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Evgeniia Gerasimovich, Svetlana Bozrova, Maria Baryshnikova, Zinaida Sokolova, Pavel Samokhvalov, Chris Guhrenz, Nikolai Gaponik, Alexander Karaulov, Igor Nabiev, Alyona Sukhanova

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Evgeniia Gerasimovich [1,2], Svetlana Bozrova [2], Maria Baryshnikova [3], Zinaida Sokolova [3], Pavel Samokhvalov [2], Chris Guhrenz [4], Nikolai Gaponik [4], Alexander Karaulov [5], Igor Nabiev [1,2,5]*, Alyona Sukhanova [1*]

[1] Laboratoire de Recherche en Nanosciences, LRN-EA4682, Université de Reims Champagne-Ardenne, 51100 Reims, France ; igor.nabiev@univ-reims.fr (I.N.), alyona.sukhanova@univ-reims.fr (A.S.)
[2] National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Laboratory of Nano-Bioengineering, 115409 Moscow, Russian Federation; svetaboz@yandex.ru (S.B.), gerasimovich.evg@gmail.com (E.G.), p.samokhvalov@gmail.com (P.S.)
[3] Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russian Federation; ma_ba@mail.ru (M.B.), zasokolova@mail.ru (Z.S.)
[4] Physical Chemistry, Technische Universität Dresden, Zellescher Weg 19, 01069 Dresden, Germany; nikolai.gaponik@tu-dresden.de (N.G.)
[5] Sechenov First Moscow State Medical University (Sechenov University), 119146 Moscow, Russian Federation; drkaraulov@mail.ru (A.K.)

* Correspondence: A.S., alyona.sukhanova@univ-reims.fr; I.N., igor.nabiev@univ-reims.fr

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Abstract: Semiconductor nanocrystals, or quantum dots, have received a great attention in the biomedical area because of their unique optical properties. However, their use in biomedical applications such as imaging, molecular tracking and drug delivery is limited due to safety concerns. The present study was conducted to assess acute toxicity of quantum dots with different chemical composition of the core and surface charge in mouse model. In addition, the immunotoxicity of CdSe/ZnS QDs was estimated. CdSe/ZnS, PbS/CdS/ZnS, $CuInS_2$/ZnS QDs were synthesized and modified with PEG derivatives following standard procedure. After solubilization the hydrodynamic diameter and surface charge of QDs were characterized. Our results showed that the acute toxicity of QDs is determined by both their surface charge and the chemical composition of the core. Immunotoxicity of CdSe/ZnS-PEG QDs was evaluated at the dose of 25 mg/kg (LD_{10}). Concentration of IL-12p70, TNF, IFN-γ, MCP-1, IL-6, IL-10 was measured using cytometric bead array. The intravenous injection of CdSe/ZnS-PEG QDs was demonstrated to cause short-term elevation of the level of two pro-inlammatory cytokines.

Keywords: quantum dots; semiconductor nanocrystals; toxicity *in vivo*; immunotoxicity; cytokine profile

1. Introduction

Quantum dots (QDs) are semiconductor fluorescent nanocrystals with a size from 2 to 10 nm [1]. Their optical characteristics such as broad excitation and narrow emission spectra, high photostability and size-depending fluorescence peak spectral position make them a promising tool in the biomedical field [2]. Possible biomedical applications of QDs include their use in bioanalytical systems, cell and tissue imaging, drug delivery and tracking *in vivo* [3–5]. However, the potential toxicity of QDs is a serious limitation to their use in living organisms. In particular, the immunotoxicity of QDs is one of the actual topics because the immune system plays an important role in protecting the body from the foreign substances and preventing diseases.

A number of studies concerning QDs toxicity clearly demonstrated that they can accumulate in various organs and tissues and cause pathological effects. The liver is shown to be one of the main organs for the accumulation of QDs [6]. In the study of hepatotoxicity of CdSe/ZnS QDs with carboxyl groups they were shown to cause hepatocytes damage and liver inflammation involving inflammosome activation and increased secretion of IL-1β cytokine [7]. Similarly, in the study of Cd/Se/Te-based QDs with ZnS shell the elevation of IL-6 and TNF-α level were shown, although no histopathological damage was observed [8]. The study of *in vivo* biodistribution of CdTe aqueous QDs stated the initial accumulation of QDs in liver and their further absorption in kidney during long-time blood circulation [9]. In addition, it was showed that Cd-containing QDs disrupted the redox balance of primary kidney cells, thus inducing their damage [10]. In conclusion, many evidences demonstrate that QDs can accumulate in major organs and cause pathological damage [11].

After the intravenous administration QDs were shown to accumulate in the spleen and thymus and maintain fluorescence ability for a long time after injection [12]. The results of immunotoxicity study of CdSe/ZnS QDs show that the majority of administered QDs are taken up by the immune organs (spleen and thymus). Lymphocytes from QD-treated mice exhibited lower viability and increase of the release of TNF-α and IL-6 [13]. The study of the acute toxicity of CdSe/CdS-MPA QDs after repeated intraperitoneal injection demonstrated the accumulation of QDs in the liver and spleen and elevated level of IL-6 in these organs and plasma [14]. The study of intraperitoneal injection of PEG-InP/ZnS QDs in mice showed the increase of the percentage of neutrophils and IL-6 levels in mouse PLF (peritoneal lavage fluid) and plasma, leading to the acute-phase inflammation in mice [15]. Intravenous administration of CdTe QDs resulted in the alteration of the level of several pro-inflammatory cytokines. Specifically, IL-6 level was significantly increased starting at the dose of 0.4 mg/kg, while IL-12 (p70) and TNF-α levels were elevated starting from 5 mg/kg, but showed significant reduction at the highest doses [16]. In contrast, immunotoxicity study *in vivo* demonstrated little effect of $CdInS_2$/ZnS QDs on immune organs and the level of pro-inflammatory cytokines [17]. Another study revealed the dependence of immunotoxicity on molecules used for surface coating of QDs [18]. Summing up, the immunotoxicity of QDs can be affected by QD type, surface modifications and dose in a non-linear manner.

Assessment of cytokines concentration is one of the indicative methods for the immunotoxicity studies [19]. The cytokines are divided into two groups: the pro-inflammatory such as IL-6 [20], TNF [21], IL-12 (p70) [22], IFN-γ [23] and anti-inflammatory such as IL-10 [22]. Monocyte chemoattractant protein 1 (MCP-1) is one of the key chemokines which regulates migration and infiltration of monocytes/macrophages during inflammatory response [24]. Thus, the alterations in cytokines and chemokines concentration can reflect the reaction of the immune system to the administration of potentially toxic substances.

Our study aimed to assess the acute toxicity *in vivo* of different types of QDs by calculating the LD_{50} values for all types of QDs, estimating the physiological responses of laboratory animals on the injection of QD preparations, and toxic effects of QDs on different organs. In addition, we used the most toxic CdSe/ZnS-PEG-OH QDs to investigate the immunotoxicity after intravenous administration in mice. For this purpose, six cytokines (IL-12p70, TNF, IFN-γ, MCP-1, IL-6, IL-10) concentration was measured to assess the potential inflammation response after exposure to CdSe/ZnS-PEG-OH QDs.

2. Materials and Methods

Methanol, chloroform, sodium phosphate dibasic, sodium phosphate monobasic, sodium hydroxide, DL-cysteine hydrochloride hydrate were purchased from Sigma-Aldrich, US. PEG derivatives HS-$(CH_2)_{11}$-EG_6-OH, HS-$(CH_2)_{11}$-EG_6-OCH_2-COOH, HS-$(CH_2)_{11}$-EG_6-NH_2 were purchased from ProChimia Surfaces Sp, Poland.

2.1 Quantum dots synthesis and solubilization

CdSe/ZnS (core/shell) quantum dots with a fluorescence maximum at 592 nm were synthesized as described earlier [25]. The synthesis of $CuInS_2$/ZnS and PbS/CdS/ZnS QDs was carried out by the method described in [26] for $CuInS_2$/ZnS QDs. After the synthesis quantum dots were transferred from organic phase to water solution by replacing hydrophobic surface ligands with polyethylene glycol derivatives following the procedure described earlier [25]. Briefly, quantum dots were dissolved in chloroform and precipitated with 10 mg/ml DL-cysteine solution in methanol. The excess of DL-cysteine was washed off with methanol. The precipitate was dried and then dissolved in a weak alkaline solution.

At the next stage, DL-cysteine ligands were replaced with PEG derivatives with hydroxyl group. We used three types of PEG derivatives: HS-$(CH_2)_{11}$-EG_6-OH, HS-$(CH_2)_{11}$-EG_6-OCH_2-COOH, and HS-$(CH_2)_{11}$-EG_6-NH_2. For the modification of CdSe/ZnS QDs mixtures of PEG derivatives were used at the following ratios: 70% of HS-$(CH_2)_{11}$-EG_6-OH/30% of HS-$(CH_2)_{11}$-EG_6-OCH2-COOH, 70% of HS-$(CH_2)_{11}$-EG_6-OH/ 30% of HS-$(CH_2)_{11}$-EG_6-NH_2, and 100% of HS-$(CH_2)_{11}$-EG_6-OH. The specified amounts of ligands were added to working mixtures with the corresponding pH values: 0.1 M sodium phosphate buffer solution (pH 7.2) if the HS-$(CH_2)_{11}$-EG_6-OH ligand was used, 0.1 M sodium phosphate buffer solution (pH 8.0) in the case of the mixture of 70% of HS-$(CH_2)_{11}$-EG_6-OH / 30% of HS-$(CH_2)_{11}$-EG_6-OCH_2-COOH, and 0.1 M sodium phosphate buffer solution (pH 6.6) in the case of 70% of HS-$(CH_2)_{11}$-EG_6-OH/30% of HS-$(CH_2)_{11}$-EG_6-NH_2. After that, the mixtures were incubated at a temperature of 4°C overnight.

After the incubation, the samples were purified from unbound PEG derivatives using Amicon Ultra-15 centrifugal filter units with a 10 kDa cut-off (Millipore) by centrifugation following the adding 15 mL of 0.1 M sodium phosphate buffer (pH 7.2) in the case of Hs-$(CH_2)_{11}$-EG_6-OH, 0.1 M sodium phosphate buffer (pH 8.0) in the case of the mixture of 70% of HS-$(CH_2)_{11}$-EG_6-OH 30% of HS-$(CH_2)_{11}$-EG_6-OCH2-COOH, and 0.1 M sodium phosphate buffer solution (pH 6.6) in the case of 70% of HS-$(CH_2)_{11}$-EG_6-OH/30% of HS-$(CH_2)_{11}$-EG_6-NH_2. The centrifugation was performed three times at the room temperature at 4000 rpm for 10 min. Then, the obtained QDs preparations were additionally purified by gel exclusion chromatography on PD MiniTrap 25G columns (GE Healthcare) according to the manufacturer's protocol. For this purpose, 500 μL of a QD solution was applied onto a column preliminarily equilibrated with a 0.1 M sodium phosphate buffer solution with the corresponding pH. After that, 1 mL of the same buffer solution was used for elution. The fractions containing QDs were collected into a separate test tube. The purification procedure was performed twice. The resultant solution was sequentially filtered through Millex-GV 0.22 μm and Whatman Anotop 0.1 μm filter units. The solution of PEG derivatives with different functional groups (PEG filtrate solutions) were prepared by centrifugation of QDs solution using Amicon Ultra-4 centrifugal filter units with a 10 kDa cut-off (Millipore).

2.2 Quantum dots characterization

For the determination of QDs concentration at the final solution we used a weight method. The aliquot of 35 μL of the final preparation of the QDs solution was placed into a preliminarily weighted 0.5-mL low-bind test tube (Eppendorf) and then dried in the Concentrator Plus (Eppendorf, Germany) for 3 h at the temperature of 30°C. After that, the test tube was weighted again. The quantity of QDs contained in 35 μL of the original QD solution was calculated by subtracting the initial weight of the empty test tube from the final weight of the test tube containing the QD preparation after drying. The QD quantity per milliliter of solution was calculated to obtain the mass concentration. The hydrodynamic diameter and zeta potential of solubilized QD were measured by dynamic

laser scattering and laser Doppler electrophoresis, respectively, by using Zetasizer Nano-ZS device (Malvern Instrument Ltd., UK).

2.3. Estimation of acute cytotoxicity of QDs in vivo

The analysis of the acute *in vivo* toxicity of the QD preparations, including the estimation of their median lethal doses (LD_{50}), was performed in experiments on female BALB/c mice obtained from the vivarium of the Blokhin Russian Cancer Research Center of the Ministry of Health of Russia. Standard animals weighing 18–22 g were used in the study. All the animals were healthy; they were kept in special roomy cages at room temperature (20–23°C), a relative humidity of 60–65%, natural illumination, and forced air supply, on a litter of wood shavings sterilized in a hot-air oven. The mice were fed on the standard commercial certified pelletized feed for rodents with a known expiry date. The mice had free round-the-clock access to pure drinking water.

The mice were divided into five groups (four mice each). $CuInS_2$/ZnS, PbS/CdS/ZnS, and CdSe/ZnS QDs modified with the thiol-containing HS-$(CH_2)_{11}$-EG_6-OH ligand, as well as CdSe/ZnS QDs modified with the mixture of 70% of HS-$(CH_2)_{11}$-EG_6-OH / 30% of HS-$(CH_2)_{11}$-EG_6-OCH_2-COOH, the mixture of 70% of HS-$(CH_2)_{11}$-EG_6-OH / 30% of HS-$(CH_2)_{11}$-EG_6-NH_2, or 100% HS-$(CH_2)_{11}$-EG_6-OH, were administered at doses from 100 to 300 mg/kg as a single injection into the caudal vein. The substances studied were dissolved in sterile 0.1 M sodium phosphate buffer solution (pH 7.2). Sterile 0.1 M sodium phosphate buffer solution (pH 7.2) and sterile filtered solutions of the corresponding modifying ligands in 0.1 M sodium phosphate buffer solution (pH 7.2) served as control solutions.

The day when the preparation was injected was taken to by day 0. In that day, the mice were watched for 6 h after the injection. After that, they were examined twice a day for 15 days. The number of the animals that died was the criterion for estimating the acute toxicity of the QDs. The toxicity was measured as the lethal dose (LD), i.e., the amount of QDs causing death of a specified percentage of the animals.

2.4. Assessment of CdSe/ZnS-PEG-OH QDs immunotoxicity

Eighty BALB/c mice were treated with 25 mg/kg CdSe/ZnS-PEG QDs. A solvent solution (0.05 M Na-phosphate buffer, pH 7.2) and PEG filtrate solution with the same concentration of PEG as in the CdSe/ZnS-PEG solution were used as negative controls. At the time points of 6, 24, 48 hours and 7 days intact animals was used as an additional negative control. All solutions were injected intravenously in the tail vein. Serum samples were collected at 6, 24, 48 hours, 7 and 30 days after injection. Serum from all mice in one group were pooled to obtain necessacary amount of material and stored at -20°C until analysis.

2.5. Mouse cytokines concentration measurement

The concentration of six pro-inflammatory cytokynes was measured using cytometric bead array kit (BD™ CBA Mouse Inflammation Kit, BD Biosciences, California, USA) according to manufacturer's instrusctions. This technique is based on six bead populations with distinct fluorescence intensities coated with antibodies specific to IL-12p70, TNF, IFN-γ, MCP-1, IL-6, IL-10. The cytokine capture beads and phycoerythrin-conjugated detection antibodies were incubated with serum samples or standards to form sandwich complexes. Then samples were measured on BD FACS Canto™ II cytometer (BD Biosciences, California, USA) and FCAP Array™ 3.0 software (BD Biosciences, California, USA) was used to calculate the concentration of cytokines using fluorescence intensity data. The standard curve range for each analyte was from 20 to 5000 pg/ml.

3. Results

3.1. QD synthesis, solubilization and characterization

In the presented study, QDs with various chemical composition and surface charge were used to investigate their toxic effects in mice model. After the synthesis of QDs in the organic phase, we modified their surface with thiol-containing PEG derivatives to obtain aqueous QD preparations. Using mixtures of PEG derivatives with different functional groups for the modification of CdSe/ZnS QDs, we obtained QD solutions with different surface charge. The surface charge and hydrodynamic diameter of QDs were determined using a Zetasizer Nano ZS instrument by the electrophoretic mobility method employing the Doppler effect and by the dynamic light scattering method, respectively. Table 1 shows the sizes and charges of the QDs used in the study.

Table 1. Size and surface charge of the quantum dots used in the study.

QD composition	Size, nm	ζ-potential, mV
PbS/CdS/ZnS-PEG-OH[1]	32.04±0.87	–10.60±2.92
$CuInS_2$/ZnS-PEG-OH	16.08±0.51	–6.12±1.81
CdSe/ZnS-PEG-OH	16.74±0.28	–4.72±0.38
CdSe/ZnS-PEG-COOH[2]	15.37±0.14	–17.80±3.01
CdSe/ZnS-PEG-NH_2[3]	22.77±0.36	6.43±1.12

[1]PEG-OH denotes 100% of HS-$(CH_2)_{11}$-EG_6-OH
[2]PEG-COOH denotes a mixture of 70% of HS-$(CH_2)_{11}$-EG_6-OH and 30% of HS-$(CH_2)_{11}$-EG_6-OCH_2-COOH
[3]PEG-NH_2 denotes a mixture of 70% of HS-$(CH_2)_{11}$-EG_6-OH and 30% of HS-$(CH_2)_{11}$-EG_6-NH_2

3.2. Acute in vivo cytotoxicity of quantum dots

The mice were injected with the QDs preparations into the caudal vein, after which we watched the animals carefully for 24 h. After that, the number of surviving animals was recorded.

We estimated the *in vivo* acute toxicity of QDs with different chemical compositions of the core (Figure 1) and with different surface charges (Figure 2). The data obtained in this experiment were used to calculate the LD_{50} values for all types of QDs studied (Table 2). Our results showed that $CuInS_2$/ZnS QDs had less toxic effect on mice than PbS/CdS/ZnS and CdSe/ZnS QDs, despite their surface charge were relatively similar due to the same PEG derivative used for their modification (Fig. 1). Furthermore, the obtained means of LD_{50} for QDs with the same chemical composition of the core (CdSe/ZnS) but carried different surface charge varied considerably. CdSe/ZnS-PEG-OH QDS with low negative charge exhibited the highest toxic effect on mice, while the LD_{50} of CdSe/ZnS-PEG-NH2 QDS with low positive charge turned out to be approximately two times higher. CdSe/ZnS-PEG-COOH QDs were showed to cause the weakest toxic effect on mice.

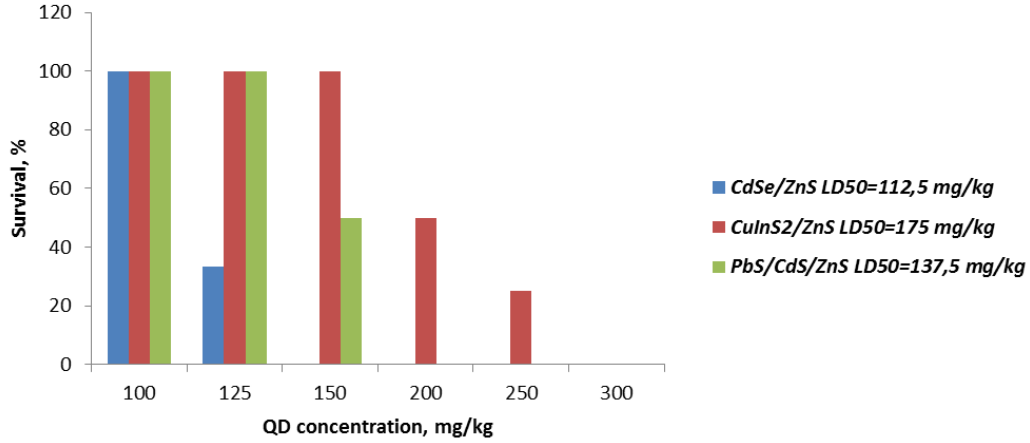

Figure 1. Survival rate of BALB/c mice as dependent on the quantum dot concentration and core composition.

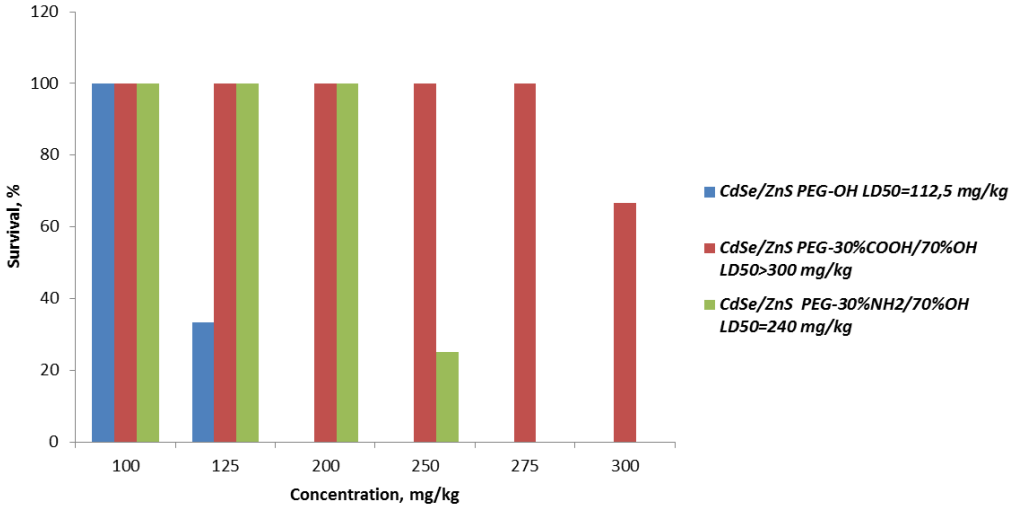

Figure 2. Survival rate of BALB/c mice as dependent on the quantum dot concentration and charge.

Table 2. LD_{50} values of different quantum dots for BALB/c mice.

QD type	LD_{50}, mg/kg
CdSe/ZnS-PEG-OH	112.5
$CuInS_2$/ZnS-PEG-OH	200
PbS/CdS/ZnS-PEG-OH	150
CdSe/ZnS-PEG-COOH	>300
CdSe/ZnS-PEG-NH_2	240

3.2 Cytokine profile in mice after injection of QDs

In three experimental groups mice were treated with 0.1 M Na-phosphate buffer, PEG filtrate solution, and CdSe/ZnS-PEG solution at the dose of 25 mg/kg. The concentrations of six cytokines (IL-12p70, TNF, IFN-γ, MCP-1, IL-10, IL-6) in mice serum determined by CBA assay are shown in Table 3. The data show that 24 hours after administration of CdSe/ZnS-PEG QDs the level of IL-12 was slightly elevated compared to control group and groups treated by buffer and PEG solutions, but starting from 48 hours the level of this cytokine was low in all groups of animals. The concentration of

another pro-inflammatory cytokine, TNF, is raised in group treated with CdSe/ZnS-PEG QDs after 48 hours after injection. In addition, the level of IL-6 was somewhat higher 6 hours after injection in the groups treated with buffer, PEG and CdSe/ZnS-PEG QDs solutions compared to intact animals, but after longer time periods it remained at the basic level. The level of anti-inflammatory cytokine, IL-10, was below the limit of detection in samples from all experimental groups. The level of MCP-1, pro-inflammatory chemokine, was elevated in groups treated with buffer and CdSe/ZnS-PEG QDs 6 hours after injection. However, 30 days after the start of the experiment the level of MCP-1 was twice higher in groups treated with PEG and CdSe/ZnS-PEG in comparison with group treated with buffer.

Table 3. Concentration of cytokines in mouse serum after the injection of CdSe/ZnS QDs.

Cytokine concentration, pg/ml	Time	control	Na-phosphate buffer	PEG solution	CdSe/ZnS-PEG
IL-12p70	6 h	10.26±2.75	10.36±7.06	7.38±7.81	0.00±0.00
	24 h	3.56±0.30	2.37±0.74	0.00±0.00	10.27±1.25
	48 h	1.50±0.50	0.00±0.00	0.00±0.00	1.85±0.00
	7 d	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	30 d	N/A	0.00±0.00	0.00±0.00	0.00±0,00
TNF	6 h	21.71±0.74	25.39±10.42	24.51±9.18	17.14±5.23
	24 h	24.69±4.47	18.19±6.72	26.09±0.99	24.87±1.24
	48 h	26.27±7.69	18.91±0.25	21.18±3.97	36.62±0.99
	7 d	5.25±2.29	4.72±0.90	4.66±0.33	5.70±0.66
	30 d	N/A	2.49±3.22	5.41±0.25	4.84±1.38
IFN-γ	6 h	2.68±0.57	3.12±0.51	3.55±1.34	2.84±0.34
	24 h	2.01±0.48	3.00±0.34	2.56±0.06	2.26±0.83
	48 h	3.59±1.4	2.84±0.57	2.88±0.40	2.05±0.54
	7 d	0.59±0.00	0.31±0.21	0.00±0.00	1.11±0.11
	30 d	N/A	0.67±0.06	0.81±0.00	0.42±0.13
MCP-1	6 h	32.06±5.21	79.47±12.2	49.05±5.71	77.72±5.59
	24 h	35.54±0.28	28.98±3.25	20.08±0.00	32.49±3.46
	48 h	20.62±7.36	26.03±1.51	24.73±2.76	21.88±1.26
	7 d	11.44±3.02	13.79±1.22	17.13±2.91	14.48±4.08
	30 d	N/A	7.51±3.52	13.68±0.16	12.7±0.93
IL-10	6 h	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	24 h	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	48 h	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	7 d	0.00±0,00	0.00±0,00	0.00±0,00	0.00±0,00
	30 d	N/A	0.00±0.00	0.00±0.00	0.00±0.00
IL-6	6 h	1.54±0.13	6.20±2.83	3.68±0.74	8.56±1.85
	24 h	1.51±2.13	2.27±0.65	0.00±0.00	0.00±0.00
	48 h	0.00±0.00	0.00±0.00	0.00±0.00	1.33±0.43
	7 d	0.62±0.69	0.00±0.00	0.00±0.00	0.78±0.42
	30 d	N/A	0.33±0.47	0.00±0.00	0.00±0.00

4. Discussion

Semiconductor nanocrystals, or quantum dots, have a great potential in the field of biomedicine, but their use is restricted by potential toxic effects on living organisms. The *in vivo* toxicity of CdSe/ZnS QDs has been reported in several studies. However, a more systematic evaluation of negative effects of QDs on living organisms is necessary for safe human use. One of the important aspects is an immunotoxicity as the immune system functions include protection from foreign substances.

We have estimated the LD_{50} values for different types of QDs, physiological responses of laboratory animals on the injection of QD preparations, and toxic effects of QDs on different organs. Such experiments could help to improve the safety testing standards for nanotechnological products based on nanocrystals.

The QD preparations were injected into the caudal vein of BALB/c mice, after which we carefully watched the mice for 24 h and then examined them twice a day for 15 days. We estimated the *in vivo* toxicity of QDs with different chemical compositions of the core (Fig. 1) and those with different surface charges (Fig. 2). The results were used to calculate the LD_{50} values for all types of QDs studied (Table 2). Our data showed that the CdSe/ZnS-PEG-OH QDs with a low negative surface charge were the most toxic. The QDs with a high negative surface charge were the least toxic. It was also demonstrated that the QDs with $CuInS_2$ cores were less toxic than the QDs whose cores contained heavy metals.

In addition, our experiments on toxicity showed that intravenous injections of QDs with different chemical compositions provoked different physiological responses. The administration of CdSe/ZnS and PbS/ZnS QDs caused an increase in the respiration and heart rates, paresis of the legs, convulsive retching movements, and spasm of the caudal vein. After several hours, the surviving animals completely recovered. After the injection of $CuInS_2$/ZnS QDs, the mice felt well for 5–10 min, after which leg paresis, rapid and shallow breathing and general paralysis were observed. These transitory disorders suggest the formation of metastable QD aggregations in the blood, which either were broken up due to opsonization and absorbed without affecting considerably the general state of health or, in more acute situations, led to death of the mice. There are published data that QDs may cause lung thrombosis [27], which explains the condition of the mice in our study. The mechanism of QD-induced thrombosis is unknown; however, it is assumed [27] that QDs are capable of triggering the clotting process in blood. A different physiological response to $CuInS_2$/ZnS QDs suggests that the toxic effect of these QDs is underlain by a different mechanism, apparently determined by copper, which, unlike lead and cadmium, is not a heavy metal [28].

Our results clearly demonstrate that the QDs surface charge also influences their toxicity. Indeed, the QDs with a low negative surface charge have proved to be the most toxic, and those with a higher negative charge, the least toxic. The observed effect is most likely to be determined by the differences in the distribution and accumulation of QDs in the mouse body and the differences in the interactions with the components of biological fluids. For example, negatively charged QDs may rapidly accumulate in lymph nodes [29], and electrically neutral QDs are less prone to electrostatic interaction with proteins, which interferes with the formation of the "protein corona" and promotes their degradation [30].

After the most toxic type of QDs was revealed, we performed an additional experiment in order to assess the effect of these QDs on the immune system. For this purpose, we estimated the effect of CdSe/ZnS-PEG-OH QDs at the dose of 25 mg/kg, or LD_{10}, on cytokine profile in mice. Buffer solution (0.1 M Na-phosphate, pH 7.2) and PEG-OH solution were used as controls in the study. The concentration of IL-12p70, TNF, IFN-γ, MCP-1, IL-10, and IL-6 was determined using cytometric bead array kit (Table 3). The results demonstrated a slight increase in the production of two pro-inflammatory cytokines, IL-12 (p70) and TNF, 24 and 48 hours after injection, respectively. The level of MCP-1 was elevated in the groups treated with both buffer solution and CdSe/ZnS-PEG

QDs solution 6 hours after administration, but after 30 days it was relatively higher in groups treated with PEG solution and CdSe/ZnS-PEG QDs solution. Our results might evidence that injection of CdSe/ZnS-PEG QDs can cause short-term inflammatory reaction in mice. However, after 7 and 30 days after injection of QDs the level of the majority of pro-inflammatory cytokines was similar to that of control groups. Based on these results, we can suggest that immune reaction to CdSe/ZnS QDs at the dose of 25 mg/kg is reversible, but this issue needs further investigation.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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