Toxicity and Safety Study of Cd-based and Cd-free Quantum Dots in Third-Gen PV and Scaled-up Processing Platforms

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***Abstract* — Quantum dots (QDs) are being incorporated at an accelerated rate into Third-Gen photovoltaic (PV) and scaled-up PV processing platforms for production of high efficiency devices. As a result, studies are needed to examine QD toxicity in workplace environment. Herein, we report on a rapid and sensitive detection methods to examine risk of QD exposure in PV processing. QD-associated toxic elements were detected in slight amounts using gold nanoparticles (Au NPs) probe, followed by photoluminescence and Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses, which indicated the possibility of QD aerosolization during deposition, transferring and testing of the QD film. Cytotoxicity effects of different type QDs were also studied using cell culture viability. The results indicate that QD material and their coating are important factors in producing cytotoxicity effects. It was also demonstrated that CIS QDs have less cytotoxic effects on HeLa and CHSE cells than CdSe QDs, and may be considered non-toxic in comparison.**

I. Introduction

As trends in research move toward the nanoscale, use of nanotechnology can be found in every field of scientific research, from device engineering to medicine. Nanomaterials have also moved beyond the realm of research and into marketplaces. However, knowledge of the health and safety issues of nanomaterial use and exposure is inadequate. Currently, there are no comprehensive protocols or guidelines specifically designed for nanomaterial safety and handling [1].

Among nanoparticles, quantum dots (QDs) are of particular interest as QD properties can be tuned during synthesis, making them applicable for a wide range of functions. As most nanotoxicology research of QDs focus on biological toxicity, there are a lack of studies on the toxicity effects of QD exposure to workers and researchers that handle QDs for non-biomedical purposes.

Despite cadmium's known toxicity, cadmium-based QDs are particularly prevalent materials in photovoltaic (PV) devices [2]. Due to emerging concerns on the applicability of QDs based on toxic materials, focus in the research community has shifted to investigation of so-called non-toxic QDs. Among these, copper indium sulfide (CIS) has attracted particular attention due to their straightforward high throughput synthesis and applicability for Third-Gen and large scale PV devices. However, these QDs can still be toxic due to their nanoscale size. Also heavy metal ions, the commonly encountered toxic substances in the QDs, can pose significant health hazards if expose to the work place environment. Since such QDs are being accelerated in incorporation into scaled-up PV processing platforms for production of high efficiency devices, studies are needed to develop a rapid and sensitive method for detection of toxic element and aerosolized QDs.

Gold nanoparticles (Au NPs) with various functionalities have been used as an adsorbent sensitive material in sensing technology. The high surface area and a foundation for surface modification chemistry of Au NPs have been utilized to create high-affinity material. As a result, Au NPs have been used as colorimetric sensors using the appropriate functional group that can react specifically with heavy metal cations. The color changes associated with nanoparticle aggregation, and/or local refractive index change, have been exploited as optical sensing methods for the detection of toxins, heavy metals and other environmental pollutants [3]. Another approach to use Au NPs as chemical probe is monitoring the shift in the surface plasmon resonance (SPR) peak of Au NPs [4]. We used this approach for our detection.

In our previous study, we qualitatively showed the presence of small traces of aerosolized QDs components adsorbed onto Au NPs using absorption and photoluminescence (PL) spectroscopy [5]. Although the escaped particles were weakly detected, the health risk they pose to the human body is significant. The low detection is partly due to the precision of the measurement methods, as well as low QD dosage. The use of appropriate detection methods can give more reliable and quantitative results for QD toxicity.

Here, we report on the studies performed to investigate risk of exposure and potential cytotoxicity effects associated with CdSe- and CIS-based QDs used in PV processing environments. Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) and photoluminescence techniques were used for aerosolized QDs detection during QD processing, coating, and handling in both small and large scale. Cell viability studies were performed on HeLa and CHSE to study the potential cytotoxicity effects of QDs on mammalian cells [5, 6]. The purpose of these studies is to further understand the health risks associated with QDs.

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II. Aerosolized QD Detection with Inductively Coupled Plasma-Mass Spectroscopy

In this study, amine-functionalized gold nanoparticles were used as indicators to detect the possibility of QDs becoming airborne in a PV processing environment. The Au NPs were drop-cast onto a glass substrate and allowed to dry, forming a thin film. These Au NPs slides were placed into various environments where QDs are processed and handled.

Photoluminescence and absorption spectroscopy was first performed to determine the presence of adsorbed QDs on Au NPs [5]. Shift in SPR peak of Au NPs indicated absorption of toxic elements. Based on results from optical spectroscopy, five samples were chosen for ICP-MS. In the first two samples, the QDs and Au NP layers deposited side-by-side on the same substrate. QDs with chalcogenide and oleic acid (OA) ligands were used for sample 1 and sample 2, respectively. Sample 3 was prepared by placing Au NPs film into a closed container with a film of OA-ligated QDs, each deposited on a separate substrate. All samples were exposed to UV illumination for 1 hour and ambient environment for 3 weeks. The final two samples were formed by placing a monitoring sample in proximity to a dip coater and spin coater used for QD film formation, respectively.

As shown in Figure 1, ICP-MS results show the presence of cadmium (Cd) and selenium (Se), in the samples used as indicators. The detection of Cd was quite high for the samples were placed in vicinity of adsorbent Au NPs, with a maximum of 655 µg detected over the entire sample, 22×22 mm^2 in size. Selenium was also detected on the samples made with adsorbent Au NPs. Detection of Cd and Se on the dip coater and spin coater were low, although Cd was still detected in some amounts.

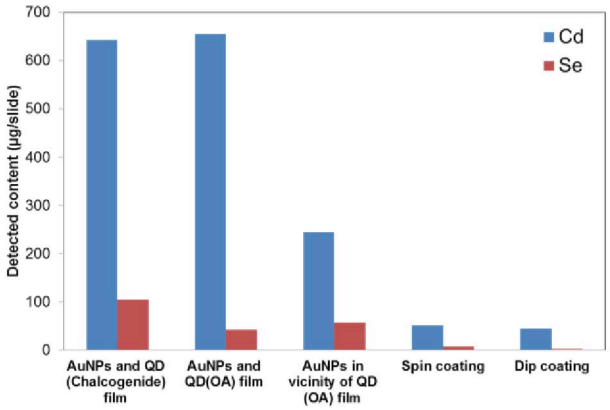


Fig. 1. ICP-MS detection of Cd, and Se on Au NPs indicators exposed to CdSe QD films during processing and testing.

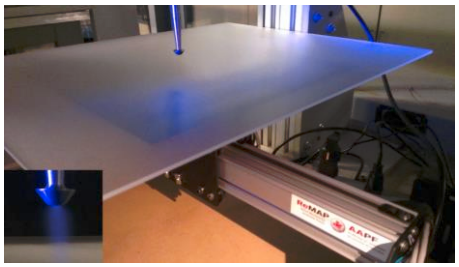
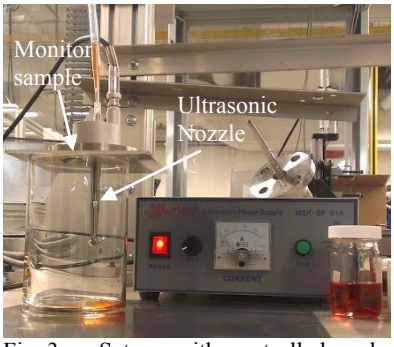


Fig. 2. Ultrasonic spray coating prototype developed for QD layer formation in scaled-up processing.



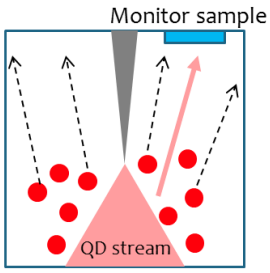


Fig. 3. Set up with controlled enclosure simulates spray coating condition for detection of possible aerosolized QDs during large area processes with spray coating.

Results from ICP-MS show that when placed in proximity of adsorbent Au NPs, some traces of aerosolized QD adsorbed onto Au NPs can be detected. Therefore, aerosolization of QDs is a concern for scientists and researchers that handle QDs, or work in environments for QD synthesis, processing, and testing.

Since the risk of QD aerosolization during scaled-up QD processing and film formation is high, results were obtained for toxicity risk during ultrasonic spray coating within a controlled enclosure (Fig. 2). The majority of the aerosolized QDs are expected to travel downwards due to the gravity during the spray coating. We simulate a similar setup to detect any possible aerosolized QDs traveling to the opposite directions of the QD stream (see Fig 3). The set up contains an enclosed area. The monitor sample was mounted on the top lid in the opposite direction of the spraying nozzle. The CIS QDs were delivered to the nozzle and an ultrasonic power supply was controlled the spray coating. The photoluminescence of the monitor sample was measured after 100s of spray coating. The PL result reported in Fig. 4 show no traces of the aerosolized QDs. The ICP results show a very slight but twice the amount of In to Cu and pretty much no detection of S (<0.01µg). Overall, not much detection was observed, but it is enough to confirm toxicity (see Fig. 5).

Figure 6 shows the doctor blade coating prototype we developed for formation of large-area smart solar glass and lamination materials. For detection of aerosolized QDs during coating, a similar condition was simulated in a controlled enclosure. Low amounts of Cd and Se were detected with ICP-MS. Very little detection of toxic element was also

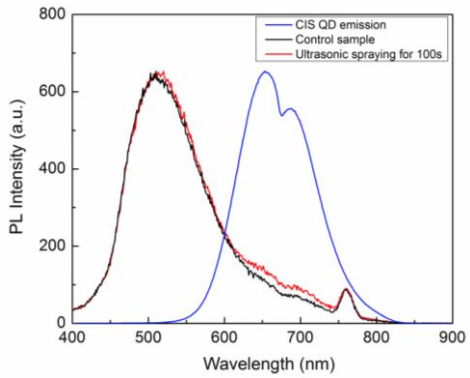


Fig. 4. PL spectra of the monitor sample before and after spray coating of CIS QDs for 100s

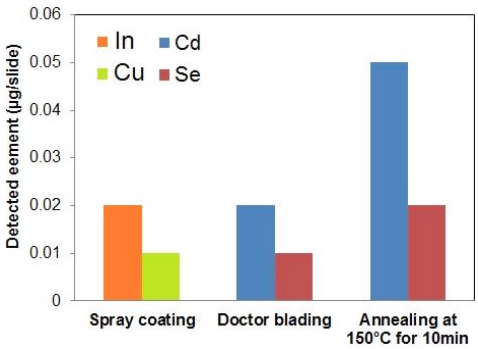


Fig. 5. ICP-MS detection of toxic elements during QD scaled-up processing and film formation

observed with ICP after annealing of the QD film at 150°C for 10min, but twice the amount before annealing (see Fig. 5).

The QD layers deployed on different surfaces will be transferred between different zones in manufacturing lines. Another experiment was designed to examine if any QDs aerosolized and detached from a deposited film during handling and transfer steps. As shown in Fig. 7 the setup uses air turbulence close to the area covered with the QD-film to amplify the real condition that might cause spread of QDs in the environment. The monitor sample was mounted in the place to receive enough amount of the air flow. No Traces of aerosolized QDs was detected on a monitor sample after running the experiment for three days using PL measurement (see Fig. 8). ICP-MS results only show a very small content of toxic elements in the monitor sample (<0.01µg/slide). So toxicity shouldn't be a concern in this case.

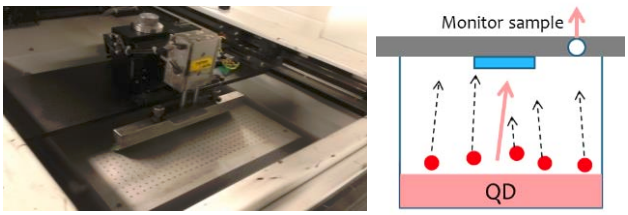


Fig. 6. a) Doctor blade coating prototype developed for QD layer formation, and b) schematic of setup used for detection of aerosolized QDs in scaled-up processing.

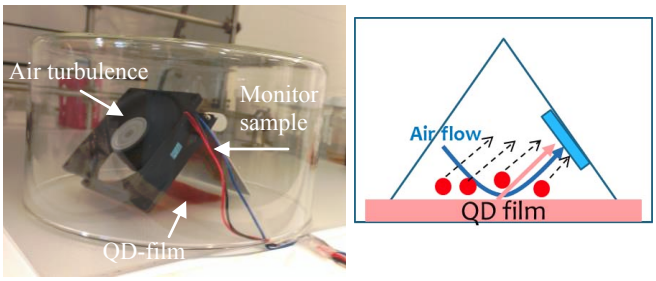


Fig. 7. Set up used for detection of possible aerosolized QDs spread in the environment during handling and transfer of QD-films.

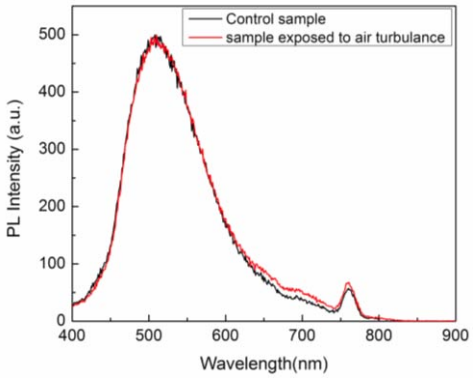


Fig. 8. PL spectra monitor sample before and after exposure of QD-film to air turbulence.

III. QD CYTOTOXICITY VIA CELL CULTURE VIABILITY TESTS

To further examine the health risk of QDs, cytotoxicity of QDs was examined via cell culture viability test. This study used HeLa and CHSE cells as an *in vitro* model to investigate the potential cytotoxicity effects of various QDs on mammalian cells.

HeLa cells were exposed to QDs of varying compositions, ligands, sizes, and coatings. The typically non-polar solvents used to disperse QDs are toxic to HeLa cells, and incompatible with the polar media used to grow and store HeLa cells. Therefore, this study was designed to remove the effect of solvent by evaporation prior to HeLa exposure.

CdSe QDs in toluene were drop-cast onto circular glass microscope slide covers. To confirm that residual toluene did not interfere with the study, one set of substrates were prepared with only toluene. After drying a film of QDs formed on the glass slides, they were subsequently placed QD-side up into 24-well plates. HeLa cells were prepared using the method previously reported by the authors [5, 6] prior to exposure, and added to the plates. Each well was seeded with approximately 200,000 cells in a total of 0.5 mL media. The cells were incubated with the QD layer for 24 hours, at 37° C, with 5% CO_2-95% air.

A. Comparing the cytotoxicity effects of core and core/shell CdSe QDs on HeLa cells

To compare the cytotoxicity effects of core and core/shell QDs, the HeLa cells were exposed to core CdSe and core/shell

CdSe/ZnS QDs. The CdSe and CdSe/ZnS QDs, with emissions of 520 nm and 540 nm, respectively, were of comparable sizes; both had oleic acid ligands. The results of this experiment showed that CdSe/ZnS killed nearly all of the exposed cells at the highest concentration of 25 µg/slide over the entire slide, while lower concentrations showed either low or negligible cell death. Cell death was confirmed by optical microscopy; as seen in Fig. 9a, healthy HeLa cells grow as a uniform monolayer in the well bottom, while Fig 9b shows floating cell debris as a result of QD-induced death.

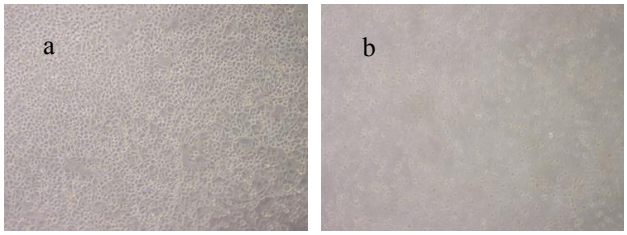


Fig. 9. Optical images of HeLa cells grown in a) nutrient media without QDs and b) 25 µg/slide of CdSe/ZnS QDs.

Resazurin metabolic activity assay was used to more accurately investigate the effects of QDs on HeLa cells viability. A fluorescent plate reader was used to measure the fluorescence of a well to determine cell viability within the well. Fig. 10 shows the core QDs, which do not have a passivating ZnS shell, induced significant cell death at 0.25 µg/slide, a concentration hundred-fold lower than the death-inducing CdSe/ZnS QDs.

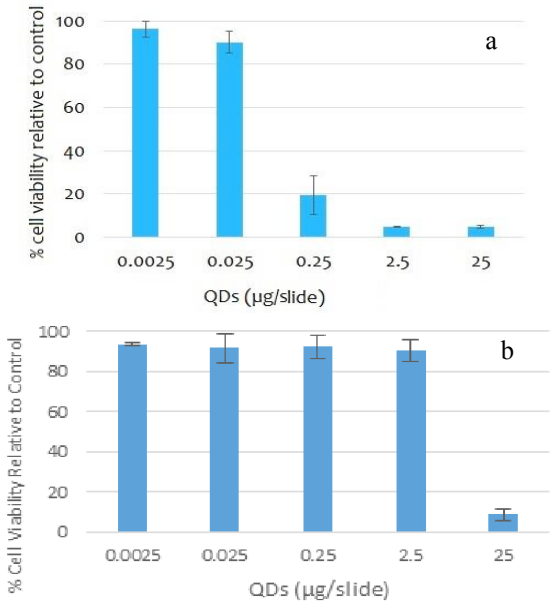


Fig. 10. Relative viability of HeLa cells exposed to a) CdSe QDs with 520 nm emission, and b) CdSe/ZnS QDs with 540 nm emission.

B. Effects of QD ligand and Size on the cytotoxicity effects of core CdSe QDs on HeLa cells

The effects of ligands were also studied using CdSe QD with emission at 520 nm with chalcogenide ligand. The chalcogenide-ligated QDs caused death at a higher concentration (25 µg/slide) than oleic acid, showing oleic acid to have more cytotoxic effects. Finally, the effect of QD size on cytotoxicity was studied. CdSe QDs with chalcogenide ligands and emissions of 480 nm and 560 nm were prepared, and the result of their effects on HeLa was compared to the aforementioned 520 nm CdSe chalcogenide-ligated QDs. All QD exposures caused cell death at between 2.5 µg/slide and 25 µg/slide. Although further tests were performed to more accurately determine the fatal concentration, the results of exposures for concentrations between 2.5 µg/slide and 25 µg/slide did not follow a clean trend. It was proposed that this is caused by the borderline cytotoxicity effects of this range of concentrations, resulting in the death of some, but not all cells.

C. Cytotoxicity effects of silica coated core/shell CdSe/ZnS QDs on HeLa cells

HeLa was exposed to 10-fold dilutions of silica-overcoated CdSe/ZnS QDs, with both TOPO and ODA ligands and emission wavelength of 550nm. From the optical microscopy images shown in Fig 11, and Cytofluor fluorescent plate reading results shown in Fig 12, it can be seen that the effect of 25 µg/slide CdSe/ZnS/Silica on HeLa toxicity is less fatal than 25 µg/slide of a similar CdSe/ZnS QDs without silica passivation. However, some death can still be observed, showing that, while silica overcoating can mitigate toxicity, it does not completely passivate the toxic core.

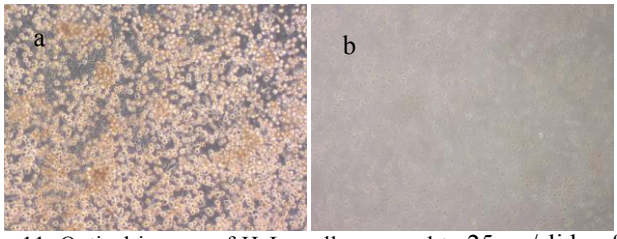


Fig. 11. Optical images of HeLa cells exposed to 25 µg/slide of a) silica coated CdSe/ZnS QDs, b) CdSe/ZnS QDs

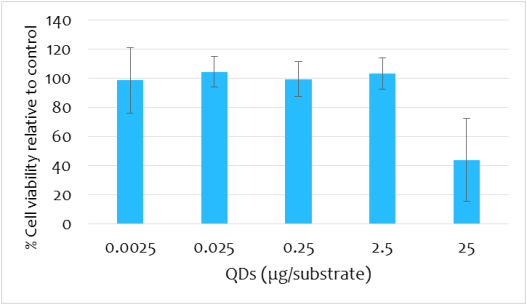


Fig. 12. Relative viability of HeLa cells exposed to silica coated CdSe/ZnS QDs.

C. Cytotoxicity effects of core CIS QDs on HeLa cells

To investigate CIS cytotoxicity, HeLa cells were exposed to CIS QDs at the same concentrations as CdSe QD exposures. In the CdSe QD experiments on HeLa, the concentration that typically induced cell death was between 0.25 to 25 µg/slide. When HeLa cells were exposed to CIS QDs at higher concentrations, relative cell viability did not significantly decrease compared to the results of CdSe exposure. As seen in Fig. 13, at a CIS QD exposure of 250 µg/slide, thousand-fold higher than the death-inducing concentration of 0.25 µg/slide for core-only CdSe QDs, the HeLa cells remained more viable than those exposed to CdSe QDs. It can be seen in Fig. 14 that the cells maintain a uniform monolayer at the bottom of the well when exposed to 250 µg/slide of CIS QDs, similar to the unexposed, healthy HeLa cells.

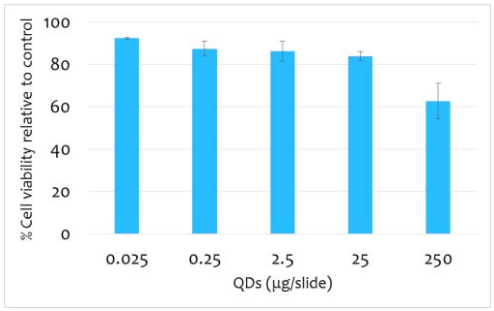


Fig. 13. Relative viability of HeLa cells exposed to CIS QDs.

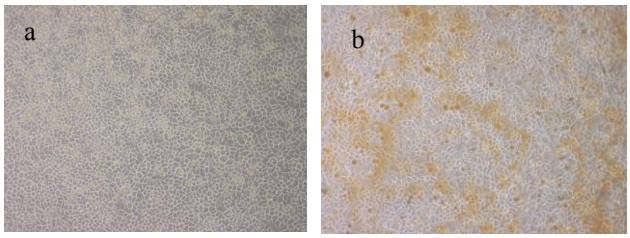


Fig 14. Optical images of HeLa cells grown in a) nutrient media without QDs, b) 250 µg/slide of CIS QDs.

C. Cytotoxicity effects of core/shell CdSe/ZnS QDs and core CIS QDs on CHSE cells

In vitro viability of fish cells (CHSE) when exposed CdSe/ZnS and CIS QDs were also examined using the similar method described above. The results from Cytofluor fluorescent plate reading presented in Figure 15 demonstrate that the CHSE cells are more sensitive than Hela cells when exposed to QDs, resulting in more cell death with a little too much variation. Also less CHSE cell death observed when CIS QDs were used.

IV. Conclusions

In this work QD associated toxic elements were detected in slight amounts using Au NPs probe and ICP-MS technique.

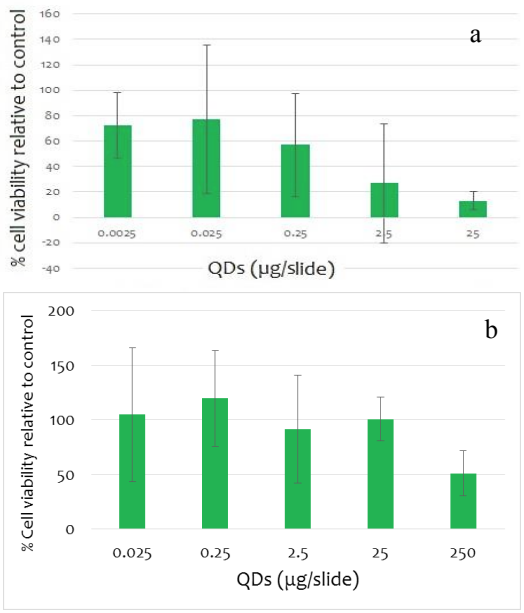


Fig. 15. Relative viability of CHSE cells exposed to a) CdSe/ZnS QDs, and b) CIS QDs.

Toxicity risk in scaled-up processing platform was also examined. QD health risk was further investigated by testing the cytotoxicity of HeLa and CHSE cells exposed to CdSe- and CIS-based QDs. Our studies indicate that QD material and their coating are the most important factors in producing cytotoxicity effects. It was also demonstrated that CIS QDs have less cytotoxic effects than CdSe QDs, and can be considered non-toxic in comparison.

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