Biodistribution and Systemic Effects in Mice Following Intravenous Administration of Cadmium Telluride Quantum Dot Nanoparticles

Kathy C. Nguyen,*†∥ Yan Zhang, ‡ Julie Todd,‡ Kevin Kittle,‡ Dominique Patry,‡ Don Caldwell,‡ Michelle Lalande,§ Scott Smith,‡ Douglas Parks,‡ Martha Navarro,§ Andrey Massarsky,§ Thomas W. Moon,*†∥ William G. Willmore,*∥ and Azam F. Tayabali,*†∥

†Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, 50 Colombine Driveway, Ottawa, Ontario, Canada K1A 0K9
‡Bureau of Chemical Safety, Health Products and Food Branch, 251 Sir Frederick Banting Driveway, Health Canada, Ottawa, Ontario, Canada K1A 0K9
§University of Ottawa, Department of Biology, Centre for Advanced Research in Environmental Genomics and the Collaborative Program in Chemical and Environmental Toxicology, 75 Laurier Avenue East, Ottawa, Ontario, Canada K1N 6N5
∥Department of Biology and Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario Canada, K1S 5B6

ABSTRACT: Quantum dots (QDs) are engineered nanoparticles (NPs) of semiconductor structure that possess unique optical and electronic properties and are widely used in biomedical applications; however, their risks are not entirely understood. This study investigated the tissue distribution and toxic effects of cadmium telluride quantum dots (CdTe-QDs) in male BALB/c mice for up to 1 week after single-dose intravenous injections. CdTe-QDs were detected in the blood, lung, heart, liver, spleen, kidney, testis and brain. Most CdTe-QDs accumulated in the liver, followed by the spleen and kidney. At high doses, exposure to CdTe-QDs resulted in mild dehydration, lethargy, ruffled fur, hunched posture, and body weight loss. Histological analysis of the tissues, upon highest dose exposures, revealed hepatic hemorrhage and necrotic areas in the spleen. The sera of mice treated with high doses of CdTe-QDs showed significant increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin levels, as well as a reduction in albumin. CdTe-QD exposure also led to a reduced number of platelets and elevated total white blood cell counts, including monocytes and neutrophils, serum amyloid A, and several pro-inflammatory cytokines. These results demonstrated that the liver is the main target of CdTe-QDs and that exposure to CdTe-QDs leads to hepatic and splenic injury, as well as systemic effects, in mice. By contrast, cadmium chloride (CdCl₂), at an equivalent concentration of cadmium, appeared to have a different pharmacokinetic pattern from that of CdTe-QDs, having minimal effects on the aforementioned parameters, suggesting that cadmium alone cannot fully explain the toxicity of CdTe-QDs.

1. INTRODUCTION
Nanomaterials (NMs) possess novel properties that have led to their widespread use in biotechnology applications and consumer products. The heightened production and use of NMs will result in greater exposure to consumers and workers. Therefore, the hazards associated with NMs need to be assessed. Quantum dots (QDs) are a class of engineered nanoparticles (NPs) that range from 2 to 100 nm in diameter.¹ Owing to their unique optical and electronic properties, including broad absorption and narrow emission, QDs are widely used in electronic and medical applications.²,³ These NPs are also used in different technologies, including solar cells, light emitting devices, quantum computing, and security inks. In addition, QDs hold great promise as an important tool in medical imaging, cancer detection, and targeted drug
behavior and to measure the toxicity of QDs and systemic effects in various tissues to determine the principal tissue targets. Several studies have reported toxicity of cadmium QDs in mice and rats including altered locomotor activity, oxidative stress, and genotoxicity, reports of QD toxicity in rodents are inconclusive. A few studies reported toxicity of cadmium QDs in mice and rats including altered locomotor activity, pulmonary vascular thrombosis leading to death, and injuries to the liver, spleen, and kidneys. Furthermore, Wang and colleagues demonstrated that the liver and kidney toxicity of cadmium QDs was time-dependent and reversible. By contrast, several studies reported no observable toxicity induced by cadmium QDs in rodents or in primates. This discrepancy in mammalian toxicity of cadmium QDs may be explained by the variation in particle size, coating, dose, mode of administration, and other exposure conditions that were used in the studies. Consequently, despite the effort by many research groups to characterize the behavior and to measure the toxicity of QDs in vivo, little can be concluded about the biological effects of these NPs in relevant animal models or during human exposure.

This study aimed to investigate the biodistribution of CdTe-QDs and their toxic effects in BALB/c mice after intravenous administration. The presence of CdTe-QDs was monitored in various tissues to determine the principal tissue targets. Several toxicological endpoints were employed to investigate tissue and systemic effects. Cadmium chloride (CdCl₂) was also used to compare the distribution and effects of cadmium to those of CdTe-QDs. The data provide important information on the behavior of CdTe-QDs within an animal model and their potential health effects, which should be helpful to assess the human health risk of these NPs.

2. MATERIALS AND METHODS

2.1. Materials. The stock of CdTe-QDs used in this study was purchased from Nano Impex Canada (Mississauga, ON) and provided at a concentration of 5 mg/mL in water containing 20% (w/w) of cadmium. These CdTe-QDs were mercaptobenzonionic acid (MBA) capped CdTe-QDs with a size of 5 nm and a spectral emission of 540 nm, as described by the manufacturer. CdCl₂, hematoxylin, eosin, and Giemsa were obtained from Sigma-Aldrich (St. Louis, MO). Biochemical kits, including alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, total bilirubin, and creatinine, were purchased from Fisher Scientific (Ottawa, ON). Serum amyloid A (SAA) kit was obtained from Life Technologies (Carlsbad, CA). Bio-Plex cytokine kits and reagents were purchased from Bio-Rad Laboratories (Hercules, CA).

2.2. Characterization of CdTe-QDs. In this study, transmission electron microscopy (TEM), dynamic light scattering (DLS), and spectrophotometry were used to characterize CdTe-QDs. For TEM and DLS, the characterizing methods were previously described in Nguyen et al. Characterization of CdTe-QDs using spectrophotometry involved measuring fluorescence spectra of CdTe-QDs at 340 nm excitation and emission wavelengths from 450 to 650 nm. The methods employed 100 μL (125 μg/mL) of CdTe-QD solutions placed in a 96-well opaque plate and scanned with a SPECTRAMax GEMINI XS microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). A standard curve for QDs was obtained by plotting fluorescence intensities (measured at 550 nm) against concentrations, using three replicates of serial dilutions ranging from 0 to 250 μg/mL.

2.3. Animal Treatments and Sample Collection. All procedures involving animals in this study were approved by the Health Canada Animal Care Committee and adhere to the published guidelines of the Canadian Council on Animal Care. The mouse model used in this study was BALB/c. The mice (male, ∼25 g or ∼8 weeks) were obtained from Charles River Laboratories Inc. (Saint-Constant, QC). A total of 96 mice were used in the entire study. The mice were housed in cages under a 12-h light/dark cycle in a controlled temperature (20 ± 2 °C) and relative humidity (50 ± 20%) environment, given access to food and water ad libitum, and allowed to acclimatize for 1 week before treatments.

CdTe-QDs or CdCl₂ was administered into mice via intravenous injection into the tail vein. Physiological saline was used as the vehicle for the study and for diluting the stocks of CdTe-QDs and CdCl₂. Eight mice were used for each control and treatment group. Mice were regularly monitored for physical activity, behavior, and body weight right after injection until necropsies. Blood and tissues, including lung, heart, liver, spleen, kidney, brain, and testis, were collected at necropsy. The study was divided into two sets of experiments as described in Figure 1.

In the first set of experiments, the dose effects of CdTe-QDs were investigated by treating mice by single injections with a range of CdTe-QD doses (0.4, 2, 5, 6, 7, and 10 mg/kg body weight, bw) and examining the effects 24 h later. The threshold dose, the dose at which there were no observable changes in mouse behavior and physical activity caused by CdTe-QD exposure to mice from the first set of experiments, was determined and used in the second set of experiments. CdCl₂ was also used in this first set of experiments to compare cadmium effects to those of CdTe-QDs. A concentration of 1.95 mg/kg bw of CdCl₂ was used to treat the mice. This concentration of CdCl₂ contains 1.2 mg/kg bw (61.3%) of cadmium, which should be helpful to assess the human health risk of these NPs.
which is equivalent to the cadmium concentration in 6 mg/kg bw of CdTe-QDs since these NPs contain 20% of cadmium (Figure 1).

In the second set of experiments, the threshold dose of CdTe-QDs (5 mg/kg bw, determined in the first set of experiments) was used to treat the mice. Different postinjection sampling times of 2 h, 24 h, 3 days (d), and 1 week (wk) were employed to examine the progression of effects and the recovery of the mice (Figure 1).

2.4. Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Detection and quantification of cadmium from CdTe-QDs and CdCl₂ in the blood and tissues were carried out using ICP-MS. Multielement standard and multi-internal standard were obtained from Agilent (Santa Clara, CA). Nitric acid for trace metal analysis was purchased from Sigma-Aldrich (St. Louis, MO). Standards and reagents were prepared using Milli-Q (Millipore, Etobicoke, ON) water. Samples were digested following the DigiPrep application for tissue digestion adapted from USEPA methods 200.2 revision 2.8. Briefly, blood and tissue samples were digested with 2 mL of 10.3 M HNO₃ for 60 min at 95 °C, utilizing a digestion system (DigiPREP MS, SCP Science, France). Samples were brought to a final volume of 5 mL with Milli-Q water. For QA/QC, blanks and certified reference material were used. Samples were then analyzed using an Agilent 7700x ICP-MS.

2.5. Spectrofluorometry and Confocal Microscopy. The presence of CdTe-QDs in the blood and tissues was analyzed using spectrofluorometry and confocal microscopy. CdTe-QDs in blood were assessed using whole blood samples from the control and treated mice smeared on microscope slides, dried, and examined with a Nikon TE2000 microscope attached to a C1 confocal unit (Nikon Canada Inc., Mississauga, ON). Other tissues (100 mg of each tissue) were homogenized in 500 μL water, and the homogenates were then diluted and measured for fluorescence intensity (FI) using CdTe-QD excitation and emission wavelengths (340 and 550 nm, respectively), in a SPECTRAmax GEMINI XS microplate spectrofluorometer.

Another separate set of tissues was sectioned and examined with a confocal microscope (Nikon Canada Inc., Mississauga, ON) for evaluation of tissue distribution of CdTe-QDs.

2.6. Pathology/Histology. Small tissue pieces (approximately 3 mm³ in size) were fixed with 10% formaldehyde, embedded in paraffin, sectioned into 5 μm thick sections, and mounted on glass microscope slides using standard histopathological techniques. The tissue sections were stained with Hemotoxylin and Eosin (H&E) and examined with a light microscope. Blood smears were stained with Wright’s stain and also examined with a light microscope.

2.7. Biochemical Tests. Liver function biomarkers, including serum albumin, ALT, AST, total bilirubin, albumin, and kidney function biomarker, creatinine, were measured using a Clinical Chemistry benchtop analyzer (Horiba Medical, Irvine, CA). Assays were conducted as described by the manufacturer.

2.8. Haematology. Following anesthesia with isoflurane, 500–1000 μL of blood was immediately collected by cardiac puncture and transferred to tubes containing ethylenediaminetetraacetic acid (EDTA). Blood was analyzed for hematological parameters using an automated hematology analyzer (Sysmex XT-2100, Kobe, Japan) as described by the manufacturer. Parameters examined included WBC, RBC, platelet, and hemoglobin indices, as well as leukocyte differentials.

2.9. Serum Amyloid A (SAA) Assay. SAA levels were determined using a mouse SAA ELISA kit (Life Technologies, Carlsbad, CA). The assay was performed according to the manufacturer’s instructions. Briefly, serum samples were diluted 1:200 in the provided sample diluent buffer. To each well of a 96-well plate precoated with SAA antibody, 50 μL of anti SAA/HRP conjugate was added followed by 50 μL of standard or sample. After a 1 h incubation at RT, the plate was washed four times with kit washing buffer. After washing, 100 μL of TMB substrate was added to each well and the plate was incubated for 15 min. Stop solution (100
Table 1. Cadmium Concentrations (μg/g tissue) in the Blood and Tissues as Detected by ICP-MS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control 2 h</th>
<th>24 h</th>
<th>3 d</th>
<th>1 w</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLOOD</td>
<td>0.0 ± 0.0</td>
<td>4.0 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>LUNG</td>
<td>0.0 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>HEART</td>
<td>0.0 ± 0.0</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>LIVER</td>
<td>0.0 ± 0.0</td>
<td>6.5 ± 0.2</td>
<td>9.3 ± 0.8</td>
<td>14.3 ± 0.9</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>0.0 ± 0.0</td>
<td>2.6 ± 0.2</td>
<td>3.2 ± 0.3</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.0 ± 0.0</td>
<td>1.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>BRAIN</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>TESTIS</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0/0</td>
<td>0.6 ± 0.0</td>
</tr>
</tbody>
</table>

*Mice were treated with a single CdTe-QD dose of 5 mg/kg bw, and cadmium was measured in various tissues at different time points after exposure (n = 8).

3. RESULTS

3.1. Characterization of CdTe-QDs. Size characterization using TEM showed that the average size of the CdTe-QDs when diluted in water was 15.25 ± 0.34 nm (Figure 2A). Agglomeration of test CdTe-QDs was demonstrated with TEM examination. DLS analysis indicated average sizes of CdTe-QDs were 3.96 ± 0.34 nm (Figure 2A). Dilution of known concentrations of CdTe-QDs were used to create standard curves of the NPs by plotting fluorescence intensities (measured at 550 nm) against concentrations. The results showed that the standard curves of CdTe-QDs in water and in saline were linear with \( R^2 = 0.994 \) in water and \( R^2 = 0.992 \) in saline, respectively (Figure 2C).

3.2. Detection of Cadmium in Blood and Tissues Using ICP-MS. The biodistribution of the IV-injected CdTe-QDs in blood and tissues of BALB/c mice was examined using ICP-MS (Table 1). At 2 h, the level of cadmium was the greatest in the liver, but blood, spleen, and kidney also had substantially higher cadmium levels than other tissues. At 24 h with an administered dose of 5 mg/kg bw, ICP-MS results showed that CdTe-QDs distributed to all organs examined, including the lung, heart, liver, spleen, kidney, brain, and testis (Table 1). From 2 h to 1 w of exposure, the levels of cadmium decreased over time in the blood and heart while they increased in the liver, spleen, kidney, and testis. The levels of cadmium in the liver, spleen, and kidney reached the highest levels at 3 d and remained at similar levels at 1 w, while those in the lung peaked at 24 h postinjection and remained approximately the same at 3 d and 1 w (Table 1). Accumulation of CdTe-QDs appeared greatest in the liver over time after exposure (Table 1).

A comparison of the distribution of CdTe-QDs and CdCl₂ in tissues was made using 6 mg/kg bw of CdTe-QDs and 1.95 mg/kg bw of CdCl₂, which contain equivalent cadmium concentrations. Cadmium was measured at 24 h postexposure using ICP-MS (Table 2). The cadmium level from CdCl₂ was very low in the blood at 24 h compared to that of CdTe-QDs, which was substantial. Cadmium from CdCl₂ appeared greatest in the liver and kidney but was low in other tissues. Although equivalent cadmium concentrations were used for both CdTe-QDs and CdCl₂, the levels of cadmium from CdCl₂ appeared significantly lower than those from CdTe-QDs in all tissues (Table 2).

3.3. Effects of CdTe-QDs on Behavior and Physical Appearance of Mice. At 24 h, the high doses (6–10 mg/kg bw) of CdTe-QD caused the general malaise of the mice. The treated mice appeared slow in activity and showed signs of distress including mild irregular respiration, mild dehydration, lethargy, ruffled fur, and hunched posture. Treatment with 10 mg/kg bw CdTe-QDs also led to an approximate 10% body weight loss (data not shown). Lower CdTe-QD doses (0.4–5 mg/kg bw) and CdCl₂ did not cause changes in apparent animal physical activity or appearance.

3.4. Detection of CdTe-QDs in Blood and Tissues. Confocal microscopy and spectrofluorometry were used to confirm the presence of CdTe-QDs in the blood and body tissues. The blood and tissues showed high background fluorescence intensities when measured by spectrofluorometry and confocal microscopy due to tissue autofluorescence.
However, at the same tissue concentrations, the homogenates of the lung, heart, liver, spleen, kidney, brain, and testis from CdTe-QD-treated mice showed significantly greater fluorescence intensity than the control homogenates (Figure 3).

Confocal microscopy of blood and tissue sections showed fluorescent bright green structures that were not observed in the control tissues, suggesting the presence of CdTe-QDs in the blood, lung, liver, spleen, and kidney (Figure 4A, B, C, D, and E) but not in the heart, brain, or testis (Figure 4C, G, and H).

3.5. Effects of CdTe-QDs on Tissue Morphology. The necropsies revealed visible macroscopic changes in the liver and spleen of CdTe-QD-treated mice. At a dose of 6 mg/kg bw and higher, these organs appeared dark red in color compared to those from the control (data not shown). Hematoxylin and eosin (H&E) stained sections from these tissues showed areas of hemorrhage and necrosis (Figure 5 and 6). Specifically, treatments of CdTe-QDs at 6 mg/kg bw and higher induced liver hemorrhage that was observed in the periportal areas of the hepatic lobules accompanied by apoptotic and necrotic liver cells in those areas (Figure 5B, C, F, and G). Similarly, bleeding and disruption to the normal structure of marginal regions in the spleen were observed with CdTe-QD treatments at 6 mg/kg bw and higher (Figure 6B, C, F, and G). There were no changes in the appearance and morphology of the lung, heart, kidney, brain, and testis (data not shown). Mice treated with CdCl₂ at an equivalent cadmium concentration to 6 mg/kg bw CdTe-QDs demonstrated none of these macroscopic or histological alterations in either the liver or spleen (Figure 5D and H; Figure 6D and H) or in any of the other tissues examined (data not shown).

3.6. Changes in Biochemical Parameters. Biochemical tests of serum samples 24 h after exposure showed that high CdTe-QD doses induced decreases in albumin level and increases in ALT, AST, and total bilirubin levels (Figure 7A, B, C, and D). However, CdTe-QDs did not affect creatinine levels (Figure 7E). Treatment with CdCl₂ (containing an
equivalent concentration of cadmium to 6 mg/kg bw) did not affect these biochemical biomarkers (Figure 7A, B, C, and D).

3.7. Changes in Hematology. Haematology analysis showed that CdTe-QD injection at 24 h resulted in an increased number of white blood cells (WBC) at the highest dose (10 mg/kg bw) (Figure 8A). However, the increased percentages of blood monocytes and neutrophils were observed at 5 mg/kg bw and above (Figure 8B and C). At 24 h postinjection, CdTe-QDs also reduced the numbers of platelets at 5 mg/kg bw (25%), 6 mg/kg bw (60%), 7 mg/kg bw (80%), and 10 mg/kg bw (100%) (Figure 8D). However, in mice treated with 5 mg/kg bw, the platelet numbers appeared to recover to the levels that were comparable to those of the control at 3 d and 1 w (Figure 8E). Blood smears also revealed an increased number of reticulocytes (immature red blood cells) in the blood of mice treated with 10 mg/kg bw CdTe-QDs (Figure 8F). CdCl₂ also caused an increase in the percentage of monocytes but did not result in any observable

Figure 5. Tissue histological images stained with H&E obtained from livers of mice treated with saline (A and E), 6 mg/kg bw CdTe-QDs (B and F), 10 mg/kg bw CdTe-QDs (C and G), and 1.95 mg/kg bw CdCl₂ (D and H). The H&E stained sections were examined with light microscopy at magnification of 10× (A, B, C, and D) and 60× (E, F, G, and H). The arrows indicate the areas of hemorrhage and apoptotic and necrotic events.

Figure 6. Tissue histological images stained with H&E, obtained from the spleen of mice treated with saline (D and E), 6 mg/kg bw CdTe-QDs (B and F), 10 mg/kg bw CdTe-QDs (C and G), and 1.95 mg/kg bw CdCl₂ (D and H). The H&E stained sections were examined with light microscopy at magnification of 20× (A, B, C, and D) and 40× (E, F, G, and H). The arrows indicate the areas of hemorrhage and necrotic events.
changes in other hematology parameters tested (Figure 8A and B).

3.8. Pro-inflammation and Acute Phase Responses.
CdTe-QD treatments led to changes in the level of the acute phase response protein, SAA. At 5 mg/kg bw and higher, CdTe-QDs significantly increased serum SAA levels (Figure 9A and B). Treatment of CdCl2 also resulted in significantly elevated SAA levels (Figure 9A). However, the CdCl2 effect was much less than that of 6 mg/kg bw CdTe-QDs that contain an equivalent cadmium concentration (Figure 9A).

In addition, both CdTe-QDs and CdCl2 altered the levels of several serum pro-inflammatory cytokines. Specifically, starting at 0.4 mg/kg bw, CdTe-QDs caused significantly increased IL-6 levels. Elevations of KC (keratinocyte chemoattractant), IL-12 (p70), and TNF-α levels were observed with treatments of CdTe-QDs at 5 mg/kg bw and higher (Figure 10A, B, C, and D). However, significant reductions in IL-12 (p70) and TNF-α occurred at the highest doses of 7 and 10 mg/kg bw (Figure 10B). Treatment with CdCl2 also resulted in increased IL-6, KC, and TNF-α (Figure 10A and B). The elevations of these cytokines were also observed in mice treated with 5 mg/kg bw of CdTe-QDs at longer time points of 3 d and 1 w (Figure 10C and D).

4. DISCUSSION
Due to the increases in the production and use of cadmium QDs, more information on their behavior and biological effects is necessary for safe human use. This study examined the distribution of CdTe-QDs and their toxic effects in male BALB/c mice after an intravenous injection. In terms of the study design, BALB/c mice were chosen as the animal model for the study. This is because this strain has been employed in several in vivo QD studies and is widely used in toxicological studies due to the fact that these mice are sensitive to carcinogens and to development of different types of tumors following treatments of toxic drugs and xenobiotics, making them a good candidate for cancer and hepatotoxicity research. In addition, these mice have the characteristics of easy breeding and minimal weight variations between males and females. This study employed intravenous injection to administer QDs; this is a relevant exposure route for CdTe-QDs due to their potential use in medical imaging for diagnostics and therapeutics. Furthermore, the doses used were within the range of those used for in vivo imaging and targeting applications of quantum dots and comparable to reported doses from other in vivo studies.

In selecting the QD for study, we focused on MPA-capped QDs, which are among the most commonly studied for imaging applications. MPA is also relatively nontoxic compared to other capping agents such as mercaptacetic acid, so direct toxicity from the capping agent would be minimal. Furthermore, our results demonstrated that the MPA-capped QDs fluoresced for 24h, which suggests that they retained their stability. This was substantiated by Loginova and colleagues, who demonstrated that MPA-capped QDs persisted in tissues for 22 days.
The distribution of CdTe-QDs in the blood and tissues was assessed by measuring cadmium concentrations using ICP-MS. The results showed that CdTe-QDs partitioned into the lung, heart, liver, spleen, kidney, brain, and testis. The liver, spleen, and kidney exhibited the highest levels of cadmium, suggesting these tissues are preferential sites of CdTe-QD accumulation. However, the greatest accumulation of CdTe-QDs over time was in the liver, suggesting that this organ is the primary target when these NPs are administered by an intravenous injection. These findings agreed with previous studies showing that after an intravenous injection into the tail vein, various types of QDs accumulated in the liver and spleen, and their excretion occurred through the kidney. It has been suggested that the uptake of NPs into the reticuloendothelial (RE) tissues, such as liver, spleen, and lungs, was due to the nonspecific NP clearance from the blood by cells of the mononuclear phagocyte system. However, similarly to the report by Haque et al. (2012), in this study, the distribution of CdTe-QDs in the lungs was much less than that in the liver and spleen, and the level of CdTe-QDs in the lungs decreased over time. This suggests that the specific distribution of CdTe-QDs to the liver and spleen is not consistent with the RE system and could be a result of other factors. The observed accumulation of CdTe-QDs in the liver and spleen could depend on the injection site which was at the tail vein in this present study consistent with that of Haque and colleagues. In contrast, Salykina et al. (2011), who used the same MPA-coated QDs as in this study, reported that the primary accumulation of these QDs was in lung since their QDs were injected in the jugular vein. Furthermore, tissue distribution of QDs has been shown to depend on the surface coating. It has been reported that negatively charged QDs such as MPA-coated QDs used in this study may interact with and adsorb to proteins in the blood. Such protein binding plays a key role in delivery of QDs from plasma into the liver, which appeared to be the main site of accumulation of CdTe-QDs in this study, as the role of this organ in the metabolism and degeneration of a multitude of xenobiotics is well described.

CdCl₂ was used as the control for comparing Cd²⁺ distribution and effects at the tissue level, since our in vitro toxicity data suggested that the pattern of CdTe-QD toxicity was essentially related to cadmium. To permit this comparison, CdCl₂ was exposed to mice at a concentration equivalent to the cadmium within 6 mg/kg bw CdTe-QD. Based on the cadmium concentration in the blood and examined tissues measured by ICP-MS at 24 h postinjection, the distribution pattern of CdCl₂ appeared to be different from that of CdTe-QDs. At 24 h postexposure, the cadmium level from CdCl₂ was very low in the blood while a certain level of cadmium from CdTe-QDs still remained in the blood. Although an equivalent cadmium concentration was used for both CdTe-QDs and CdCl₂, in all tissues, the levels of cadmium from CdCl₂-treated mice were significantly lower than those from CdTe-QDs.
CdTe-QDs-treated mice, suggesting that CdCl₂ has a shorter retention time in the animal than CdTe-QDs and its pharmacokinetic profile appeared to be different from that of CdTe-QDs. The findings agreed with previous reports showing that cadmium accumulated primarily in the liver and kidney after an intravenous injection. ⁶⁴,⁶⁵,⁶² It has been reported that cadmium, after being introduced into the systemic circulation, binds to albumin in the blood plasma, and is transported to the liver, where the complex cadmium–metallothionein is formed and transported to the kidneys for excretion. ⁶³,⁶⁶ These findings also align with a previous study by Yeh and colleagues, ⁶⁵ who showed that QDs and CdCl₂ had very different kinetics in distribution and metabolism after intravenous injection. In this previous study, the authors found that QDs were retained in plasma and organs longer than CdCl₂, and that QDs could be detected in the brain during early exposure. ⁶⁵

Characterization and detection of NPs inside a biological system have been challenging in nanotoxicological research due to limited methods and techniques available for measurement and detection of NPs in tissues. In this study, the fluorescent property of CdTe-QDs permitted their detection in the blood and tissues using confocal microscopy and spectrofluorometry. Although autofluorescence control tissues and blood autofluorescence were relatively high, significantly greater fluorescence was observed in tissues and blood of CdTe-QD-treated mice, suggesting the presence of CdTe-QDs in these tissues. These results qualitatively corresponded to tissue levels of cadmium measured by ICP-MS. Confocal microscopy demonstrated the presence of CdTe-QDs in the blood, lung, liver, spleen, and kidney, but not in the brain, heart, and testes, suggesting that confocal microscopy has limitations associated with structural tissue interference, autofluorescence, and/or concentration threshold.

Kato and colleagues (2010) demonstrated that CdSe/ZnS QDs (13.5 nm) were able to cross the blood–brain barrier, as evidenced by the presence of these NPs in the parenchyma (neurons and glial cells) of the brain at 6 h after intraperitoneal administration. ⁶⁶ In the present study, the presence of CdTe-QDs in the brain was detected by ICP-MS and spectrofluorometry. Since the particles used in this study are less than 5 nm (by DLS), it is possible that some CdTe-QDs crossed the barrier. However, levels detected in the brain using these techniques were minimal compared to those detected in the preferential sites of accumulation, and CdTe-QDs were not observed in the brain sections using confocal microscopy. In addition, the levels of cadmium from CdTe-QDs in the brain decreased over time. This finding suggests that CdTe-QDs detected in the brain were associated with the blood vessels, but detailed distribution patterns of CdTe-QDs within the brain need further investigation.

CdTe-QDs were also observed in the heart, and their levels decreased over time, suggesting a similar kinetic to that of the blood. Interestingly, the levels of CdTe-QDs in the testes increased over time, suggesting that this organ could be an important tissue for chronic exposures. Certainly a more detailed study of CdTe-QDs distribution in these tissues needs to be conducted.

Further examination of the preferential sites of accumulation demonstrated pathological and toxicological effects. Histological examination revealed changes in the liver and spleen tissue morphology but not other tissues examined. Liver and spleen showed hemorrhage at the highest doses. Bleeding within the liver was localized to the perportal areas of the lobules, which may have resulted from damage to the endothelial cells of the portal vein, leading to leakage of red blood cells into these areas. Furthermore, CdTe-QDs significantly increased ALT and AST activities, increased bilirubin concentrations, and decreased albumin in the serum, which are all known biomarkers of liver injury. ⁶⁷ These results support previous studies, showing liver toxicity caused by QDs in vivo. Tiwari and colleagues ⁴⁸ reported increased serum AST and ALT activities induced by intravenous injection of QDs in rats. Similarly, a study by Lin and colleagues ⁴⁷ reported that intravenous injection of Cd/Se/Te-based QD 705 in mice resulted in significant elevations in ALT and AST activities at 12 and 16 wk postinjection. Although the kidney was one of the sites of QD accumulation in the present study, there were no observable alterations in its histopathology. In addition, biochemical analyses showed no changes in the level of creatinine, which is an important indicator of renal injury. CdCl₂ containing an equivalent cadmium concentration to a CdTe-QDs dose that induced liver injury, did not cause changes in either the liver or the kidney in this study. This may be due to differences in either in vivo pharmacokinetics or mechanisms of action between QDs and CdCl₂. In addition, the CdCl₂ dose used here is much lower than the toxic dose to mice as reported in previous studies. ⁴⁶,⁴⁷ The results also suggested that CdTe-QDs are more toxic than CdCl₂ when
injected into the animal, and this finding agrees with previous in vitro studies.70,71

Systemic effects of CdTe-QDs were also investigated. Increased numbers of WBC were observed following exposure to the highest test dose. Specifically, the increases in blood monocytes and neutrophils were detected, indicating an immunological response. In addition, Wright’s stained blood smears revealed an increased number of reticulocytes, suggesting acute anemia caused by hemorrhage. This result corroborated the hepatic hemorrhage observed from histological analysis. CdTe-QDs also induced significant dose-dependent reduction in the number of platelets. Cadmium QDs such as CdSe/ZnS are shown to cause aggregation of platelets, as well as a drop in the circulating platelet count.26 The latter study suggested that activation of platelets with aggregates retained in the pulmonary microvasculature eventually led to pulmonary thrombosis. Moreover, the same study also showed that QDs enhanced adenosine-5'-diphosphate-induced platelet aggregation in vitro.26 In the present study, although the platelet numbers significantly decreased, there were no effects observed in the lungs as these organs were not the main targets of the test CdTe-QDs. The difference between the present study and the Geys study could be due to the different types of QDs used. Furthermore, the platelet number in animals and humans is controlled by thrombopoietin, which is produced mainly in the liver.72 It has been reported that a decrease in platelet numbers was associated with both acute and chronic hepatitis due to either impaired synthesis or increased degradation of thrombopoietin.73,74 Therefore, in the present study, the decreased platelet number induced by CdTe-QDs could be a result of the combination of platelet aggregation, as discussed above, and liver injury that led to a decrease in the synthesis of thrombopoietin. CdCl2 did not cause any observable changes in hematology, and again this is due to the fact that the concentration of CdCl2 used in this study was much lower than the CdCl2 concentrations required to induce toxicity in animals.69,75

Serum cytokines and SAA levels were also employed as indicators of systemic effects of CdTe-QDs. SAA represent a family of acute phase proteins and are expressed in response to inflammatory stimuli. Their hepatic expression is regulated by the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α.76 Thus, in the current study the increased levels of SAA may result from the elevated IL-6 and TNF-α. It is shown that during liver injury, resident Kupffer cells and monocyte/macrophages initiate an immune response by producing pro-inflammatory cytokines such as TNF-α and IL-6 that initiate the acute-phase response and inflammation. Such acute inflammation is characterized by the rise in concentration of numerous plasma acute-phase proteins including SAA.77 The observed inflammation induced by CdTe-QDs agrees with the increased number of WBC, monocytes, and neutrophils in the blood of the treated mice. It is noteworthy that the elevation of SAA and cytokines, as well as monocytes and neutrophils, occurred at the threshold or lower doses. In addition, the effects on these end points were also observed with CdCl2. This indicates that SAA and serum cytokines are sensitive biomarkers of systemic effects of QDs and CdCl2 and inflammation appeared to be an early event of CdTe-QD toxicity. In this study, the increased SAA and serum cytokine levels were also observed at longer exposures, and these increases can be explained by the accumulation of QDs in the liver and spleen over time as previously discussed. At the

Figure 10. Effects of CdTe-QDs and CdCl2 on selected serum cytokines. Levels of (A) IL-6 and KC, (B) IL-12 (p70) and TNF-α in mice treated with saline, different concentrations of CdTe-QDs, and 1.95 mg/kg bw of CdCl2, which contains an equivalent cadmium concentration to 6 mg/kg bw CdTe-QDs, for 24 h. Levels of (C) IL-6 and KC, (D) IL-12 (p70) and TNF-α in mice treated with 5 mg/kg bw CdTe-QDs for different time points. The asterisks (*) indicate statistically significant differences compared to the control (p < 0.05). The hash mark (#) indicates statistically significant difference compared to the 6 mg/kg bw CdTe-QD-treated group (p < 0.05).
highest doses of CdTe-QDs, the levels of IL-12(p70) and TNF-α decreased and this might be associated with excessive injury of target organs, including liver and spleen, and the detailed mechanisms of these inhibitory effects at high doses need to be further investigated.

In summary, this study investigated the biodistribution and toxic effects of MPA-coated CdTe-QDs in BALB/c mice. The results demonstrated that these NPs distribute to all examined tissues, but liver is the primarily affected target. CdTe-QDs injection resulted in hepatic and splenic injury and induced systemic effects. Further studies are required to determine the underlying mechanisms of these effects. At an equivalent cadmium concentration, CdCl₂ appears to have different pharmacokinetics and to be much less toxic than the tested QDs. While the distribution of CdTe-QDs observed in this study aligned with many previous studies, the toxic effects of CdTe-QDs in mice observed here disagreed with several studies. The disagreement could be due to several factors, including doses, surface coating of QDs, and test animal models. This suggests that the toxicity of QDs in animals needs to be assessed in a case-by-case basis before making conclusions on the impacts on human health. While the study revealed important information on the target organs and the overall effects of CdTe-QDs, a detailed investigation of the fate of the NPs was not done. Thus, a detailed pharmacokinetic study to examine QDs fate within a biological system is recommended for a comprehensive risk assessment of these NPs.

**REFERENCES**


