Current Topics in Toxicology
Vol. 16, 2020

Original Article

Size-dependent toxicity of CdTe quantum dot aggregates in trout and human hepatocytes

C. André[1], B. Lachance[2], P. Turcotte[1], F. Gagné[1], C. Gagnon[1] and C. Emond[3]

[1]Aquatic Contaminants Research Division, Environment and Climate Change Canada, 105 McGill, Montreal, QC H2Y 2E7; [2]Biotechnology Research Institute, National Research Council-Canada, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2; [3]Environmental Health and Occupational Health Department, Faculty of Medicine, University of Montreal, Canada.

ABSTRACT

The objective of this study was to compare the cytotoxicity of monomeric and aggregated cadmium telluride quantum dots (CdTe QD) in human hepatoma (HepG2) and rainbow trout hepatocytes (RTH). Hepatocytes were exposed to concentrations of monomeric CdTe QDs (4 nm diameter) and isolates of different size aggregates for 48 h. The results revealed that the added Cd concentration in the cell culture media increased with the additions of both the monomeric and aggregated QDs where most (72%) of the total Cd was between 100 and 450 nm diameter size range as determined by ultrafiltration. CdTe QDs were cytotoxic to both cell types with an estimated 48 h-EC50 of 3.6 and 7.3 mg/L Cd for monomeric CdTe QDs for the HEPG2 and trout hepatocytes respectively. For the aggregated QDs, analysis of the concentration-response slopes revealed that HepG2 cells were able to significantly discriminate between 2 size ranges: nanoparticles < 4.6 nm and aggregates between 4.6 and 450 nm with the < 4.6 nm group being more toxic than the latter. The RTH model discriminated between 3 distinct size ranges in decreasing order of toxicity: 6.8 nm and smaller > 6.9-50 nm > 50-450 nm. In all cases, the toxicity of QD aggregates decreased with increasing size of the aggregates.

KEYWORDS: cadmium telluride quantum dots, size distribution, size-related toxicity.

INTRODUCTION

Over the last decade, manufactured nanoparticles have gained more and more commercial attention [1, 2]. Nanoparticles are operationally defined as fine particles with at least one dimension between 1-100 nm. At this size range, these products display unique (quantum) properties compared to larger particles in the µm range [3]. Indeed, the high surface area to particle volume ratio provides new physico-chemical, electronic and reactive properties [4]. These properties offer considerable commercial interest in the area of optics, electronics, energy and medical applications [5, 6]. It is anticipated that the global market of nanoparticles will reach 54.2 billion US dollars even amid the COVID19 crisis in 2020 [7], hence a basis of concern towards human and environmental health. On the one hand, consumers could be exposed to the nanoparticles from pharmaceutical and personal care products, food packaging and clothes. On the other hand, the inadvertent release of these products in the environment is likely to contaminate the ecosystems through sewage [8, 9].

Nanoparticles with low surface charges tend to aggregate due to surface charge cancellation by salts and other organic ions in surface waters. Indeed, when charges at the surface of the nanoparticles are canceled by the presence of counterions in the water, aggregation is thermodynamically favored. The Zeta potential is a measure of surface charge density of nanoparticles

providing information on aggregation susceptibility: nanoparticles with low Zeta potential (< 50 mvolts) will aggregate even at low salt concentrations of freshwater [10]. Aggregation also depends not only on the Zeta potential of the nanoparticles but also on the surface water properties such as pH, ionic composition, and the composition and concentration of the organic carbon. The behavior of nanoparticles is therefore complex since it can form aggregates of various sizes. It is therefore expected that nanoparticles occur in their aggregated state in the environment. The toxicity of nanoparticle aggregates of different size is largely unknown although common sense suggests that toxicity is more significant with smaller particles. The toxicity of nanoparticles is associated to four basic properties of nanoparticles [11]: 1) the release of free ions from the backbone, 2) the size/form, 3) surface reactivity (coatings and surface charges) and 4) vectorization. For example, CdTe QDs (Zeta potential of -45 mvolts at 10 mM NaCl according to [12]) are composed of Cd and if free Cd ions are liberated from the QDs, this will contribute to toxicity [13, 14]. CdTe QDs are nano crystalline semi-conductors usually in the 2-25 nm diameter size range. The optical properties (fluorescence, luminescence) are stable and provide the basis of commercial application such as sun-powered batteries and diagnostic medical dyes [15, 16].

In the present study, thioglycolate-coated CdTe QDs were used giving a net negative charge at the surface of the nanoparticle. However, this coating binds to the surface of Cd QDs by electrostatic interaction only, which can be exchangeable with other counterions. Based on equal mass, smaller nanoparticles are more toxic than their equivalent higher size counterparts [17]. Lovrić *et al*. [18] showed that the PC12 and microglia cells exposed to different sizes of CdTe QDs responded differently, in part towards the size of the QDs, the smaller particle leading to greater toxicity. However, the toxic properties of nanoparticles with respect to aggregate size are not well understood presently, hence its relevance to examine in the environmental context given the likelihood of the aggregation process in field conditions [19]. It is hypothesized, by inference from comparison between dissolved Cd and Cd-based QD monomers, that larger aggregates would be less bioavailable to cells even though cells could ingest various particles by pinocytosis [20].

The objective of this study was therefore to evaluate the cytotoxic potential of CdTe QDs and to determine whether the size of aggregates could also influence toxicity towards human hepatoma cell line (HepG2) and primary cultures of rainbow trout hepatocytes. The null hypothesis is that the size of QD aggregates has no influence on cytotoxicity. The QDs were suspended in cell culture media containing salts at the range of salts in estuarine environments and the aggregates were isolated by ultrafiltration. An attempt was made to relate the intensity of toxicity responses of Cd from QDs and different size ranges of QD aggregates.

METHODS

Preparation and fractionation of CdTe-QD aggregates

Thioglycolate-coated QDs were purchased from American Dye Source (Québec, Canada). The manufacturer's reported particle size was 4 ± 1 nm with a characteristic green fluorescence at 485 nm excitation and 530 nm emission. The QDs were diluted 1/50 in MilliQ water, mixed by inversion for 30 min and passed through a 0.45 µm pore membrane (FHLC04700 0.45 µm, Millipore) to obtain a final concentration of 49 mg/mL as determined by inducted-coupled plasma (ICP) emission spectroscopy (ICP ES, Optima 5300 DV PerkinElmer). The filtrate (40 mL) was then passed through each of the 3 following membrane size filter devices (MF-Millipore, cellulose: 100, 50, 25 nm pore size). The filtrate was also passed through ultrafiltration membranes (cellulose acetate, Amicon 400, Millipore): 100 (6.8 nm), 30 (4.6 nm), 10 (3.2 nm) and 1 (1.5 nm) kDa pore size. The nitrogen pressure was set at 32 psi for all ultrafiltration membranes with the exception for the 1 kDa where the applied pressure was 64 psi. The total Cd fraction from each filtrate and materials retained on the ultrafiltration membranes was determined by ICP-emission spectrometry as described above.

Preparation and exposure of liver cells to QDs and filtrate fractions

Primary cultures of rainbow trout hepatocytes were prepared using the double perfusion

methodology with some modifications [21, 22]. Sexually immature *Oncorhynchus mykiss* trout (10-20 cm) were collected at a local hatchery (Aquamerik, St-Nicolas, Québec). The fish were maintained in 1000 L of aerated UV and charcoal-treated water at 15 ^{o}C under 16 h/8 h light and dark cycle and fed each day. Hepatocytes were prepared from N = 4 fish to limit any inter-individual variation. Fish were anesthetized in 100 mg/L Tricaine for 5 min and placed on ice. The abdominal cavity was opened and an incision was done on the hepatic artery. The liver was perfused with phosphate buffered saline (125 mM NaCl, 5 mM KH_2PO_4, 5 mM $NaHCO_3$, 1 mM glucose and 10 mM Hepes-NaOH, pH 7.4) containing 10 mM citrate and 0.5% albumin. The rinsed livers were removed, minced into small slices in the same PBS-citrate-albumin media (75 mL) and gently mixed for 30 min at room temperature. After this period, the liver suspension was gently passed through a 100 μm sieve, centrifuged at 125 × g for 5 min and resuspended in 5 mL PBS. Viable cells were counted on a hemocytometer in 0.004% trypan blue in PBS after 5 min, which was > 95% viability. The cells were then transferred in PBS media without albumin and citrate and kept at 4 ^{o}C until plating the same day.

Hepatocytes were plated (1 × 10^6 cells/mL) in sterile 24-well clear microplates for cell culture (Primaria, Becton Dickinson Labware, Oxnard, CA, USA) containing 1 mL of sterile L-15 media (containing antibiotics and mycotics) without serum. The cells were exposed to 20, 10, 5 and 2.5-fold dilutions of each CdTe QD fraction in the L15 media in triplicates and incubated at 15 ^{o}C for 48 h in a humidified incubator. The HepG2 cell line (HB-8065, American Type Culture Collection, Rockville, MD) was grown and maintained in RMPI media containing 10% fetal bovine serum and 2 mM glutamine at 37 ^{o}C with 5% CO_2. Cells were plated in 96-well microplates at a density of 270 000 cells per well (200 μL) in RPMI media with 3% serum. After a 24h pre-incubation step at 37 ^{o}C in 5% CO_2, cells were exposed to the various fractions of QDs (80, 40, 20 and 10 fold dilutions of each CdTe QDs fractions) for 48 h in RPMI media but with 3% of FBS in the presence of 10 μg/mL penicillin G and streptomycin. Cells were incubated for 48 h at 37 ^{o}C in the presence of 5% CO_2 atmosphere.

Cell viability assessment

For trout hepatocyte, cell viability was determined by the 5-carboxyfluorescein diacetate (acetoxymethyl ester) retention test as described elsewhere [22]. The relative amounts of retained fluorescein in viable cells were determined by fluorometry at 485 nm excitation and 520 nm emission (Chameleon-II, Bioscan, USA). Cell viability data was expressed as a percentage of viability relative to the control cells. For HepG2 cells, cell viability was determined using the neutral red uptake assay as described previously [23]. The exposure media were removed by aspiration and cells were resuspended in 100 μL PBS and the cell density measured at 600 nm. A volume of 20 μL of cell suspension was mixed with 180 μL of neutral red at 0.001% in PBS. After a 2 h incubation at 37 ^{o}C, the media was removed, washed in 100 μL PBS and cells attached at the bottom of wells were resuspended in 100 μL of 50% methanol containing 1% acetic acid. The absorbance at 540 nm was measured and the data was expressed as the absorbance (540 nm)/cell density. Cell viability was expressed as percentage relative to the untreated control viable cells.

Data analysis

The data were expressed as the effective Cd concentration that inhibits either neutral red or fluorescein in HepG2 and rainbow trout hepatocytes, respectively, by 50% (EC50). The EC50 values were calculated using the Regtox program (Excel compatible, version 6.3) and expressed in mg/L. This algorithm is based on the Hill equation and was developed by Vindimian *et al*. [24]. The IC50 values were estimated from 4 exposure concentration means from N = 3 replicates. The confidence interval was estimated using the boostrap non-parametric approach (500 simulations). Significant difference from controls were determined using analysis of variance (ANOVA) followed by the post hoc Tuckey HSD test. In the attempt to determine interactions between the size of the Cd particles and the exposure concentration, regression analysis was used to derive the slopes, which were compared using the homogeneity test for slopes (Statistica version 6). Significance was set at $\alpha = 0.05$.

RESULTS

Characterization of CdTe QDs

The size distribution of the nanoparticles differs greatly in water and the cell culture media (Figure 1). According to the manufacturer, the initial diameter of the QDs is 4 ± 1 nm. In distilled water, 17% of the total Cd concentration was found in the 3.2-4.6 nm size range and 37% was found in the 4.6-6.9 nm size range. About 2% of the total Cd concentration was found below 1.5 nm, which is considered the dissolved fraction of Cd (i.e., ionic Cd). Most of the Cd was found in the 4.6-6.9 nm size range while less than 34% of the Cd formed aggregates. When suspended in cell culture media, only 6% of the total Cd remained at 3.2-4.6 nm size range. The proportion of Cd in the truly dissolved fraction increased to 9% of the total Cd concentration relative to water suspensions. We found that 72% of total Cd was in the >100 nm size range and 63% of the total Cd was found at sizes > 450 nm. This suggests that QD aggregates were distributed over 2 orders of magnitude larger than the monomer.

Cytotoxicity of Cd QD monomers and aggregates

Cytotoxicity was investigated in human HepG2 cell lines (Figure 2) and in primary culture of rainbow trout hepatocytes (Figure 3) for each of the isolated size fractions of the QDs. In HepG2 cells, viability was decreased in a concentration-dependent manner of Cd in the 4.6-450 nm size range. No significant difference in toxicity was found for Cd at sizes < 4.6 nm at the (low) concentrations tested. In RTH, cell viability significantly dropped with the exposure concentration for size fractions of < 450 nm and < 100 nm. The EC50 data was calculated for all Cd size fractions (Table 1). Although the EC50 did not significantly differ based on the confidence intervals, the EC50 values were lower for Cd at < 4.6 nm. HepG2 was more sensitive (about 2 fold) than rainbow trout hepatocytes and this could be explained, in part at least, by the use of higher cell density of trout hepatocytes in the exposure media compared with HepG2.

To determine changes in toxicity, the slope of the concentration response curves was performed.

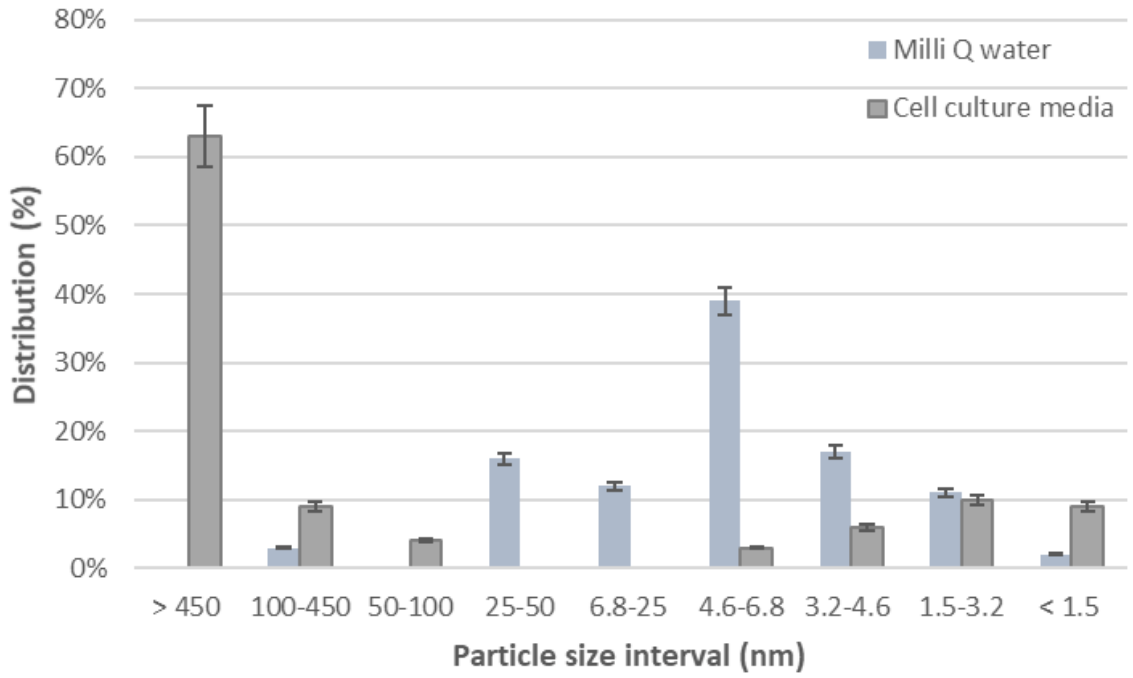
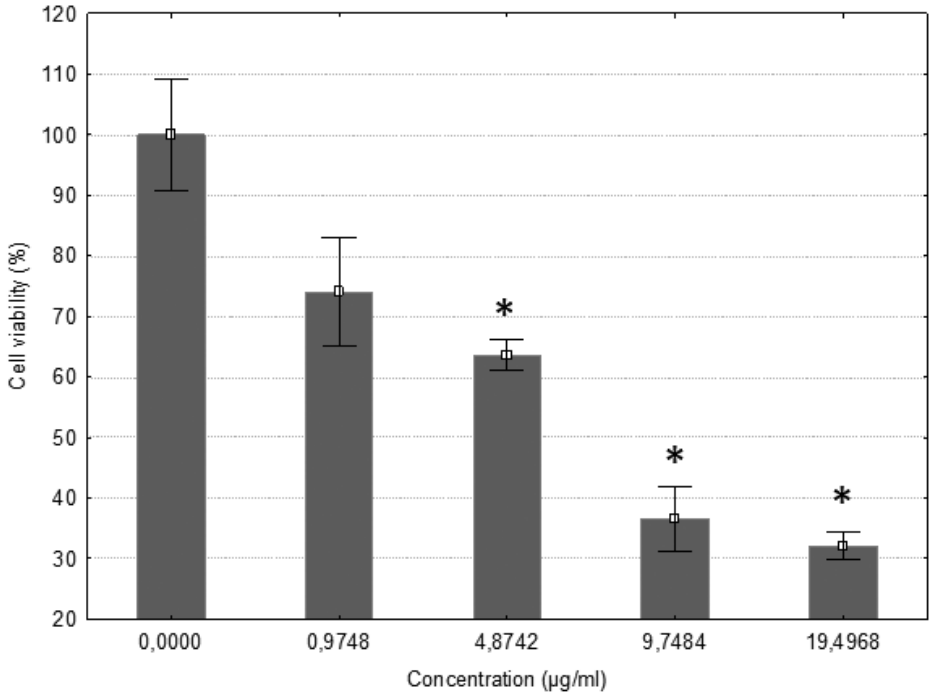
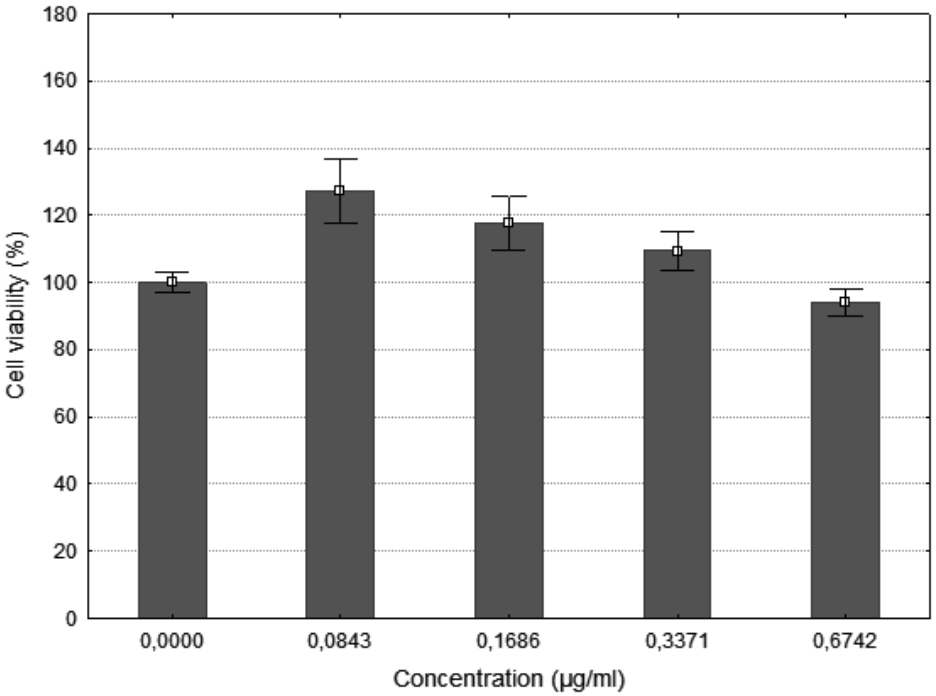


Figure 1. Particles size distribution of CdTe Qdot solution in Milli-Q water and in L-15 (Leibovitz) cell culture media. CdTe QDs were suspended in either MilliQ water or the cell culture media (L15) and passed through a series of filtration and ultrafiltration membranes. The data represent the mean with the standard deviation.

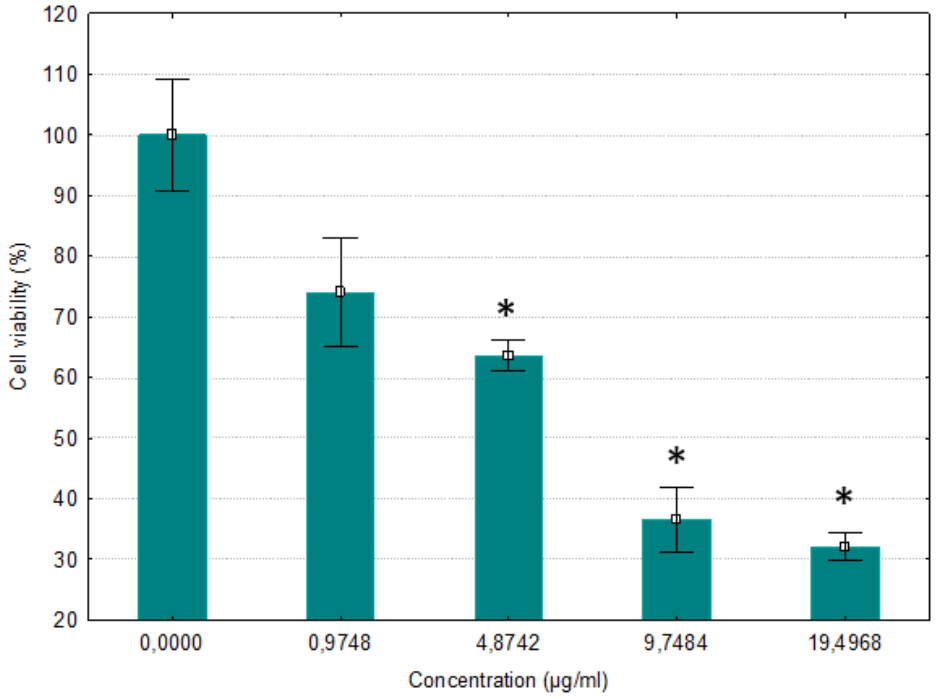


(A)

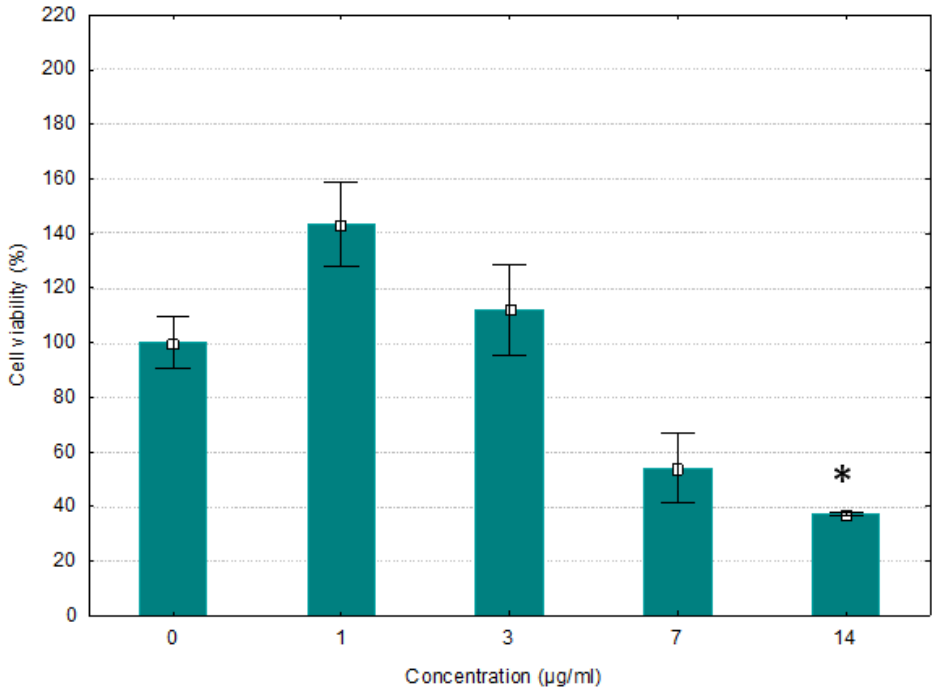


(B)

Figure 2. Representative concentration-response curve for Cd size fractions in HepG2 cells. The data for Cd at size ranges of < 450 nm (A) and < 3.2 nm (B) are shown. The data represent the mean with the standard error. The star symbol (*) indicates significance from the control group ($p < 0.05$).



(A)



(B)

Figure 3. Representative concentration-response curve for Cd size fractions in rainbow trout hepatocytes. The data for Cd at size ranges of < 450 nm (A) and < 6.8 nm (B) are shown. The data represent the mean with the standard error. The star symbol (*) indicates significance from the control group ($p < 0.05$).

Table 1. Toxic potential of isolated size fractions of CdTe QDs.

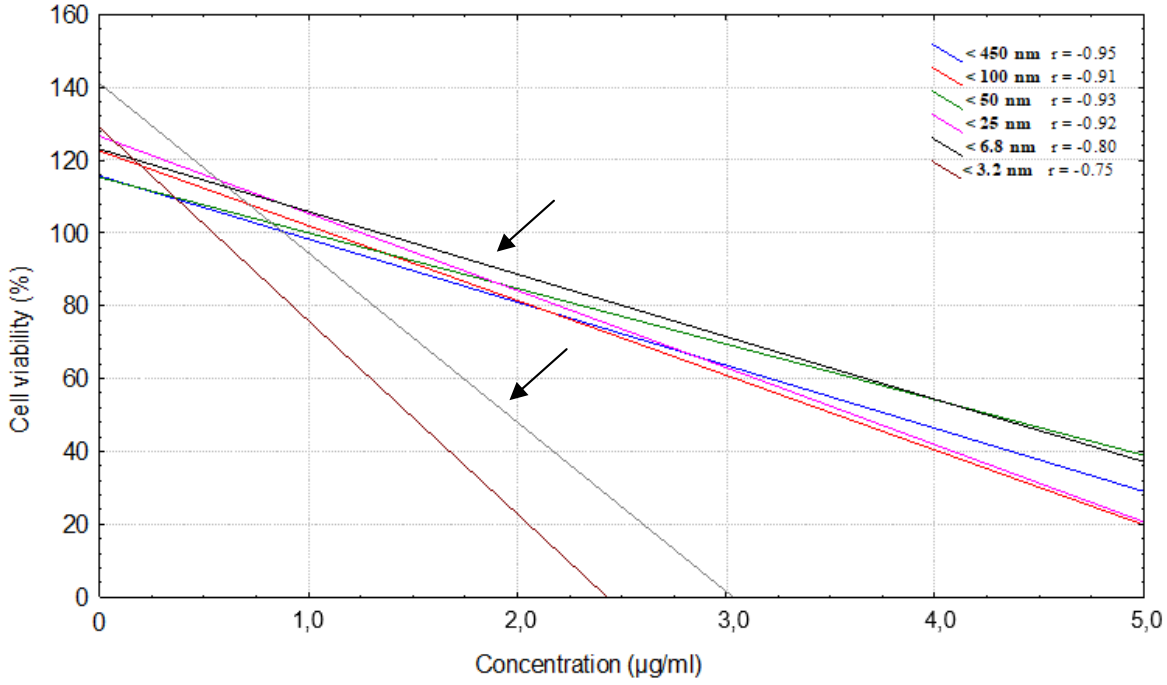
Fractions (diameter in nm)	HepG2	RTH
	EC_{50} (mg/L)	EC_{50} (mg/L)
< 450	3.4 (2.9 - 4)	6.6 (3.5 - 12.4)
< 100	2.8 (2.3 - 3.7)	14.2 (7.9 - 32)
< 50	4.1 (3.5 - 5.1)	19.9 (12 - 58)
< 25	3.4 (2.8 - 4.4)	> 15*
< 6.8	3.6 (3.1 - 4.8)	7.3 (4.6 - 10.7)
< 4.6	1.8 (1.4 - 6.2)	> 6*
< 3.2	1 (0.7 - 55)	> 2.7*
< 1.5	> 0.015*	> 0.5*
$CdSO_4$	Not determined	180 (104 - 338)

*Not toxic at the highest tested concentration.

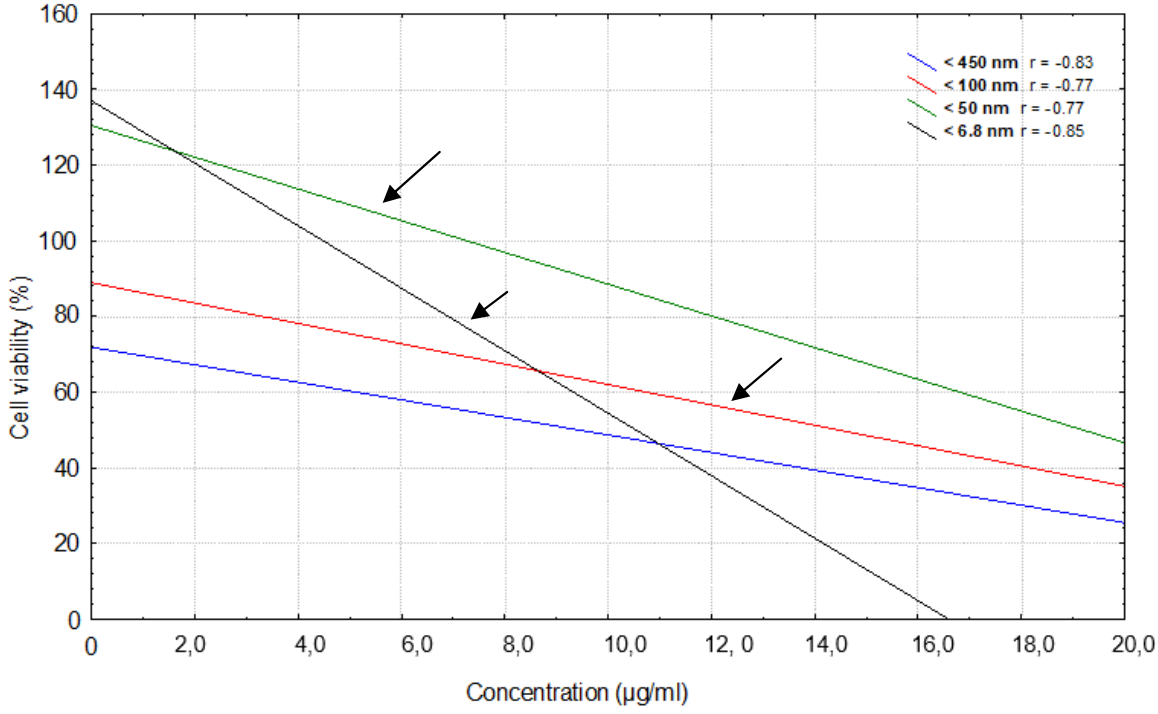
For HepG2 cells, a negative correlation was obtained between the Cd concentration in the exposure media and cell viability ($p < 0.01$) for all tested size fractions with the exception for Cd size < 1.5 nm. The slopes were not homogeneous (i.e., parallel) among the size fractions, suggesting different interactions between the size fractions and toxicity. The analysis of the slopes revealed two significantly distinct groups of toxic responses: Cd fraction size < 4.6 nm and particles between 4.6 and 450 nm (Figure 4A) where Cd from fractions < 4.6 nm was more toxic than Cd from the latter. For RTH, a negative correlation between Cd concentration of the different size fraction and cell viability was also obtained (Figure 4B). The slopes were not homogeneous suggesting again different interactions between the size fraction and toxic potential. Indeed, Cd toxicity could be separated by 3 size fractions in decreasing order of toxicity: 6.8 nm and smaller > between 6.9-50 nm > 50-450 nm.

DISCUSSION

This study sought to determine not only the cytotoxic potential of QD monomers but their aggregates where the null hypothesis was tested for invariance of size towards toxicity to human and fish hepatocytes. Nanoparticles spontaneously form aggregates in the environment when the surface charges are canceled by high inorganic and organic salts. The aggregated state of nanoparticles is an environmentally realistic form of inorganic nanoparticles. In this respect, the evaluation of the toxicity of nanoparticles in their aggregated form is warranted. The data obtained in the present study rejected the null hypothesis i.e., the size invariance of QD-based aggregates' toxicity. This supports the contention that the toxicity of nanoparticles is not only a function of the concentration but the size and form of nanoparticles/aggregates [3, 25, 26]. Aggregation will bring important changes on the surface properties (optical and electronic properties) and will change the nature of interaction at the surface of cells or tissues [27]. By using a combination of filtration and ultrafiltration techniques, the size distribution of CdTe QD particles and aggregates in the exposure media were obtained. Indeed, dissolution of monomeric QDs in the cell culture media led to important aggregation in 4.6-450 nm size range where 72% of the total Cd concentration were found at sizes between 100 and 450 nm compared to only 3% when diluted in deionised water. This was in the same range for thioglycolate-coated CdTe QDs when suspended in deionised water by dynamic light scattering analysis [26]. About 1% of the total Cd was found between 60 and 100 nm. When mixed in more



(A)



(B)

Figure 4. Concentration-response analysis of Cd size fractions of QDs suspensions. The data are shown for HepG2 cells (A) and rainbow trout hepatocytes (B). The arrow indicates a significant difference in the slopes between groups of slopes. Not all the slopes were shown for clarity purposes.

complex environments such as cell culture media, aggregation occurs, which was independent of cells in the medium [28]. In a previous study, CdTe QDs toxicity was partly attributed to the release of free Cd [13]. The release of free Cd, a very toxic form of Cd, from CdTe QDs could lead to various damages such as protein dysfunction, oxidative stress and genotoxicity [29]. Cd-induced oxidative stress was reduced but not completely by the addition of antioxidants such as N-acetylcysteine, bovine serum albumin, glutathione and cysteamine [13, 18, 30]. In another study, the binding of ionic Cd^{2+} to thiolate clusters in mitochondria led to the formation of reactive oxygen species capable of initiating cell death [2, 14]. Cd-based QDs were more toxic in the presence of oxidizing conditions and under UV radiation in rat hepatocytes, highlighting the production of reactive oxygen species at the surface of the nanoparticle [2]. In the present study, the formation of QD aggregates in cell culture media also involved dissolved Cd (< 1.5 nm). However, it is not known whether the release of Cd was involved during the aggregation process. In a previous study with the same CdTe QD preparation, a 48-h exposure led to a concentration-dependent release of free Cd in rainbow trout hepatocytes [25]. Although the release of free Cd ions is an important aspect of Cd-based QD toxicity, other factors such as aggregation size could also contribute to the observed toxicity [31].

In this study, the toxicity of Cd was higher at low molecular weights (< 4.6 nm) than Cd associated to larger sizes based on slope analysis of the concentration-effect relationships. This raises the question of the stability of different size aggregates during the exposure period and whether dissolved Cd was released more in smaller aggregates (high surface area ratio). Nevertheless, RTH showed low sensitivity to dissolved $CdSO_4$ associated with the high salt content of the L15 medium [32]. In future studies, measurement of the intracellular total and free Cd would be of value to better understand the toxicity of CdTe QDs and their aggregates.

At least two particle size groups with significantly distinct toxic responses were identified in Rainbow trout hepatocytes and HepG2 cells based on the slopes of the concentration-response data at the 4.6 nm threshold corresponding to the upper size limit of monomeric QDs. Particles larger than 4.6 nm were significantly less toxic than Cd found at <4.6 nm in size. For RTH, another size class of toxicity was found for particles > 6.8 nm and 50 nm which was less toxic than those at < 4.6 nm but more toxic than those at 50-450 nm size range. The comparative toxicity of CdTe QD aggregates and monomers were previously examined in leucocytes of trout, marine and freshwater mussels [33]. In this study, toxicity of dissolved (< 1.5 nm) Cd to trout leucocytes was not observed at concentrations up to 0.2 µg/mL, which was in agreement with the present study. In a previous study, the toxicity threshold was 35 µg/mL for dissolved Cd ($CdSO_4$) and 4.5 µg/mL for the unfitltred CdTe suspension suggesting that toxicity was greater for Cd-based nanoparticles compared to dissolved Cd ions [25]. If we take the EC50 value (7.3 µg/ml) of the most toxic QD fraction for RTH (< 6.8 nm) and assume that 9% of the total Cd is dissolved, the dissolved Cd in this fraction would then be 0.66 µg/mL, well lower than the 35 µg/mL toxicity threshold. This suggests that the observed toxicities were not mostly associated with the dissolved fraction (< 1.5 nm) but the nanoparticles/aggregates themselves. In situations where no liberation of dissolved Cd occurs, the size of nanoparticles can influence toxicity where the lower size nanoparticles are increasingly toxic [5, 34]. In a previous study, smaller green emitting CdTe QDs (2.2 nm) were found to be more toxic than larger red-emitting QDs (5.2 nm) [27] and this size-dependent toxicity seems to hold with aggregates as well based on the data in the present study. Moreover, the small QDs were found to reach the nuclei in cells while the larger QDs remained in the cytoplasm suggesting that the more toxic lower sized nanoparticles involve the nuclei. In another study with HepG2 cells, CdTe QDs of 2-4 nm diameter proved more toxic than 6 nm diameter size QDs although aggregation effects was not determined in this study [28]. The cellular uptake of CdSe/ZnS QDs was increased by smaller size QDs although slower than with Cd(II) and Se (IV) in HepG2 cells [35]. Moreover, there was evidence of release of free Cd ions since MT1A gene expression was increased at 100 nM

Cd equivalents of the QDs. The inverse relationship between the size of the nanoparticles and toxicity of CdTe QDs were also shown with CdTe QDs in *Escherichia coli* [36]. From the environmental protection perspective, nanoparticles with low Zeta potential are likely to be found in aggregates in surface waters [37] and toxicity could result depending on the size distribution of aggregates where the monomeric QDs would be the most toxic form. In this respect, aggregation mechanisms are likely to reduce the toxicity of monomeric QDs in freshwater. The natural organic matter in surface waters was shown to interact with some nanoparticles and in some cases increase the stability of the monomers which could influence the toxicity of metallic nanoparticles [38]. This complicates risk assessment of CdTe QDs and perhaps other metal-based nanoparticles since the influence of external factors such as salt, pH and the presence and type of organic matter could influence the aggregation dynamics and therefore the toxic outcomes.

CONCLUSIONS

The addition of CdTe QDs in cell culture media readily formed aggregates from > 4.6 up to 450 nm size ranges. Large aggregates (> 100 nm) represented the bulk of Cd concentration in the exposure media representing in the order of 70% of the total Cd concentration. The large aggregates were the least toxic form of Cd compared to monomeric Cd QDs. Nevertheless, all isolated fractions were cytotoxic to both rainbow trout hepatocytes and HepG2 cells in a concentration-dependent manner and the cytotoxicity of CdTe QDs was inversely related to the size of the nanoparticles and aggregates. Environmental factors that favor small aggregates or maintain the monomer could increase the toxic risk of nanoparticles.

ACKNOWLEDGMENT

This work was funded by the Chemical Management Plan of Environment and Climate Change Canada.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

1. Isigonis, P., Afantitis, A., Antunes, D., Bartonova, A., Beitollahi, A., Bohmer, N., Bouman, E., Chaudhry, Q., Cimpan, M. R., Cimpan, E., Doak, S., Dupin, D., Fedrigo, D., Fessard, V., Gromelski, M., Gutleb, A. C., Halappanavar, S., Hoet, P., Jeliazkova, N., Jomini, S., Lindner, S., Linkov, I., Longhin, E. M., Lynch, I., Malsch, I., Marcomini, A., Mariussen, E., de la Fuente, J. M., Melagraki, G., Murphy, F., Neaves, M., Packroff, R., Pfuhler, S., Puzyn, T., Rahman, Q., Pran, E. R., Semenzin, E., Serchi, T., Steinbach, C., Trump, B., Vrček, I. V., Warheit, D., Wiesner, M. R., Willighagen, E. and Dusinska, M. 2020, Small Weinh. Bergstr. Ger., 16(36), e2003303.
2. Derfus, A. M., Chan, W. C. W. and Bhatia, S. N. 2004, Nano Lett., 4(1), 11-18.
3. Murdock, R. C., Braydich-Stolle, L., Schrand, A. M., Schlager, J. J. and Hussain, S. M. 2008, Toxicol. Sci. Off. J. Soc. Toxicol., 101(2), 239-253.
4. Oberdorster, G. 2000, Philos. Trans. Math. Phys. Eng. Sci., 358(1775), 2719-2740.
5. Oberdörster, G., Oberdörster, E. and Oberdörster, J. 2005, Environ. Health Perspect., 113(7), 823-839.
6. Maysinger, D. 2007, Org. Biomol. Chem., 5(15), 2335-2342.
7. Globe Newswire. 2020.
8. Boxall, A. B. A., Tiede, K. and Chaudhry, Q. 2007, Nanomed., 2(6), 919-927.
9. Reijnders, L. 2008, J. Hazard. Mater., 152(1), 440-445.
10. Handy, R. D., von der Kammer, F., Lead, J. R., Hassellöv, M., Owen, R. and Crane, M. 2008, Ecotoxicol. Lond. Engl., 17(4), 287-314.
11. Gagné, F., Gagnon, C. and Blaise, C. 2007, Curr. Top. Toxicol., 4, 51-64.
12. Keane, P. M., Gallagher, S. A., Magno, L. M., Leising, M. J., Clark, I. P., Greetham, G. M., Towrie, M., Gun'ko, Y. K., Kelly, J. M. and Quinn, S. J. 2012, Dalton Trans., 41(42), 13159-13166.
13. Cho, S. J., Maysinger, D., Jain, M., Röder, B., Hackbarth, S. and Winnik, F. M. 2007, Langmuir ACS J. Surf. Colloids, 23(4), 1974-1980.

14. Lewinski, N., Colvin, V. and Drezek, R. 2008, Small Weinh. Bergstr. Ger., 4(1), 26-49
15. Clift, M. J. D., Rothen-Rutishauser, B., Brown, D. M., Duffin, R., Donaldson, K., Proudfoot, L., Guy, K. and Stone, V. 2008, Toxicol. Appl. Pharmacol., 232(3), 418-427.
16. Hardman, R. 2006, Environ. Health Perspect., 114(2), 165-172.
17. Ostiguy, C., Soucy, B., Lapointe, G., Woods, C., Menard, L. and Trottier, M. 2008, Les effets à la santé reliés aux nanoparticules. Montréal, Que.: Institut de recherche Robert-Sauvé en santé et en sécurité to travail du Québec. IRSST (Québec) Ed.
18. Lovrić, J., Bazzi, H. S., Cuie, Y., Fortin, G. R. A., Winnik, F. M. and Maysinger, D. 2005, J. Mol. Med. Berl. Ger., 83(5), 377-385.
19. Bundschuh, M., Filser, J., Lüderwald, S., McKee, M. S., Metreveli, G., Schaumann, G. E., Schulz, R. and Wagner, S. 2018, Environ. Sci. Eur., 30(1), 6.
20. AFSSET. 2006, Les Nanomatériaux: Effets sur la santé de l'homme et sur l'environnement. Agence française de sécurité sanitaire de l'environnement et du travail, Juillet 2006, Paris, 248 p.
21. Klaunig, J. E., Ruch, R. J. and Goldblatt, P. J. 1985, Vitro Cell. Dev. Biol. J. Tissue Cult. Assoc., 21(4), 221-228.
22. Gagné, F. 2005, In Small-scale Freshwater Toxicity Investigations: Toxicity Test Methods, C. Blaise and J-F. Férard (Ed.), pp. 453-472. Dordrecht: Springer Netherlands.
23. Borenfreund, E. and Puerner, J. A. 1985, J. Tissue Cult. Methods, 9(1), 7-9.
24. Vindimian, E., Robaut, C. and Fillion, G. 1983, J. Appl. Biochem., 5(4-5), 261-268.
25. Gagné, F., Auclair, J. and Blaise, C. 2008, Curr. Top. Toxicol., 5, 19-30.
26. Zhang, Y., Chen, Y., Westerhoff, P. and Crittenden, J. C. 2008, Environ. Sci. Technol., 42(1), 321-325.
27. Jones, C. and Grainger, D. W. 2009, Adv. Drug Deliv. Rev., 61(6), 438-456.
28. Zhang, Y., Chen, W., Zhang, J., Liu, J., Chen, G. and Pope, C. 2007, J. Nanosci. Nanotechnol., 7(2), 497-503.
29. Gagné, F., Auclair, J., Turcotte, P., Fournier, M., Gagnon, C., Sauvé, S. and Blaise, C. 2008, Aquat. Toxicol. Amst. Neth., 86(3), 333-340.
30. Peyrot, C., Gagnon, C., Gagné, F., Willkinson, K. J., Turcotte, P. and Sauvé, S. 2009, Comp. Biochem. Physiol. - C Toxicol. Pharmacol., 150(2), 246-251.
31. Nagy, A., Steinbrück, A., Gao, J., Doggett, N., Hollingsworth, J. A. and Iyer, R. 2012, ACS Nano, 6(6), 4748-4762.
32. Ibrahim, M., Oldham, D. and Minghetti, M. 2020, Comp. Biochem. Physiol. Part C Toxicol. Pharmacol., 236, 108816.
33. Bruneau, A., Fortier, M., Gagne, F., Gagnon, C., Turcotte, P., Tayabali, A., Davis, T. L., Auffret, M. and Fournier, M. 2013, Environ. Sci. Process. Impacts, 15(3), 596-607.
34. Wang, L., Nagesha, D. K., Selvarasah, S., Dokmeci, M. R. and Carrier, R. L. 2008, J. Nanobiotechnology, 6, 11.
35. Peng, L., He, M., Chen, B., Wu, Q., Zhang, Z., Pang, D., Zhu, Y. and Hu, B. 2013, Biomaterials, 34(37), 9545-9558.
36. Fang, T-T., Li, X., Wang, Q-S., Zhang, Z-J., Liu, P. and Zhang, C-C. 2012, Toxicol. Vitro Int. J. Publ. Assoc. BIBRA, 26(7), 1233-1239.
37. Moore, M. N. 2006, Environ. Int., 32(8), 967-976.
38. Grillo, R., Rosa, A. H. and Fraceto, L. F. 2015, Chemosphere, 119, 608-619.