



Published in final edited form as:

Environ Sci Technol. 2013 August 20; 47(16): 9132–9139. doi:10.1021/es304987r.

Toxicity of Oxidatively Degraded Quantum Dots

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Abstract

Once released into the environment, engineered nanoparticles (eNPs) are subjected to processes that may alter their physical or chemical properties, potentially altering their toxicity vis-à-vis the as-synthesized materials. We examined the toxicity to zebrafish embryos of CdSe_{core}/ZnS_{shell} quantum dots (QDs) before and after exposure to an *in vitro* chemical model designed to simulate oxidative weathering in soil environments based on a reductant-driven Fenton's reaction.

Exposure to these oxidative conditions resulted in severe degradation of the QDs: the Zn shell eroded, Cd²⁺ and selenium were released, and amorphous Se-containing aggregates were formed. Weathered QDs exhibited higher potency than did as-synthesized QDs. Morphological endpoints of toxicity included pericardial, ocular and yolk sac edema, non-depleted yolk, spinal curvature, tail malformations, and craniofacial malformations. To better understand the selenium-like toxicity observed in QD exposures, we examined the toxicity of selenite, selenate and amorphous selenium nanoparticles (SeNPs). Selenite exposures resulted in high mortality to embryos/larvae while selenate and SeNPs were non-toxic. Co-exposures to SeNPs + CdCl₂ resulted in dramatic increase in mortality and recapitulated the morphological endpoints of toxicity observed with weathered QD exposures. Cadmium body burden was increased in larvae exposed to weathered QDs or SeNP + CdCl₂ suggesting the increased potency of weathered QDs was due to selenium modulation of cadmium toxicity. Our findings highlight the need to examine the toxicity of eNPs after they have undergone environmental weathering processes.

INTRODUCTION

Increased production and use of engineered nanoparticles (eNPs) makes their release into the environment inevitable. Once released into the environment, eNPs may be subjected to processes that alter their physical and chemical properties in ways that affect their toxicity.¹⁻⁴ These processes may be collectively termed “weathering”. Aggregation/

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Supporting Information. Text and figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

agglomeration of eNPs is recognized as an important process affecting eNP fate and has consequences on their toxicity.⁵⁻⁷ Depending on eNP composition and that of intentional coatings, eNPs may also be altered by simple dissolution, redox transformations, photolysis, and the loss or acquisition of organic or inorganic coatings.^{6, 8-15} These transformations may impact eNP suspension stability.^{6,12,14,16} Environmentally induced alterations to eNPs have the potential to influence their bioavailability, uptake, and toxicity.¹⁷

Most current knowledge about eNP toxicity stems from research focused on intact, as-synthesized NPs.^{2,18} However, organisms are unlikely to be exposed to NPs in their as-synthesized forms.¹ Environmental alterations of eNPs is expected to alter uptake and toxicity. For example, exposure of CdSe quantum dots (QDs) to acidic or alkaline conditions increases toxicity to bacteria due to release of Cd²⁺ and SeO₃²⁻.⁸ Dissolution of ZnO NPs (i.e., release of Zn²⁺) has been shown to explain NP toxicity to bacteria,⁶ but release of Zn²⁺ alone could not explain toxicity to *Daphnia magna*.¹⁹ As zero-valent iron NPs oxidize to magnetite (Fe₃O₄) and maghemite (Fe₂O₃), the antimicrobial capacity of the particles diminishes as the oxidation state of Fe increases.²⁰ The same trend has been observed in rodent microglial cells, where more oxidized forms of iron induce lower levels of oxidative stress.²¹ As a further example, exposure to an aged TiO₂ nanocomposite (T-Lite™) used in sunscreens has a small effect on developing zebrafish (*Danio rerio*).²² To our knowledge, aside from this latter example, effects of weathered eNPs on aquatic vertebrates have not been reported.

We previously developed an *in vitro* chemical model based on the extracellular chemistry of lignolytic fungi^{23,24} to allow investigation of the oxidative weathering of eNPs under conditions representative of oxic soil environments.⁹ The model consists of a methoxyhydroquinone (MHQ)-promoted Fenton's reaction leading to the production of hydroxyl radicals (H₂O₂ + Fe²⁺ → •OH + OH⁻ + Fe³⁺) and other reactive oxygen species (ROS). Exposure of PEGylated CdSe_{core}/ZnS_{shell} quantum dots (QDs) to this model system caused dissolution of the QDs, releasing Cd²⁺ from the nanoparticle core and producing amorphous Se-containing aggregates.⁹ The toxicity of the as-synthesized CdSe_{core}/ZnS_{shell} QDs has been investigated,²⁵ but that of the products of oxidative weathering has not yet been reported.

The objectives of this study were to (1) evaluate changes in toxicity induced by oxidative weathering vis-à-vis as-synthesized QDs; and (2) provide insight into the causes of observed changes in toxicity. To accomplish these objectives we employed the zebrafish embryo as a model organism. The zebrafish embryo is a well-established and widely used vertebrate model in developmental toxicity,^{26,27} and has recently been shown to have utility in assessing the toxicity of eNPs.^{25,28-33} Oxidative weathering of QDs results in a complex mixture of products, complicating the determination of mechanisms of toxicity. We therefore adopted a reductionist approach and examined the toxicity to zebrafish embryos of Se nanoparticles, dissolved cadmium, dissolved selenium species, and their mixtures.

MATERIALS AND METHODS

Chemicals

The following chemicals were purchased from Sigma-Aldrich Chemicals (Milwaukee, WI): CdO (99.5%), trioctylphosphine oxide (TOPO, technical grade, 90%), trioctylphosphine (TOP, technical grade, 90%), zinc stearate (technical grade), sulfur powder (99.98%), selenium powder (100 mesh, >99.5%), tetramethylammonium hydroxide solution (TMAH, ~2.2 M) in methanol, ZnCl₂ (>98%), and CdCl₂ (99%). Ferrous sulfate (ACS certified), hydrazine (99%), selenous acid (98%), sodium selenate (99%), sodium selenite (99%), and selenium tetrachloride (>98%) were obtained from Fisher Scientific (Waltham, MA). We

procured 2,5-dimethoxyhydroquinone (97%) from Alfa Aesar (Ward Hill, MA) and PEG₅₀₀₀-OCH₃ (>96%) from RAPP polymer (Germany).

Nanoparticle Syntheses

Synthesis of the CdSe_{core}/ZnS_{shell} QDs was described previously.^{9,25} The CdSe core diameter was 2.6 ± 0.1 nm; the ZnS shell thickness did not exceed 1 nm.⁹ Coordinating ligands from the synthesis were exchanged with 5000-Da PEG-thiol (PEG₅₀₀₀) in chloroform. After functionalization, the solvent was evaporated, and QDs were suspended in ultrapure water (18 M Ω -cm resistivity, Barnstead NANOpure Ultrapure Water System, Dubuque, IA, USA). In the exposure medium (*vide infra*) the hydrodynamic diameter and ζ -potential of the PEG₅₀₀₀-QDs were 21 ± 1 nm and -2.0 ± 0.6 mV.²⁵ After 24 h in exposure medium dissolved Cd concentrations were $28 \mu\text{M}$.²⁵ The characterization of the QDs used in this study are detailed elsewhere.^{9,25}

Amorphous SeNPs were synthesized via hydrazine (N₂H₄) reduction of selenous acid (H₂SeO₃) in ultrapure water similar to published methods.³⁴ To keep the SeNP solutions similar to the QD solutions, we used PEG-thiol as the coordinating ligand rather than bovine serum albumin. Briefly, a 0.35 M N₂H₄ solution (5 mL) was reacted in 20 mL ultrapure water at room temperature for 10 min. To this solution, 10 mL 0.07 M H₂SeO₃ and excess PEG₅₀₀₀-thiol was added. The solution was stirred for 2 h then stored at 4 °C.

Oxidative Weathering of CdSe_{core}/ZnS_{shell} QDs

PEGylated QDs were subjected to the MHQ-driven Fenton's reaction.⁹ Solutions containing 2 μM QDs (7×10^{15} QD·L⁻¹), 200 μM H₂O₂, 20 μM Fe²⁺ and 20 μM MHQ in 10 mM acetate buffer (pH 4.1) were allowed to react overnight (16-18 h) in the dark. The reaction was quenched by adjusting pH to 6 with NaOH. Oxidative weathering of the PEG₅₀₀₀-CdSe_{core}/ZnS_{shell} QDs by the MHQ-promoted Fenton's reaction resulted in the erosion of the ZnS shell, release of Cd from the core, and formation of amorphous selenium aggregates ~10s to 100s of nm in diameter (as determined by transmission electron microscopy, selected area electron diffraction, and energy dispersive X-ray spectroscopy).⁹ These findings are reported in detail elsewhere.⁹ Amounts of dissolved and (nano)particulate Cd, Zn, and Se present after weathering are provided in Table S1 in the Supporting Information. We operationally defined dissolved and (nano)particulate species as those passing and retained by a 10 kDa MWCO filter (pore size ~2.8 nm).^{9,25} Solutions were diluted to the desired QD concentrations for zebrafish experiments in embryo medium immediately before dosing (see below for composition).

Scanning Electron Microscopy (SEM)

SeNPs were spin-cast onto doped silicon wafers for imaging by a Leo Supra55 VP SEM. Data were obtained using 2 kV incident electron energy using the standard in-lens detector.

Ultraviolet-visible (UV-Vis) Spectroscopy

UV-Vis spectra of NP suspensions were obtained using a Shimadzu PC-2401 UV-Visible spectrophotometer. The core diameter and number concentration of as-synthesized QDs was estimated from the position and absorbance of the first exciton peak centered between 525 and 530 nm.^{9,35} While the accuracy of UV-visible absorbance measurements have received criticism,³⁶ such estimates are considered acceptable for comparative analyses. Concentrations of SeNPs were determined by matching spectra from 450-600 nm to UV-Vis spectra obtained from MHQ-driven Fenton's reaction exposed QDs.⁹

Raman Spectroscopy

Raman spectra were recorded on a Thermo Scientific DXR Raman microscope using a 780 nm excitation from a diode-pumped, solid-state laser. Scans (32) were accumulated at 3 cm^{-1} resolution using 0.1-1 mW laser power. Care was taken to not alter chemical compounds due to laser power. Samples were first run at low power to assess any changes from interaction with the laser prior to increasing power to produce spectra with higher S/N ratio.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Digests of zebrafish larvae (*vide infra*) were analyzed by magnetic-sector ICP-MS (Thermo Finnigan Element 2) at the Wisconsin State Lab of Hygiene to determine Cd and Se body burden. Unexposed control larvae had Cd and Se concentrations of $0.0055 \pm 0.0004 \mu\text{g}\cdot\text{g}^{-1}$ and $0.14 \pm 0.01 \mu\text{g}\cdot\text{g}^{-1}$, respectively.

Zebrafish Exposures

Beginning 4-6 hours post fertilization (hpf) zebrafish embryos (AB strain) were exposed to graded concentrations (corresponding to 0.2 to 200 μM Cd) of as-synthesized or weathered QDs suspended in embryo medium (58 mM CaCl_2 , 0.7 mM KCl , 0.4 mM MgSO_4 , 0.6 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM HEPES, pH 7; ionic strength (I) = 0.18 M). For comparison, we also exposed fish to CdCl_2 , Na_2SeO_3 , Na_2SeO_4 , SeNPs and PEG₅₀₀₀-thiol. Exposures were conducted in a 96-well plate format with a single embryo per well. Dosing solutions (100 μL) were renewed daily, and zebrafish embryos/larvae were monitored for mortality and morphological signs of toxicity until 120 hpf. Experiments were conducted in triplicate, and data are presented as the mean \pm SE.

Morphological endpoints of toxicity were evaluated at 120 hpf following exposure to QD concentrations equivalent to 20 μM Cd, a concentration that produced consistent morphological endpoints for both weathered and as-synthesized QDs. Larvae ($n = 12$) were immobilized in 3% methylcellulose and imaged live (2.5 \times) in lateral orientation. Images were analyzed for total body length, yolk sac edema and pericardial edema.

To determine cadmium and selenium body burdens, larvae (15-20 per replicate) were collected at 120 hpf after exposure to 20 μM Cd or Se equivalents as-synthesized QDs, weathered QDs, or controls (*viz.* embryo media, CdCl_2 , Na_2SeO_3 , SeNP or SeNP + CdCl_2), euthanized with 3-aminobenzoic acid ethylester, rinsed 3 \times and transferred to pre-weighed microcentrifuge tubes. Wet weights were measured following removal of excess water, and larvae were digested overnight in 5 M HNO_3 at 60 $^\circ\text{C}$. Cadmium and selenium concentrations were determined by ICP-MS (*vide supra*).

Statistical Analyses

Statistical significance of differences between treatments and control was determined using Student's *t*-test. Mortality data at 120 hpf were used to calculate median lethal concentrations (LC_{50} values) and 95% confidence intervals ($\text{CI}_{95\%}$) by probit analysis (USEPA Probit Analysis Program, Ver 1.5). Median effects concentrations (EC_{50} values) were determined for selected morphological endpoints of toxicity from incidence data using the trimmed Spearman-Kärber method (USEPA Trimmed Spearman-Kärber Analysis Program, Ver 1.5). LC_{50} and EC_{50} values were compared among treatments for statistical significance by two-way analysis of variance (ANOVA). The level of significance for all analyses was $p = 0.05$.

RESULTS AND DISCUSSION

Toxicity of Oxidatively Weathered CdSe_{core}/ZnS_{shell} QDs

We obtained dose-response relationships for mortality induced by exposure to as-synthesized and oxidatively weathered PEG₅₀₀₀-CdSe_{core}/ZnS_{shell} QDs (Figure 1a). The LC₅₀ for the as-synthesized PEG₅₀₀₀ QDs was 42 (CI_{95%}: 24-75) μ M Cd equivalents as we previously reported.²⁵ Weathering produced a leftward shift in dose-response curves (Figure 1a) and a decline in LC₅₀ value to 14 (CI_{95%}: 9-21) μ M Cd equivalents. The mortality induced by both the as-synthesized and oxidatively weathered QDs was higher than that produced by an equivalent concentration of CdCl₂ (Figure 1a). The higher toxicity of intact QDs relative to CdCl₂ is consonant with our previous findings.²⁵

The types of morphological endpoints of toxicity observed did not differ between as-synthesized and weathered QDs (Figure 1b). Morphological endpoints included pericardial, ocular and yolk sac edema (i.e., swelling due to fluid retention), low absorption of the yolk, spinal curvature, tail malformation, opaque tissues (particularly in the head region), craniofacial malformation and failure to inflate the swim bladder. Some of these endpoints fit the profile of Cd toxicity (i.e., spinal curvature, pericardial edema, ocular edema),^{37,38} while others correspond to those associated with Se toxicity (i.e., pericardial edema, yolk sac edema, low absorption of the yolk).³⁹

Weathered PEGylated QDs displayed increased potency for inducing morphological endpoints of toxicity relative to as-synthesized QDs. That is, weathered QDs produced morphological malformations both at lower concentrations and in more larvae than did as-synthesized QDs. Exposure to weathered PEG₅₀₀₀-QDs at a concentration of 20 μ M Cd equivalents (i.e., 6.7×10^{16} QD·L⁻¹) significantly increased the incidences of pericardial edema, yolk sac malformations and craniofacial malformations relative to the as-synthesized QDs (Figure 2). Increases in incidence of pericardial edema and craniofacial malformations were also observed upon exposure to 2 μ M Cd equivalents weathered vs. as-synthesized QDs (data not shown). We calculated EC₅₀ values for pericardial edema, yolk sac malformations, and craniofacial malformations for as-synthesized and weathered QDs. For as-synthesized PEG₅₀₀₀-QDs, EC₅₀ values for pericardial edema, yolk sac malformations and craniofacial malformations were 18 (CI_{95%}: 12-28), 9 (CI_{95%}: 6-12) and 9 (CI_{95%}: 5-14) μ M Cd equivalents; for weathered PEG₅₀₀₀-QDs, the EC₅₀ values for these responses decreased to 8 (CI_{95%}: 6-11), 4 (CI_{95%}: 3-5) and 3 (CI_{95%}: 2-4) μ M Cd equivalents, respectively.

We quantified three morphological endpoints of toxicity following exposure to 20 μ M Cd equivalents as-synthesized or weathered QDs: total body length, pericardial edema and yolk sac edema (Figure 3). Total body length and pericardial edema differed between as-synthesized and weathered PEG₅₀₀₀-QDs ($p < 0.05$; Figure 3 a,c), while yolk sac malformations (i.e., edema, lack of adsorption) did not ($p > 0.05$; Figure 3b). These data indicate that exposure to the weathered QDs produced more severe effects for some morphological endpoints further demonstrating that weathered QDs were more toxic than their as-synthesized counterparts.

Toxicity of Dissolved Inorganic Selenium Species

Few prior studies have reported the toxicity of inorganic selenium compounds to zebrafish embryos/larvae.^{40,41} Furthermore, the morphological endpoints of toxicity caused by selenium compounds have not been described. Selenium was expected to contribute to the toxicity of oxidatively weathered CdSe_{core}/ZnS_{shell} QDs due to its oxidation to soluble species during the weathering process. We therefore investigated the toxicity to zebrafish

embryos/larvae of three dissolved inorganic selenium compounds (i.e., sodium selenate, sodium selenite and selenium tetrachloride).

Dose-response relationships were obtained for Na_2SeO_4 , Na_2SeO_3 and SeCl_4 at 120 hpf (Figure 4a). Sodium salts of selenium anions are water-soluble. Selenium tetrachloride decomposes in water to yield selenous acids (pH was adjusted to 7 in exposure solutions with NaOH). The most potent selenium compound tested was SeO_3^{2-} ($\text{LC}_{50} = 5.1$ ($\text{CI}_{95\%}$: 3.7-7.1) μM Se) followed by SeCl_4 ($\text{LC}_{50} = 240$ ($\text{CI}_{95\%}$: 193-280) μM Se). No mortality above baseline was observed following exposure to Na_2SeO_4 over the concentration range tested (0-200 μM Se). Prevalent morphological effects observed after exposure of zebrafish embryos/larvae included yolk sac edema, failure to adsorb the yolk, yolk discoloration, and pericardial edema. These endpoints of toxicity were produced at much lower concentrations of SeO_3^{2-} than SeCl_4 (Figure 4b). Morphological endpoints of toxicity produced by exposure to SeO_3^{2-} were similar to those described for other fishes exposed to this selenium oxyanion.³⁹ These data suggest that dissolved Se species may play a role in the toxicity. However, dissolved Se cannot account for the total toxicity of weathered QDs because, at a given dose, only a fraction (~76%) of Se was dissolved (Table S1). Additionally, larvae exposed to inorganic selenium species do not show all endpoints of toxicity of those exposed to weathered QDs.

Cadmium and Selenium Body Burdens

We measured Cd and Se body burden by ICP-MS in larvae exposed to 20 μM Cd equivalents of as-synthesized or weathered QDs. For comparison, we measured body burden in larvae exposed to CdCl_2 , Na_2SeO_3 , SeNPs and SeNP + CdCl_2 . Cadmium body burden in exposed larvae increased in the following order: as-synthesized QDs < weathered QDs < CdCl_2 < SeNPs + CdCl_2 treatment groups (Figure 5a). The difference in Cd body burden between larvae exposed to weathered and as-synthesized QDs was small but significant ($p = 0.05$). Cadmium body burden was higher in larvae co-exposed to SeNPs + CdCl_2 than in those exposed to CdCl_2 alone (Figure 5a). Selenium body burden in exposed larvae increased in the following order: weathered QDs < as-synthesized QDs < SeNPs + CdCl_2 < SeNPs < Na_2SeO_3 treatments (Figure 5b). The Se body burden was lower in larvae co-exposed to SeNPs + CdCl_2 than in those exposed to SeNPs alone. Similarly, larvae exposed to weathered QDs contained significantly less Se ($p < 0.001$) than those exposed to as-synthesized QDs. The highest Se body burden resulted from exposure to Na_2SeO_3 .

Differences between As-synthesized and Oxidatively Weathered QDs

In principle, several differences between as-synthesized and weathered QD dosing suspensions may have contributed to the higher potency of the latter. In the case of the weathered QDs dosing suspensions contained particulate and dissolved QD products and assay components. Exposures to relevant concentrations of individual assay components did not produce noticeable signs of toxicity. Assay components in combination, produced no mortality, but did cause craniofacial malformations at the highest dose examined (Figure S1). No other endpoints were observed. Degradation of PEG under oxidative conditions is known to produce potentially toxic byproducts.^{42,43} We examined the potential toxicity of oxidized PEG ligands in two ways. One product of PEG cleavage is ethylene glycol (EG). We therefore exposed zebrafish embryos to EG concentrations equivalent to the amount of PEG present ($\text{PEG}_{5000} = 113$ EG units) in QD exposures but failed to observe morphological malformations or mortality (Figure S1). Degradation products other than EG can be produced from PEG oxidation. We therefore exposed PEG-thiol to the MHQ-driven Fenton reaction and then exposed zebrafish embryos to the oxidatively degraded PEG. Exposures to oxidatively degraded PEG at concentrations relevant for the QD exposures failed to elicit noticeable toxic responses (Figure S1).

To determine the contribution to toxicity of the dissolved and (nano)particulate fractions, we filtered weathered QD solutions through centrifugal concentrators (10 kDa nominal molecular weight cutoff; pore size ~2.8 nm).⁹ Zebrafish embryos/larvae were exposed to each fraction at doses consistent with the dosing range used for QD solutions. Morphological malformations were observed when larvae were exposed to either fraction; however, exposure to the (nano)particulate fraction produced more pronounced toxicity (Figure S2). These results led to the hypothesis that the amorphous Se-containing aggregates, produced following oxidative weathering, contributed to the toxicity of weathered QDs.

Toxicity of Se⁰ Nanoparticles

Oxidative weathering of CdSe_{core}/ZnS_{shell} QDs by the MHQ-promoted Fenton's reaction produced amorphous selenium aggregates ~10s to 100s nm in diameter. Amounts of dissolved and particulate Se present after weathering are provided in Table S1. We operationally defined particulate selenium as Se not passing a 10 kDa MWCO filter (pore size ~2.8 nm).

To examine toxicity due to the selenium aggregates, we synthesized ~150- to 300-nm amorphous Se⁰ nanoparticles (SeNPs) (Figure 6a). Raman spectra of SeNPs exhibited peaks at 234 and 251 cm⁻¹ (Figure 6b) consistent with elemental selenium in a (predominately) six-membered ring network structure.⁴⁴ Selenium NP concentrations comparable to those of Se-aggregates in weathered QD solutions did not cause appreciable mortality (<10%; Figure 6c). Furthermore, few morphological endpoints of toxicity were apparent (Figure 6d). At the highest SeNP dose tested, minimal yolk absorption was evident. These data suggest that the increased potency of weathered QDs cannot be explained solely by exposure to the aggregates of selenium particles formed during simulated oxidative weathering.

Bovine serum albumin-coated elemental SeNPs (36 nm) have previously been shown to be more lethal to adult medaka (*Oryzias latipes*) than sodium selenite due to hyperaccumulation of selenium in nanoparticle form.⁴⁵ In the present study, however, we found the synthesized SeNPs to be less toxic than sodium selenite (*cf.* Figures 4a and 6c). The discrepancy in the toxicities of SeNPs most likely resulted from the differing SeNP sizes, ligands, and fish species tested.

Toxicity of Se Nanoparticles and Cadmium

Weathered QD solutions contain not only SeNPs, which we have shown to have minimal toxicity (*vide supra*), but free Cd²⁺ ions as well. We therefore examined the toxicity of co-administered CdCl₂ and SeNPs as they likely both contributed to the toxicity of weathered QDs. At completion of *in vitro* weathering, the dissolved fraction contained ~70% and ~76% of the total Cd and Se; the particulate fraction contained ~19% of the total Cd and ~25% of the total Se (Table S1). Based on these data, we used a 1:1 Cd:Se ratio in experiments examining the joint toxicity of Cd and Se. Interestingly, co-exposure of zebrafish embryos to SeNPs and CdCl₂ produced higher mortality than did either CdCl₂ or SeNPs alone (Figure 6c). The calculated LC₅₀ value for SeNP and CdCl₂ co-exposures was 27 (CI_{95%}: 17-40) μM Se (or Cd) equivalents (the units are equivalent because co-exposures were conducted using a 1:1 Se-to-Cd ratio). These values are much lower than LC₅₀ values for CdCl₂ alone (409 μM Cd).²⁵ Importantly, co-exposure to SeNPs and CdCl₂ recapitulated the profile of morphological endpoints produced by exposure to weathered QDs (*cf.* Figures 1b and 6d). A similar left-shift in dose-response curve was found in co-exposures of Cd²⁺ and SeO₃²⁻ compared to SeO₃²⁻ alone (Figure S3). No shift in mortality was observed in larvae co-exposed to Cd²⁺ and SeO₄²⁻ (data not shown). These data may partially explain the toxicity noted in larvae exposed to weathered QD filtrate (Figure S1). Based on solution

conditions, one would expect the dissolved Se to be primarily SeO_3^{2-} . The mortality and morphological endpoints of toxicity observed with zebrafish exposure to the dissolved fraction of weathered QD solutions are consistent with this expectation (Figure S2). We would not expect to note toxicity if the major dissolved species was SeO_4^{2-} . Additionally, these results indicate that weathered QD toxicity most likely results from both nanoparticulate and dissolved species.

Interactions between Cd and Se *in vivo* are complex and not fully understood. Low levels of Se can at least partially ameliorate symptoms of Cd toxicity.⁴⁷ Co-exposure to selenite leads to the redistribution of Cd to higher molecular weight proteins within tissues relative to exposures to Cd^{2+} alone.⁴⁷ The mechanism behind Se-induced redistribution of Cd is not currently understood. However, rescue of Cd toxicity by Se depends on the concentration and speciation of Se. We previously examined metallothionein (MT) expression as a marker for Cd exposure and found that MT expression correlated weakly with Cd body burden.²⁵ In that study, the QD exposures resulted in co-exposure to Se and Cd, potentially leading to a redistribution of Cd that lowered the amount of MT expressed relative to the Cd body burden.

Our results show an increase in potency and lethality of PEGylated $\text{CdSe}_{\text{core}}/\text{ZnS}_{\text{shell}}$ QDs to zebrafish embryos/larvae following simulated oxidative weathering. The increase in toxicity appears attributable in large part to Cd toxicity but is modulated by Se in the QD suspensions. The increased Cd body burden in larvae exposed to weathered QDs may be due to the adsorption of Cd to the SeNP surface enhancing uptake of Cd or reducing Cd elimination. However, since both as-synthesized and weathered QDs are more toxic than equivalent amounts of Cd, selenium appears to play a role in QD toxicity.

Environmental Implications

We have shown that weathering processes that may occur in the environment have the potential to alter eNP toxicity. In the system investigated here, oxidative weathering led to increased toxicity of PEGylated $\text{CdSe}_{\text{core}}/\text{ZnS}_{\text{shell}}$ QDs. We expect the impact of environmental weathering on toxicity to depend on NP composition, the nature of the ligands on the NPs, and the particular weathering process. Assessment of the environmental impacts of NPs should consider the effects of other environmental processes (e.g., reductive environments, photodegradation, microbial alterations) on NPs of a variety of compositions. In addition to potential changes in toxicity, environmental alterations to NPs are expected to influence their fate and transport and directly affect on the potential for environmental exposure.

Our data suggest that adsorption of metals to NPs, including NPs formed under environmental conditions, may alter uptake and subsequent toxicity of the adsorbed metal. Enhanced uptake of metals, such as Cd and As, has been shown in fish when co-exposed with TiO_2 NPs.⁴⁸⁻⁵⁰ Once released to the environment, NPs may serve as adsorbents for metal and non-metal contaminants.⁵¹⁻⁵³ Such “contaminant-loaded” NPs may exhibit toxicity differing from that of the NP alone. This is expected to be especially important for NPs employed in wastewater treatment and environmental remediation. Research directed at understanding metal adsorption to NPs is therefore warranted, particularly in relation to uptake, distribution and retention of adsorbed metals.

Our experiments with intact and weathered QDs as well as inorganic selenium species, highlights the importance of selenium speciation in toxicity to fish. While this is not surprising given previous research in birds and mammals,^{54,55} the literature contains scant information on selenium toxicity to fishes. Furthermore, Se present in solution impacted Cd toxicity. The literature on co-exposure of heavy metals (e.g., Cd, Hg) and Se focuses

primarily on the amelioration toxicity by Se. Our findings suggest the role of Se in heavy metal toxicity is more complex than this and may be a fruitful area of research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was funded by the National Science Foundation (initiated under DMR-0425880 and completed under DMR-082760). TKH was supported by grant number T32ES007015 from the National Institute of Environmental Health Sciences. Article contents are solely the responsibility of the authors and do not represent official views of the sponsors.

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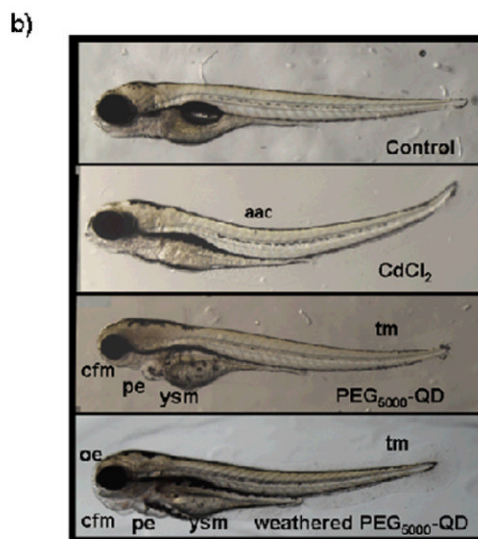
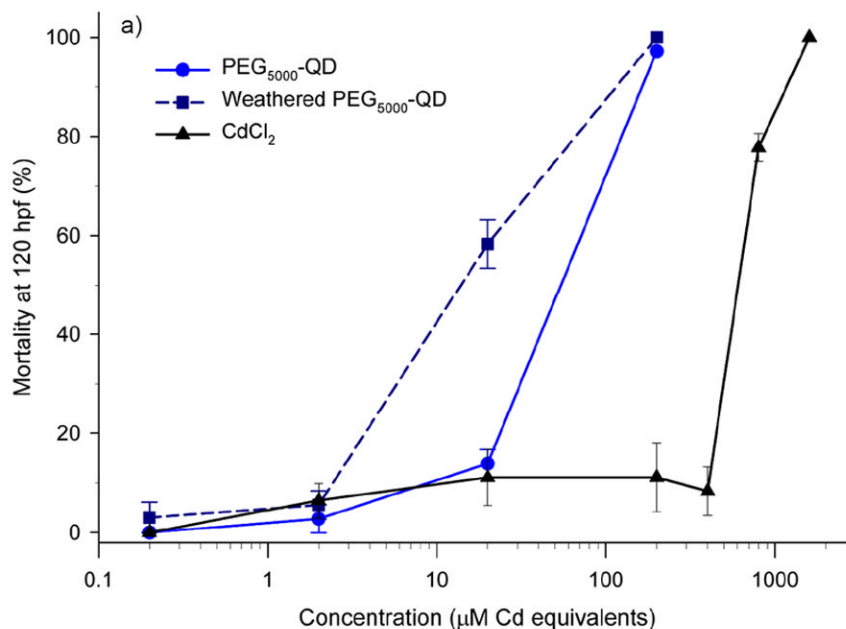


Figure 1. Toxicity to zebrafish embryos of intact and oxidatively weathered PEG₅₀₀₀-CdSe_{core}/ZnS_{shell} quantum dots (QDs)

(a) Dose-response relationships for mortality at 120 hours post fertilization (hpf) induced by intact and weathered QDs. Data for CdCl₂ shown as a reference. (b) Morphological malformations at 120 hpf resulting from exposure to 20 µM Cd equivalents of as-synthesized QDs, weathered QDs or CdCl₂. Morphological endpoints of toxicity included altered axial curvatures (aac), yolk sac malformations (ysm), pericardial edema (pe), tail malformations (tm), ocular edema (oe), opaque tissues and craniofacial malformations (cfm). In (a), calculated LC₅₀ values (95% confidence intervals, CI_{95%}) for PEG₅₀₀₀-QD, weathered PEG₅₀₀₀-QDs, and CdCl₂ were 42 (CI_{95%}: 24-75), 14 (CI_{95%}: 9-21), and 409 (CI_{95%}: 270-563) µM Cd equivalents, respectively.

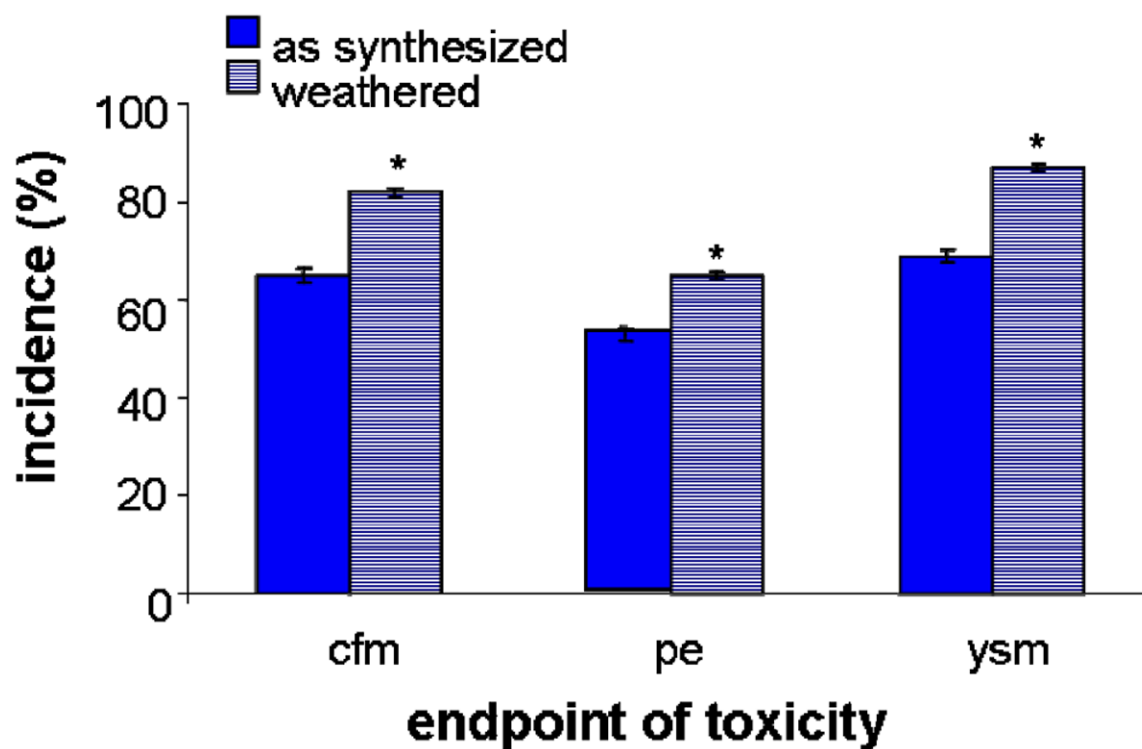


Figure 2. Incidence of selected morphological endpoints of toxicity

Bars show percent of larvae showing craniofacial malformations (cfm), pericardial edema (pe), and yolk sac malformations (ysm) after 120 h exposure to 20 μM Cd equivalents as-synthesized or oxidatively weathered PEG₅₀₀₀-QDs. *denotes incidence differs significantly ($p < 0.05$).

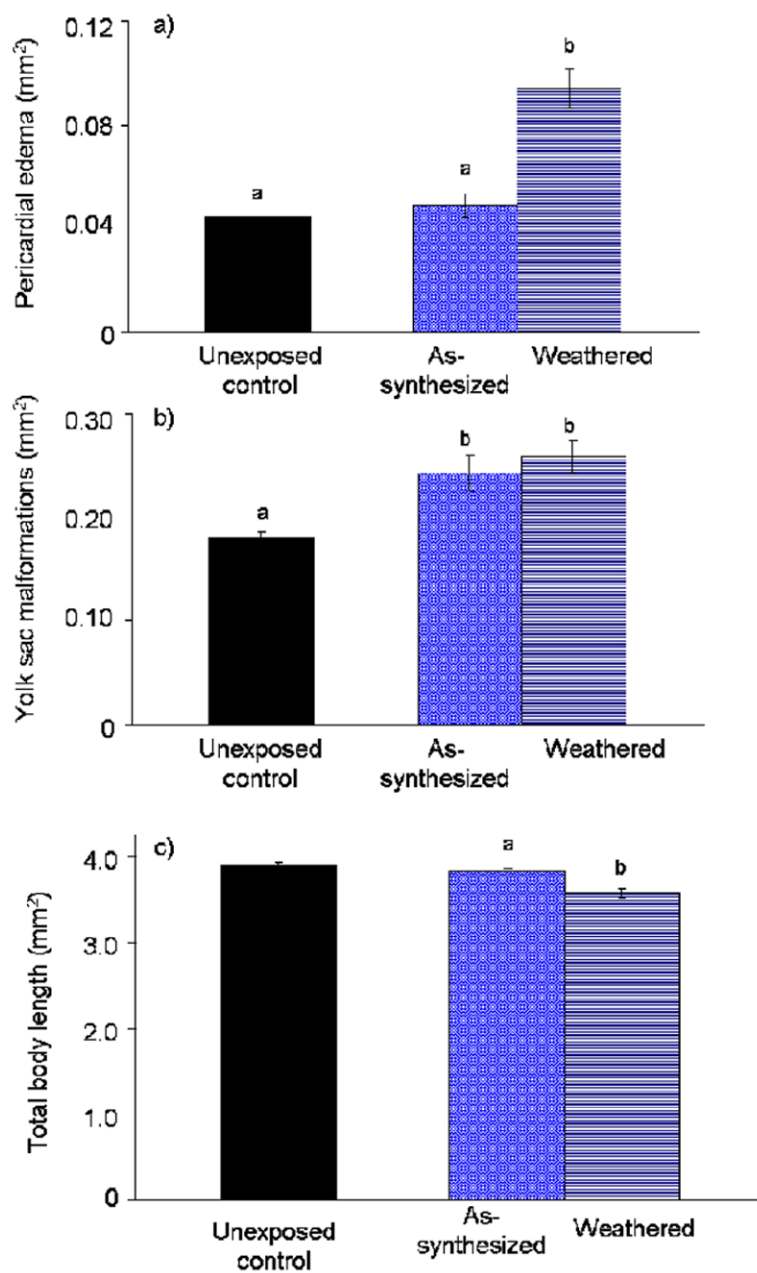
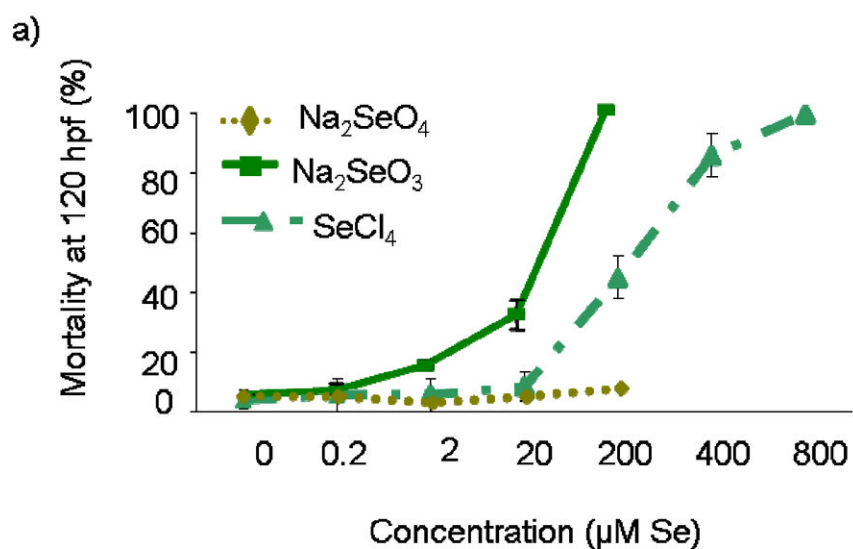


Figure 3. Severity of selected morphological endpoints of toxicity

Unexposed larvae and those exposed to 20 μM Cd equivalents as-synthesized or oxidatively weathered PEG₅₀₀₀-QDs were assessed 120 hpf for (a) total body length; (b) yolk sac area, including edema; and (c) pericardial area. Letters denote significant differences ($p < 0.05$) among treatments.



b)

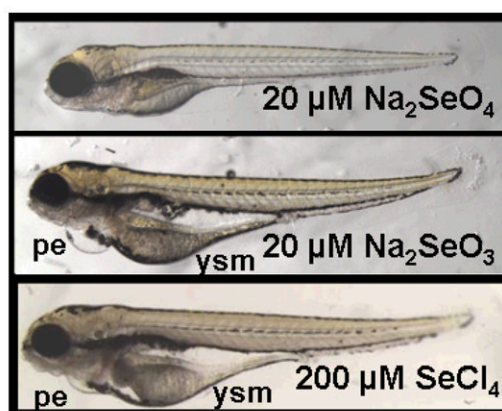


Figure 4. Toxicity of dissolved inorganic selenium species to zebrafish larvae

(a) Dose-response relationships for Na_2SeO_3 , Na_2SeO_4 , and SeCl_4 . Data are means \pm SE of 3 replicates. In some cases, error bars are smaller than the size of the symbols. (b)

Morphological endpoints of toxicity at 120 hours post fertilization (hpf) resulting from exposure to 20 μM Na_2SeO_3 , 20 μM Na_2SeO_4 or 200 μM SeCl_4 . Morphological endpoints of toxicity include pericardial edema (pe) and yolk sac malformations (ysm).

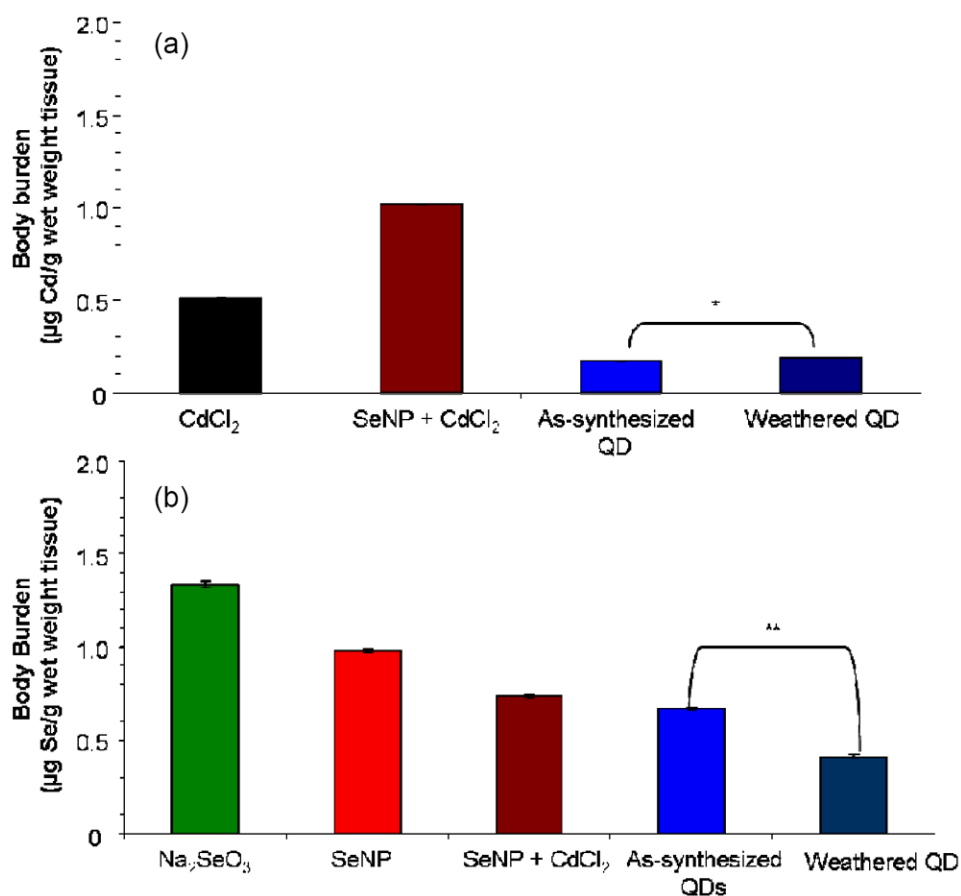


Figure 5. (a) Cd and (b) Se body burdens

Body burdens for Cd or Se were determined for larvae at 120 hpf exposed to 20 µM Cd or Se equivalents of as-synthesized or weathered PEG₅₀₀₀-QDs, CdCl₂, Na₂SeO₃, SeNPs, or SeNP + CdCl₂. Values represent mean ± SE, *n* = 3 pools of 15-20 larvae each. In some cases, error bars were too small to be visible. Difference between as-synthesized and weathered QDs: *, *p* = 0.05; **, *p* < 0.001.

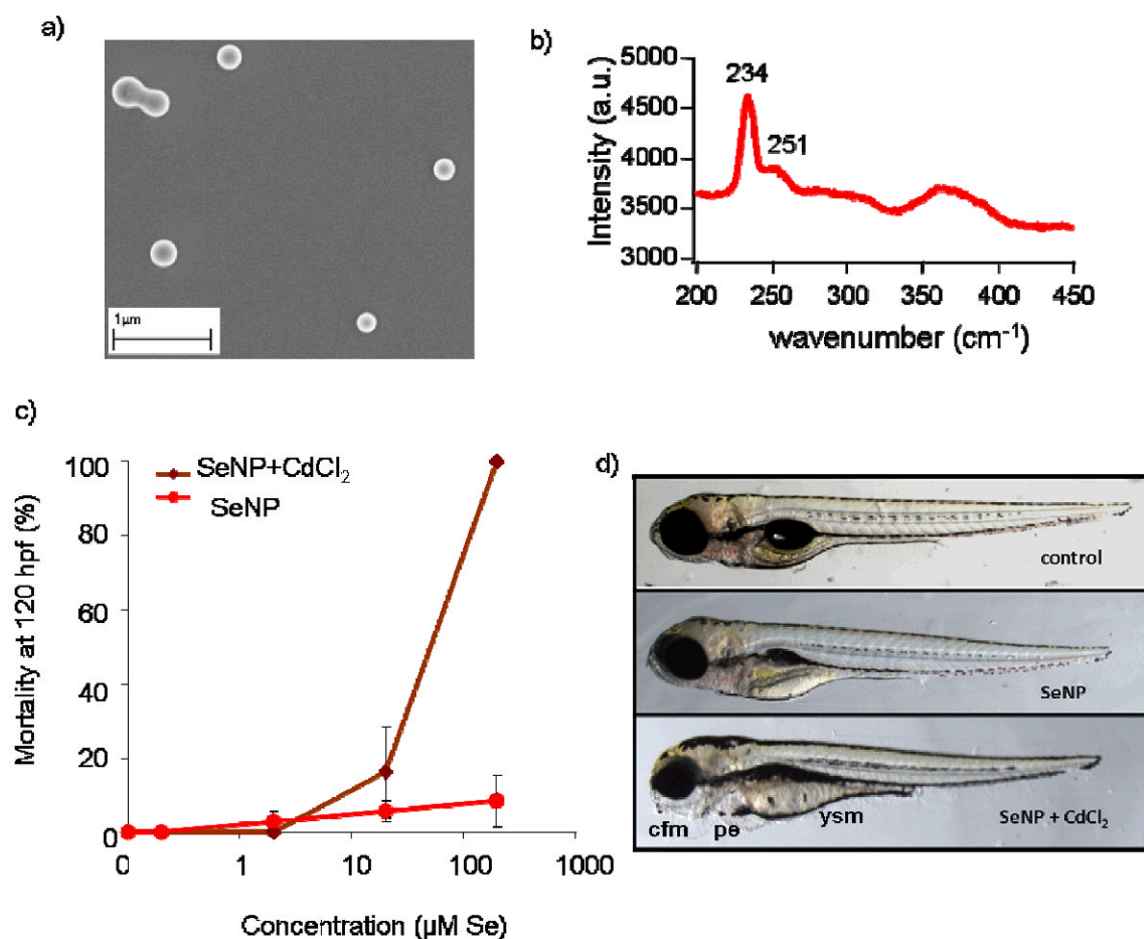


Figure 6. Characterization and toxicity of selenium nanoparticles (SeNPs). (a) Scanning electron micrograph of SeNPs. (b) Raman spectrum of SeNPs. Peaks at 234 and 251 cm⁻¹ are consistent with elemental selenium in a six-membered ring structure.⁴⁹ (c) Dose-response relationships for SeNPs, SeNPs + CdCl₂. The LC₅₀ value for SeNP + CdCl₂ was 27 (CI_{95%}: 17-40) μM Cd equivalents. In some cases, error bars are smaller than the size of the symbols. (d) Images of zebrafish larvae at 120 hpf exposed to SeNPs in the presence or absence of CdCl₂.