release of As from the particulates. As analysis detected *via* ICP-OES revealed that the trivalent form (III) was detected at a much higher percentage than the pentavalent form (V).

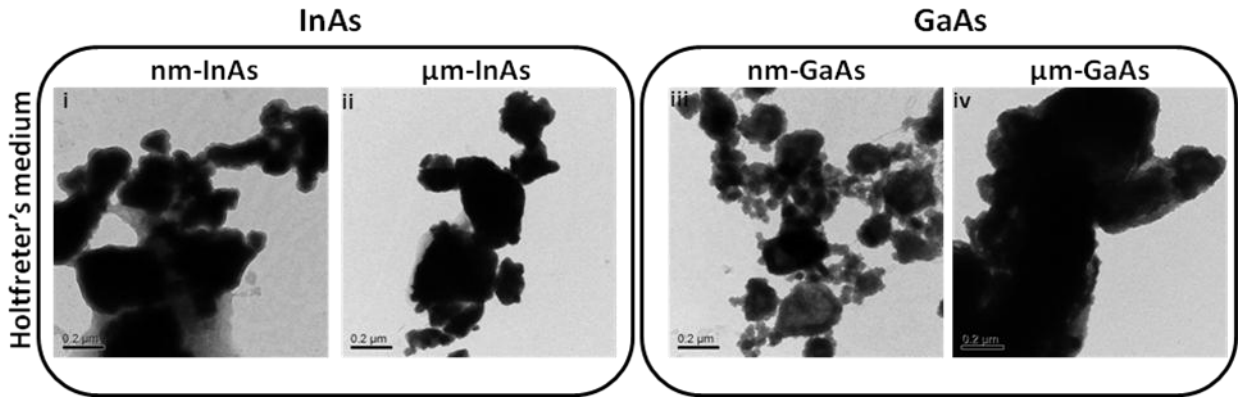

Figure S1. Physicochemical characterization of III-V particulates in HM. Representative TEM images were obtained at *t*= 4 hours to show the primary size, shape, and state of agglomeration. (i) nm-InAs; (ii) µm m-InAs; (iii) nm-GaAs; (iv) µm-GaAs.

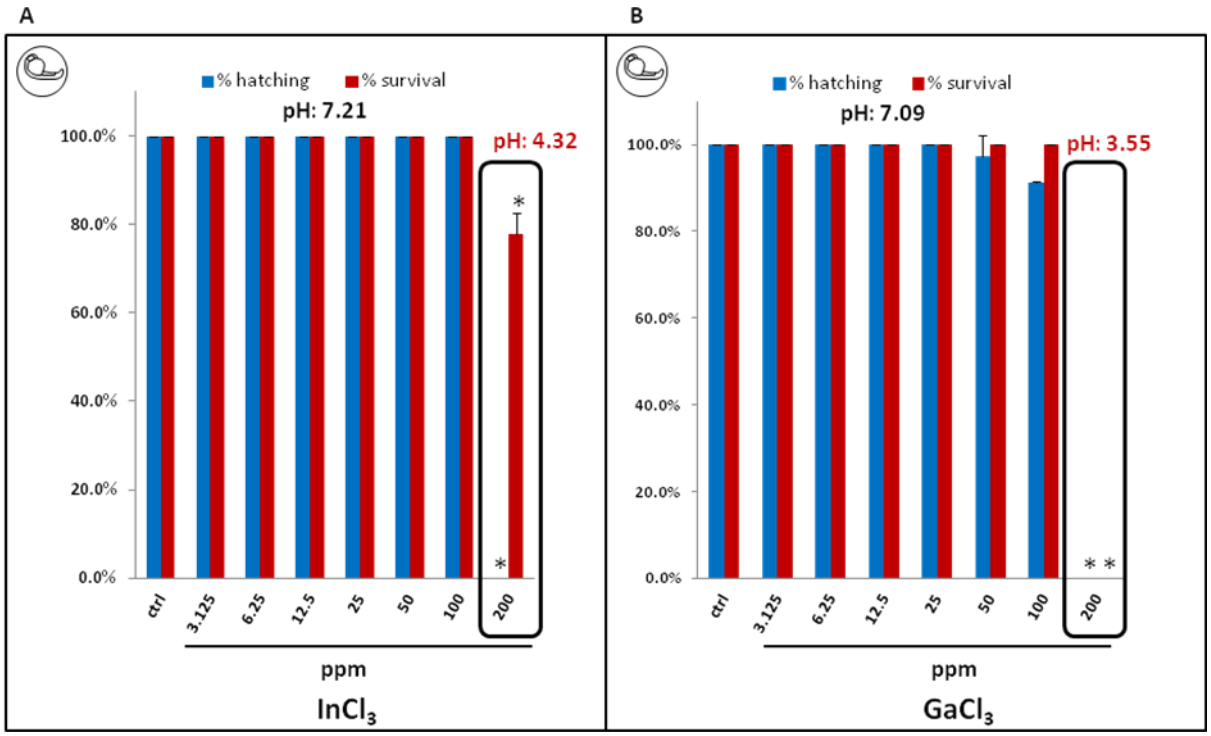

Figure S2. Statistical analysis of the survival and hatching rate interference (expressed as a percent of the number of starting embryos) using HTS embryo screening in response to $InCl_3$ (A) and $GaCl_3$ (B). The % interference in survival and hatching by $InCl_3$ and $GaCl_3$ was statistically significant (*$p<0.05$) compared to the control at 200 ppm and above. Both $InCl_3$ and $GaCl_3$ are considered as Lewis acids, implying that both compounds become acidic in solution, precipitating an abrupt pH change ~200 ppm ($InCl_3$ has a pH=4.32 and $GaCl_3$ a pH =3.55). HM do not contain any buffers.

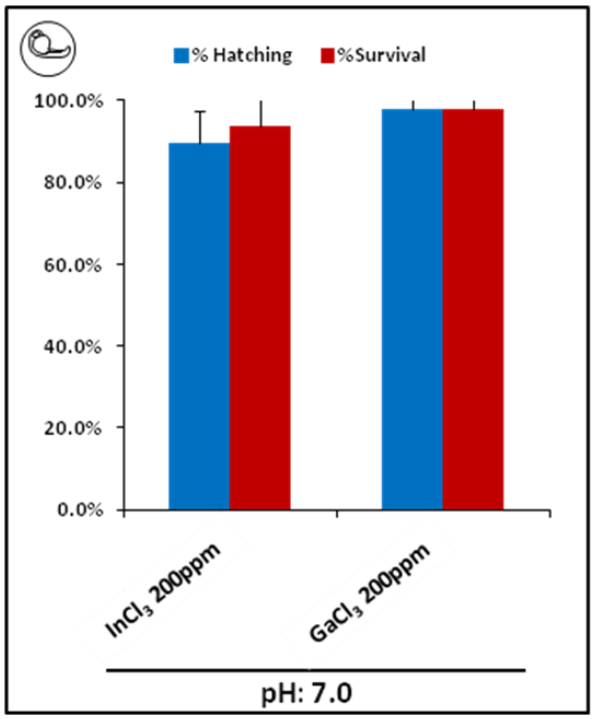

Figure S3. The addition of NaOH to restore the pH to 7 to a statistically significant improvement in survival and hatching during embryo exposure to $InCl_3$ and $GaCl_3$.

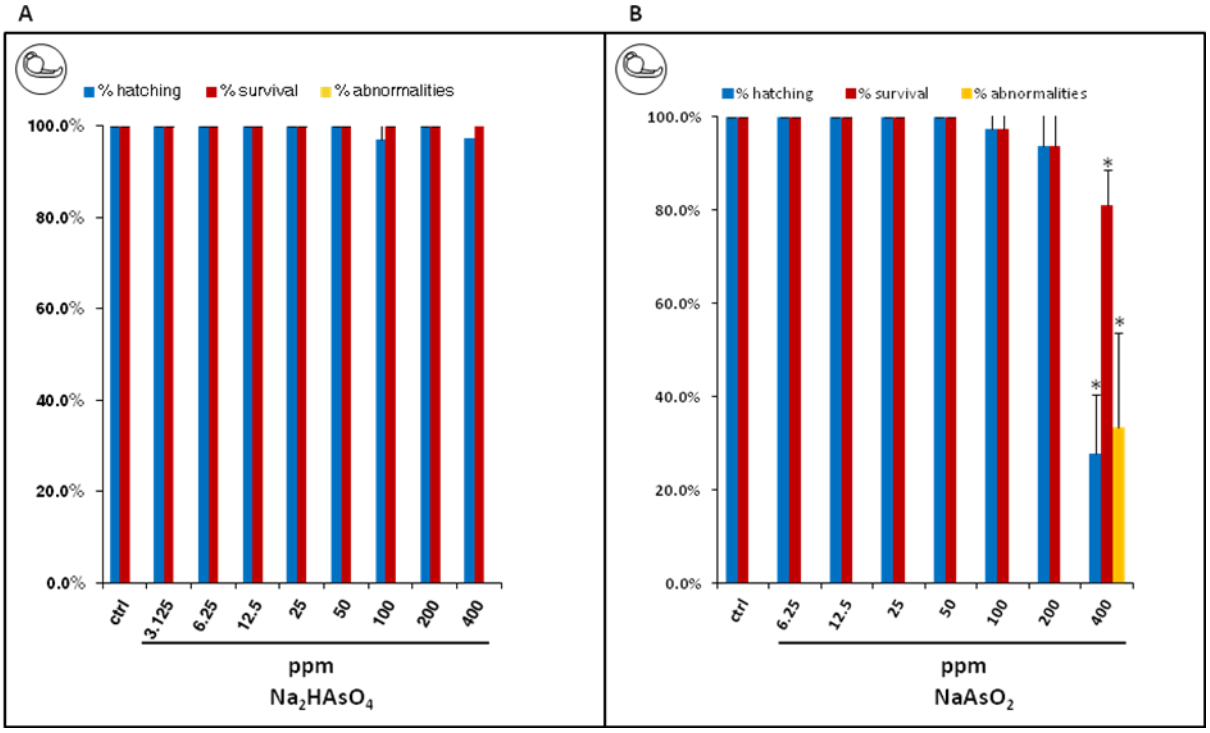

Figure S4. Statistical analysis of the comparative interference of embryo survival and hatching in response to Na_2HAsO_4 (A) and $NaAsO_2$ (B). The impact of $NaAsO_2$ on survival, hatching and morphological abnormalities was significantly different from Na_2HAsO_4 (*$p<0.05$). $NaAsO_2$ caused significantly more severe effects at 400ppm and above. Na_2HAsO_4 was devoid of these effects up to 400ppm.

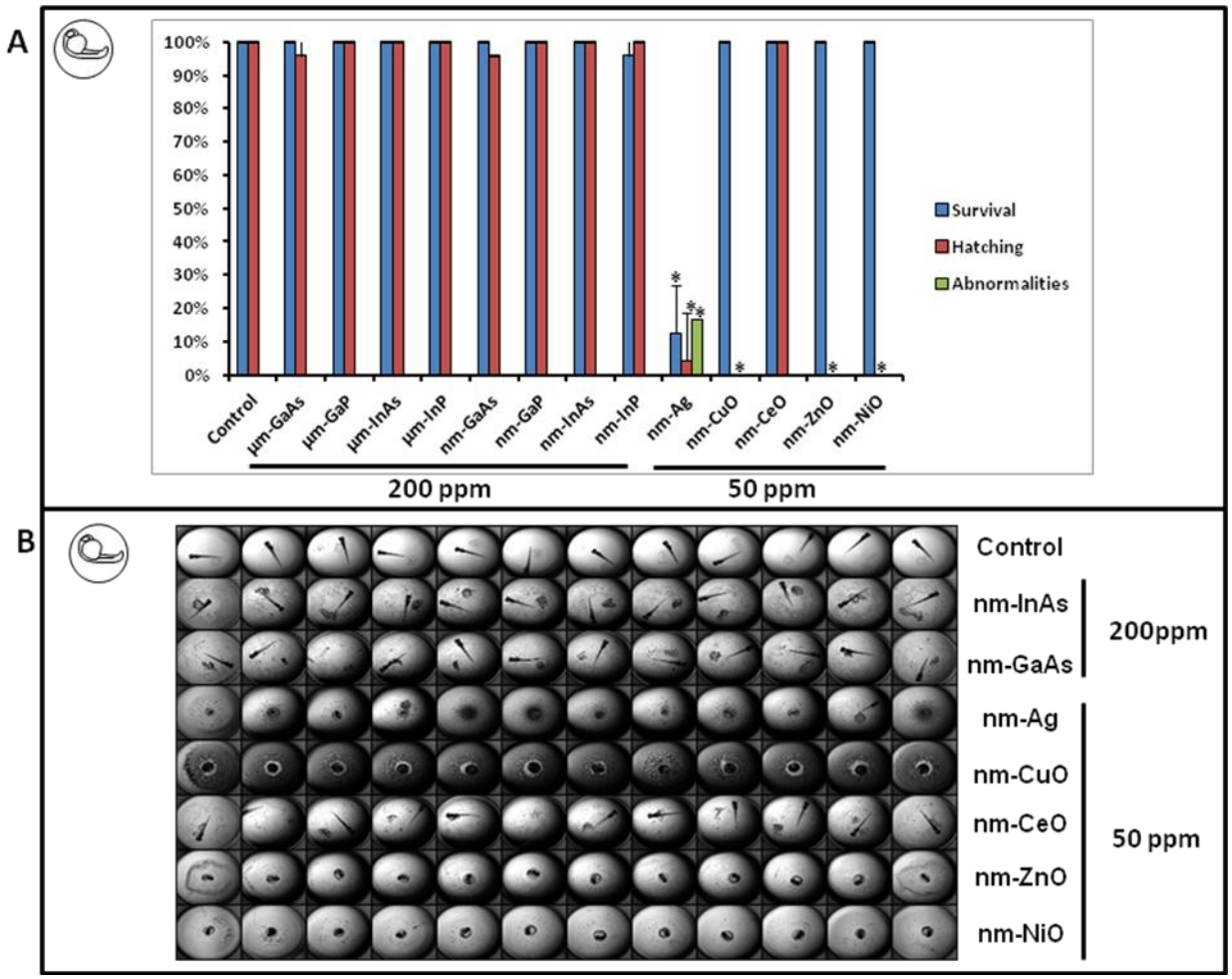

Figure S5. (A) Statistical analysis of survival rate, hatching interference and abnormalities during embryo screening in response to exposure to III-V particulates. These particulates did not exert any observable toxicity up to 200 ppm, in contradistinction to positive control, nano-Ag and metal oxide (nm-CuO, nm-ZnO, nm-NiO) nanoparticles, used at 50 ppm. nm-CeO_2, used at the same concentration, was included as a negative control. Dissolution of nano-Cu, Zn and Ni interferes with the hatching enzyme, ZHE1, while nano-Ag has a hatching interference effect through embryonal lethality. Average ± SE, * denotes $p<0.05$ (B) Representative high content images of zebrafish embryos exposed to III-V particulates (nm-InAs/nm-GaAs) at 200 ppm, in comparison to the same positive and negative control particles as in A (50 ppm). Automated bright-field imaging at 3 dpf demonstrates III-V micron and nano particulates at 200 ppm didn't exert effects on survival, hatching or abnormalities in zebrafish embryos.

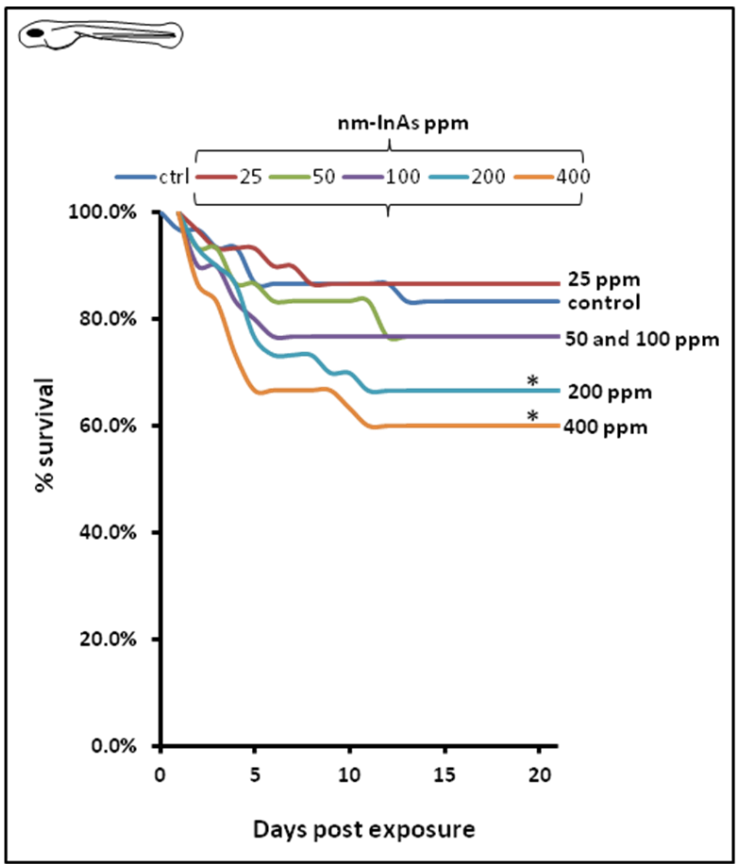

Figure S6. Graph to show the dosimetry analysis of nm-InAs in larvae at concentrations of: 25, 50, 100, 200 and 400ppm. At 200ppm and above nm-InAs exerted a significant (*$p<0.05$) decrease in survival compared to control.

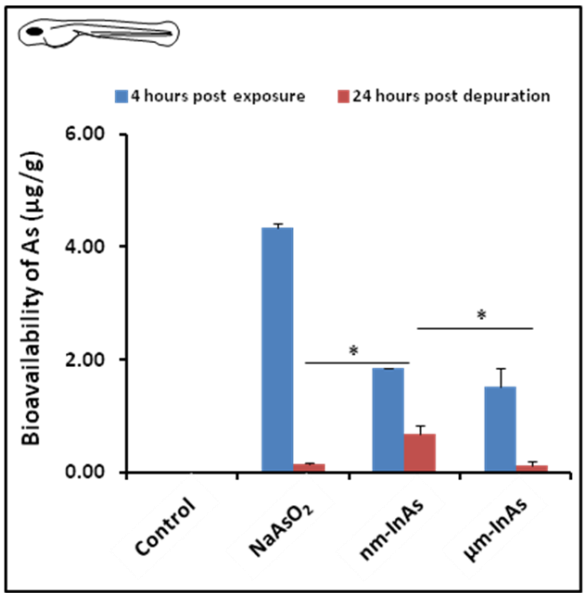

Figure S7. ICP-OES analysis to estimate the amount (µg/g) of As in the body of the larvae (*n*=60 for each treatment group) after 4 hour exposure to $NaAsO_2$, nm-InAs and µm-InAs, as well as repeat of the analysis at t= 28 hr (*i.e.*, 4 hr of exposure plus a 24 hour depuration period). Analysis revealed As retention after the depuration period only for the nm-InAs treatment group. While the $NaAsO_2$ group had a higher body content of As after 4 hr of exposure compared to the particulates, the nm-InAs treatment group showed significant (*$p<0.05$) accumulation of As following the 24 hr depuration period.

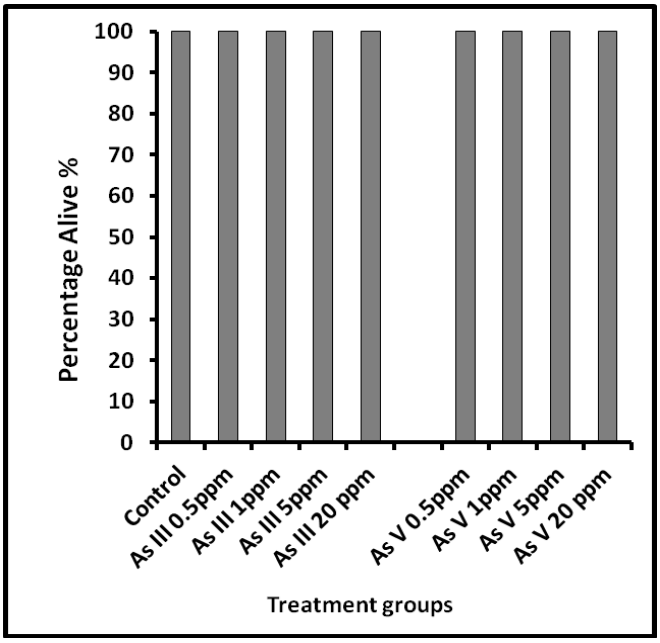

Figure S8. Graph to show the dosimetry analysis for the % of live fish (*n*=6) after a 4 day exposure of adults to 0.5, 1, 5, 20 ppm.

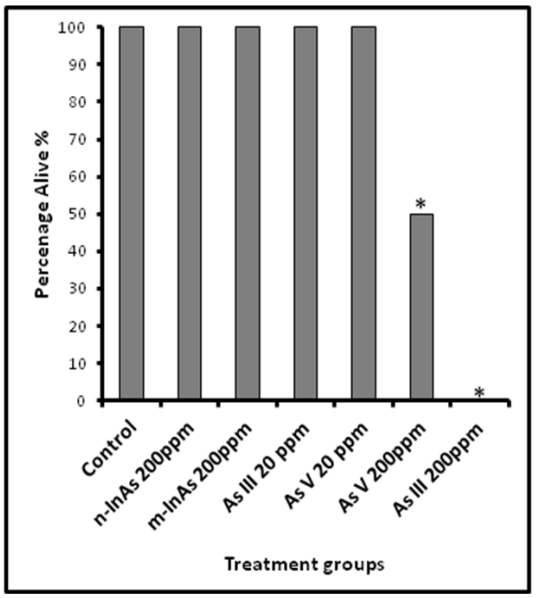

Figure S9. Dosimetry analysis, to show the % live fish (*n*=6) per treatment group after a 4 day of adults to 200 ppm nm-InAs, µm-InAs, as well as AsIII/AsV at 20ppm (to mimic the dissolution of As from particulates) and AsIII / AsV at 200ppm.

References

[1]Meng, X.; Wang, W. In *Speciation of arsenic by disposable cartridges*, Book of posters of the third international conference on arsenic exposure and health effects, Society of Environmental Geochemistry and Health Denver, Colorado: 1998.