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Potential Harm of Maltodextrin-Coated Cadmium Sulfide Quantum Dots in Embryos and Fetuses

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Abstract

Over the past years, there has been significant interest in the study of nanoparticles for clinical applications, particularly quantum dots (QDs). However, previous studies have also shown that QDs can reach the embryo through the placenta, a natural barrier for a large variety of organic substances with diverse molecular structures, and may cause developmental deformities. Due to its essential role in a toxicological profile and its relevance to human safety, knowledge regarding embryotoxicity is of great importance. Previous studies by this research group have shown that CdS-maltodextrin QDs are biocompatible and nontoxic to cells and animals; however, QDs are able to induce embryotoxic effects. Therefore, as an effort to further address the issue, we studied the effects of CdS-maltodextrin QDs on embryo and fetus development using an embryotoxicity and teratogenicity assay on chicken embryos. Chicken embryos exposed to CdS-maltodextrin QDs (0.001, 0.01, 0.1 and 1 $\mu\text{g/kg}$) *in ovo* for 72 h showed growth and developmental alterations during the early stage and at the end of their development in a dose-dependent manner. Decreased development was observed during early stages (Stages 9/10 on the Hamburger-Hamilton scale) when compared with untreated eggs (Stage 13). Chicken embryos exposed to lower CdS-maltodextrin QDs doses (0.01, 0.1 and 1 ng/kg) and incubated *in ovo* for 21 h also showed growth and development alterations during the early stages and at the end of their development in a dose-dependent manner. However, reduced development was observed at the end of the development period (21 days), and this was associated with death of the chick. Current studies have also shown that CdS-dextrin induces embryotoxicity and teratogenicity, affecting mainly the CNS, the neural tube and somites in chicken embryos. The nature of the observed abnormalities suggests that these effects could be directly associated with nanoparticle concentrations affecting somitogenesis. Therefore, according to the

results, there is a high probability that the prolonged accumulation of QDs in the maternal organism may be potentially harmful on embryo and fetus development. This study is limited to the analysis of embryotoxic and teratogenic effects induced by CdS-maltodextrin QDs.

Keywords: embryotoxicity, teratogenicity, nanoparticles, quantum dots, embryos

1. Introduction

Countless publications about of the *in vitro* and *in vivo* effects of nanomaterials have appeared over the past years [1]. It is now well known that the same properties that make nanoparticles so attractive to biomedicine may contribute to their toxicological profile in biological systems [2, 3]. Nevertheless, there are still many unanswered questions about their toxicological aspects, such as embryotoxicity and teratogenicity.

It is well known that embryos and fetuses are most sensitive to harmful factors during critical periods [4]. Not only is differentiation during organogenesis a highly susceptible period to the induction of malformations, but also the fetal/neonatal developmental phases are just as prone to certain developmental deficits [5]. Therefore, embryotoxicity and teratogenicity assays are of great importance given their indispensable role in the toxicological profile that must be established for any new active substance relevant to human safety, including nanomaterials. In recent years, different studies have explored the passage of nanomaterials through the placenta, a natural barrier for a large variety of organic substances with diverse molecular structures [6–8]. It is now known that the passage of nanomaterials through the placental barrier may affect fetal cell proliferation, embryonic growth, and organ formation [9]. Depending on the doses and time of exposure—from fertilization through the fetal period and eventually during lactation—the consequences can range from embryotoxicity to gross malformations, and a large variety of more subtle morphological, biochemical, and functional abnormalities have been detected [10–12].

However, knowledge concerning nanomaterial embryotoxicity and teratogenicity is yet limited because the toxicity of each nanoparticle depends on size, shape, and even surface cover, and so different nanomaterials may yield contradictory effects [13]. Because of this, in-depth knowledge of nanotoxicity and increased efforts devoted to the study of the toxic effects of nanomaterials on embryos and fetuses should be considered mandatory, as is with other investigational new drugs.

Our group is interested in the biomedical application of maltodextrin-coated cadmium sulfide QDs, semiconductor nanoparticles of about 3.5 nm in size with superior optical properties when compared to conventional organic dyes [14]. Although the *in vitro* studies revealed that these CdS-maltodextrin QDs produced distinct dose-dependent toxic effects, *in vivo* studies demonstrated that, when administered to rodents, CdS-maltodextrin QDs were biocompatible and nontoxic after 5 and 15 days [15]. Moreover, the pharmacokinetic data clearly showed that CdS-maltodextrin QDs were not completely cleared from *in vivo* systems after 360 h. Then,

although CdS-maltodextrin QDs appear to be nanomaterials with favorable pharmacokinetic properties to develop novel therapeutic and diagnostic modalities, according to previous results, CdS-maltodextrin QDs seem to be allowed into the body through several barriers and then pass into the blood stream, from where they can reach organs and tissues and interact with biological structures [16].

Early results by this group demonstrated that CdS-maltodextrin QDs were embryotoxic in a chicken embryo model [14]. The nature of the observed abnormalities suggests that these effects could be directly associated with nanoparticle concentrations. The observed effects indicate that prolonged accumulation of QDs in the maternal organism may increase the risk of adverse effects on embryo development. Since nanotoxicity studies targeting the reproductive and developmental aspects are rather scant, and considering that QD mechanisms of action during embryogenesis are not fully understood, this study aims at further addressing the issue. We studied the effects of CdS-maltodextrin QDs on embryo and fetus development using an embryotoxicity and teratogenicity assay on chicken embryos.

2. Materials and methods

2.1. Embryotoxicity study

Fertile White Leghorn chicken eggs were obtained from A.L.P.E. S.A. (Puebla, Mexico) and stored at 6°C. One hundred fertilized eggs were weighed, sterilized, and divided into nine groups. The first group served as a nontreated control and was considered the negative control. The second group was treated with 1 mL of Ringer solution. The third group was treated with caffeine (10 mg/mL) and was considered a positive control. The remaining six groups received CdS-maltodextrin QDs in different concentrations (0.001, 0.01, 0.1, 1 µg/kg). One of these groups received caffeine (10 mg/mL) and was considered the positive control. An embryotoxicity assay was carried out as described by Jelinek and Marthan [17]. Test solutions (1 mL) were added to the air sac under sterile conditions. Each solution was injected after drilling into the shell at the blunt end of the egg; after injection, the holes were immediately sealed with melted paraffin wax. The eggs were then transferred to and maintained in a forced draft incubator at 37.5°C with a relative humidity of 65% until the desired stage of development was reached (72 h).

Embryos in each group were fixed in buffered formal saline (pH 7.4), dehydrated, and embedded in paraffin blocks. Paraffin tissue sections of 6 µm were stained with acetocarmine for routine histological examination. The embryo was examined and staged according to morphological criteria previously outlined by Hamburger and Hamilton [18]. Malformations were considered for the following specific structures: central nervous system (CNS), lens placode, otic placode, cardiovascular system (CVS), neural tube, as well as number of somites. Embryonic stages at the time of the CdS-maltodextrin QD application varied from 14 to 16, which corresponds approximately to developed somites numbered 22–28. In order to monitor their uptake and distribution, we observed the embryos under a confocal microscope (Zeiss

LSM510, USA). CdS-Dx QDs were excited with 488 nm laser, and their signals were collected from 515 nm.

2.2. Teratogenicity assay

Fertile White Leghorn chicken eggs were obtained from A.L.P.E. S.A. (Puebla, Mexico) and stored at 6°C. One hundred fertilized eggs were weighed, sterilized, and divided into four groups. The first group served as a nontreated control and was considered the negative control. The other groups received CdS-maltodextrin QDs in different concentrations (0.001, 0.01, 0.1, 1 µg/kg). A teratogenicity assay was carried out as described by Jelinek and Marthan [17]. Test solutions (1 mL) were added to the air sac under sterile conditions. Each solution was injected after drilling into the shell at the blunt end of the egg; after injection, the holes were immediately sealed with melted paraffin wax. The eggs were then transferred to and maintained in a forced draft incubator at 37.5°C with a relative humidity of 65%. To determine the teratogenic effect of CdS-maltodextrin QDs, we allowed the chicks to fully develop until they were able to hatch by themselves). Developmental stages and the presence of malformations were measured according to the Hamburger-Hamilton scale.

2.3. Statistical analysis

Results are expressed as means + standard error of the mean (SEM). Significant differences were detected by one-way analysis of variance using GraphPad InStat V2.03 (GraphPad Software Inc., San Diego, CA). The Tukey–Kramer multiple comparisons test was used when significant variations were found. Differences were considered significant at $p < 0.05$.

3. Results

A chicken embryo model was used to assess CdS-maltodextrin QDs embryotoxicity. **Figure 1** shows a representative chicken embryo treated with different doses of CdS-maltodextrin QDs. As we can see, QD-treated chicken embryos showed developmental delays in a dose-dependent manner. Nonmalformations were found with doses of 0.001 µg/kg QDs. However, embryos treated with 0.01 µg/kg of CdS-maltodextrin QDs showed significant changes in the CNS; 38% of embryos showed malformations that included morphological alterations and anencephaly (**Figure 2**). Embryos treated with 0.1 µg/kg of CdS-maltodextrin QDs showed a significant developmental arrest at Stage 13 of the Hamburger-Hamilton scale, and 50% of embryos showed various malformations. Those embryos showed several alterations in structures including lens placodes (10%), otic placodes (10%), CNS (50%) and neural tube (29%) (**Figure 2** and **Table 1**). These embryos had a smaller number of somites than nontreated embryos. The dose that produced the most critical alterations was 1 µg/kg; it produced 50% of embryo mortality at Stage 12 of the Hamburger-Hamilton scale. Besides that, 71% of embryos had malformations, including lens placode 55%, otic placode 75%, CNS 71% (anencephaly and morphological alterations), lower number of somites, and 50% presented alterations in the neural tube (see **Figure 3** and **Table 1**).

Figure 1. Effect of CdS-maltodextrin QDs on embryogenesis. Embryos were staged in accordance with the Hamburger-Hamilton scale (S), and a dose-dependent delay in embryo development was observed. Several deformities are evident. Ot. p.: otic placode; Op. p.: lens placode; S: somites; NT: neural tube (Magnification 4 $\times$).

Figure 2. Gross abnormalities associated with CdS-maltodextrin QDs. Application of 0.01, 0.1 and 1 $\mu\text{g/kg}$ QDs in sterile chicken ring solution to chicken embryo *in ovo* induced overall growth delay, as well as defects in the brain, neural tube and somites, as opposed to embryos treated with sterile chicken ring alone (arrows). Embryos treated with 0.01 $\mu\text{g/kg}$ QDs showed evident brain defects. 0.1 $\mu\text{g/kg}$ QDs produced brain and neural tube defects; besides those defects, 1 $\mu\text{g/kg}$ QD treatments resulted in malformations. The somites formed after QD treatment lack uniform rectangular alignment and border an abnormally formed neural tube. The localization of the asterisks represents neural tube deformities. Applications of QDs caused multiple abnormalities in the otic and lens placodes. (Magnification 4 $\times$).

Figure 3. Fluorescence microscopic images showing the distribution and localization of CdS-maltodextrin QDs in embryos. These images correspond to embryos treated with 0.1 and 1 µg/kg. The distribution and localization of CdS-MDx QDs were identified by a bright green imaging in the analyzed embryos. There is an evident presence of abnormalities in the brain, placodes, neural tube and somites. (Magnification 4×).

Parameter	Controls			CdS-maltodextrin QDs (µg/kg)			
	Control ¹	Ringer	Caffeine	0.001	0.01	0.1	1
Viability (%)	100	100	100	100	100	100	50
Stage ²	16 ± 0.9	16 ± 0.5	12 ± 0.8*	15 ± 1.0	14 ± 1.2	13 ± 1.1*	12 ± 2.6*
Malformations (%)	0	0	100	0	38	50	71
Type of malformations							
Optical placode (%)	0	0	100	0	0	10	55
Otic placode (%)	0	0	100	0	0	10	75
CNS (%)	0	0	100	0	100	100	100
Somites (#)	26 ± 1.1	25 ± 1.5	20 ± 2.1	25 ± 1.8	24 ± 1.9	20 ± 4.3	15 ± 2.1
Heart (%)	0	0	100	0	0	0	0
Neural tube (%)	0	0	100	0	13	29	50

N = 14; CNS = central nervous system.

¹Nontreated embryos.

²Hamburger-Hamilton scale.

*p < 0.05 as compared to control group.

Table 1. Effect of CdS-maltodextrin QDs on embryos viability and development.

The fluorescent properties of CdS-maltodextrin QDs allowed us to monitor their uptake and distribution directly by observing the bright green light they emit. CdS-maltodextrin QDs uptake and bioimaging experiments were performed with a fluorescent microscope. **Figure 3** shows CdS-maltodextrin QD distribution in chicken embryos. The uniform distribution of QDs in embryonic tissues was evident. The presence of malformations on the CNS, placodes and somites with doses of 0.1 $\mu\text{g/kg}$ was evident too. However, more severe malformations were witnessed with doses of 1 $\mu\text{g/kg}$, such as absence of head, brain, otic placode and somites. Somites, mainly the ventral ones, were affected in size, morphology and number in a dose-dependent manner (**Figures 2 and 3**).

The teratogenic effect of CdS-maltodextrin QDs was also evaluated using a chicken embryo model. Thus, we observed several malformations in embryos with QD doses of 0.01–1 $\mu\text{g/kg}$; for the teratogenicity study, we employed lower doses (0.01, 0.1 and 1 ng/kg) in order to allow for chick development. However, although lower doses were used as compared with the embryotoxicity study, the lack of development and birth defects was clear, and even with the same doses there were variations in embryo development. For example, embryos treated with 0.1 ng/kg QDs were at Stages 23 and 41, and embryos treated with 1 ng/kg were at Stages 20 and 32.

Figure 4. Morphological aspects of chicken after CdS-maltodextrin QD treatment. Chicken treated with QDs showed different deformities. Full development was only observed with the lowest dose (0.01 ng/kg). Severe malformations were observed with 0.1 and 1 ng/kg. Effects on fetus development at Stages 20 and 23 were also observed.

These results showed that the treatment of chicken embryos with CdS-maltodextrin QDs for 21 days caused reduced chicken viability (**Figure 4 and Table 2**), and a dose-dependent

developmental delay was also observed. Only 35% of the chicks were alive and able to hatch when they were treated with 0.01 ng/kg of CdS-maltodextrin QDs. The chicks were able to hatch by themselves and did not show apparent malformations, but these chicks did not attain full development (32–36 on the Hamburger-Hamilton scale) and had a lack of lower limb motor coordination. Mortality was as high as 70% among chicks treated with 0.1 ng/kg of QDS. Of the chicks that were alive, only 20% were able to hatch by themselves, and 25% showed malformations consisting of lack of abdominal wall closure. The group of chickens treated with the highest doses (1 ng/kg) resulted in 100% mortality. These chicks showed a very early developmental stage (26–32 of the Hamburger-Hamilton scale), and it was not even possible to detect specific organs. Chicks in advanced stages presented anencephaly and a lack of abdominal wall closure (**Figure 4** and **Table 2**).

Parameters	Controls		CdS-maltodextrin QDs (ng/kg)		
	Control ¹	Ringer	0.01	0.1	1
Viability (%)	100	100	35	18	0
Hatch (%)	100	100	67	20	0
Stage (S) ²	46	45	42 ± 3*	30 ± 7*	26 ± 6*
Malformations (%)	0	0	0	25	44.4
Type of malformations					
Malformations	None	None	Nonmalformations Lack of development Lack of motor coordination of lower limbs	Lack of closure of abdominal wall with exposed viscera	Lack of closure of abdominal wall with exposed viscera Anencephaly

N = 20.
¹Nontreated embryos.
²Hamburger-Hamilton scale.

*p < 0.05 as compared to control group.

Table 2. Effect of CdS-maltodextrin QDs on chicken viability and development.

4. Discussion

Over the past years, there has been significant interest in the study of nanoparticles for clinical applications, particularly quantum dots (QDs). One of the most valuable QD properties is their

fluorescence spectrum, which renders them optimal fluorophores for bioimaging applications [19]. Due to their fluorescent features, QDs can be conjugated with bioactive moieties (e.g., antibodies or receptor ligands) to target specific biological events and cellular structures [20]; however, due to their small size and physical resemblance to physiological molecules, particular attention has been focused to potential risks of human beings. Understanding quantum dot potential toxicity should require a fundamental grasp of QDs properties. However, the most important problem is that each individual type of QD possesses its own unique physicochemical properties, which in turn determine its potential toxicity or lack thereof [21, 22].

Many research groups have contributed with evidence that supports the application of a precautionary approach when creating products containing nanoparticles, such as QDs [23–25]. According to their reports, there are several aspects to be considered. Firstly, QD size down to the nanoscale (between 1 and 100 nanometers); a small size allows nanoparticles to enter the body through several cellular barriers and pass into the bloodstream, from where they can reach organs and tissues and fully interact with biological structures, thus damaging normal functions in different ways [26]. Secondly, a potential source of confusion in assessing QD toxicity is that QD toxicity depends on multiple factors derived from both individual QD physicochemical properties and environmental conditions: QD charge, concentration, outer coating bioactivity (coating material, functional groups), as well as oxidative, photolytic, and mechanical stability have each been shown to be determining factors in QD toxicity [27]. The third aspect is that most nanomaterials enter the market without a toxicity analysis, and currently available testing is not suitable for a thorough assessment of its potential risks. The fourth aspect derives from several *in vitro* studies conducted on animals, which have shown that certain nanomaterials are toxic to subjects, and most likely are so to humans [28, 29]. The fifth and last aspect is that there are no regulations for nanomaterial synthesis, handling, use, and proper disposal in all countries.

We recently synthesized maltodextrin-coated cadmium sulfide QDs (CdS-maltodextrin) [14]. Although the *in vitro* studies revealed that these CdS-maltodextrin QDs produced distinct dose-dependent toxic effects, *in vivo* studies demonstrated that, when administered to rodents, CdS-maltodextrin QDs were biocompatible and nontoxic after 15 days of exposure. Nevertheless, the CdS-maltodextrin QD pattern of biodistribution and accumulation in tissue was different after repeated doses [15]. The pharmacokinetic study of those QDs clearly showed that CdS-maltodextrin QDs were not completely cleared from *in vivo* systems after 360 h after a single dose [16], suggesting that QDs may remain for long periods of time in some organs.

It is well known that biological barriers play an important role to determine QD biodistribution [30, 31]. This group has demonstrated that CdS-maltodextrin QDs are able to cross the blood–brain barrier (BBB) and spread into the brain after repeated doses in rodents without inducing morphological and functional changes [15]. However, the pharmacokinetic analysis showed that CdS-maltodextrin QDs could remain in the brain for a very long period of time. On the other hand, we have also demonstrated the presence of CdS-maltodextrin QDs in the blood–testis barrier (BTB) adjacent seminiferous tubules. The BTB was found to be intact and functional after a single dose as well as after repeated doses in rodents [15, 16]. Those results

suggest that CdS-maltodextrin QDs might also be able to cross the placental barrier, affecting embryos or fetuses. Therefore, concerned about the safety of CdS-maltodextrin QDs and as an effort to further address the issue, the present work studied the effects of CdS-maltodextrin QDs on the development of embryos and fetuses by using a chick embryo model. We used this model because it has long been appreciated that studying the embryonic chick *in ovo* provides a variety of advantages, including the potential to control the embryo's environment and its movement independently of maternal influences.

Exposure to chemicals during different stages of development—such as the preconceptional, periconceptional, embryonic, fetal and perinatal periods—has a varying impact on health. However, the embryonic period is the most critical period for embryogenesis, when mortality or different congenital anomalies are highly possible. NP direct embryotoxicity firstly depends on the ability of the compound to cross the maternal–fetal barrier, how much nanomaterial is accumulated in embryonic tissues, and its ability to induce damage [32]. Several reports have demonstrated contradictory effects of nanomaterials on embryos or their development. A decrease in embryonic weight after QD injection on the sixth day of embryogenesis (CdSe/ZnS QDs, 9.6%; CdTe QDs, 6.2%) has been reported [33], whereas others have found that nanomaterials did not produce embryotoxic or teratogenic effects during embryogenesis [34]. It has been shown that NPs (nSP and TiO₂s 70 and 35 nm in diameter, respectively) can cross the placental barrier in pregnant mice and cause neurotoxicity in their offspring, and then remain in the placenta, fetal liver, and fetal brain [35]. Some authors argue that some NPs (CdSe and CdTe/CdS) in different sizes, at different dosages, and with different outer capping materials can increase the rate of early-stage blastocyst death in mice and can be potentially transferred across the placenta to the fetus [32, 36]. On the other hand, some researchers have reported that the embryotoxic effects of some NPs can be modified if they are coated with silanes or by using a gold shell [37].

In the present study, we used different doses of 3.5-nm-sized CdS-maltodextrin QDs, and the dose-dependent embryotoxic effect was evident. Observation by fluorescence microscopy revealed the homogeneous distribution of QDs into the embryos. A significant concentration-related decline in embryonic growth was observed, as well as an increase in developmental defects, including various neuronal abnormalities. It has been reported that some nanomaterials induce neuronal alteration, which suggests an NP capacity to interfere with normal neurotransmission pathways [35, 38]. Recent studies have demonstrated QD-related neurotoxicity in the CNS, as well as synaptic transmission and plasticity impairments, and deteriorated brain functions in tested animals [39–41]. Another important finding from the present study was the presence of marked anomalies on somites. Somites are transient embryonic structures of the paraxial mesoderm that give rise to all of the striated muscular tissue in the adult body, the axial skeleton, and dermis during later embryogenesis. We found a significant reduction in the size of the somites in embryos treated with CdS-maltodextrin QDs, which correlates with the overall body shortening observed. When we analyzed the size of each somite separately, we observed that the somites in treated embryos tended to have a different size, morphology or absence, contrary to controls.

Birth defects result from errors during embryonic development. Normal development involves careful orchestration of multiple events, including changes in gene transcription, cell shape, cell proliferation and tissue morphogenesis. Alterations in the developmental program may have dramatic effects on the organism. Sometimes, these effects are so drastic that lethality occurs early in the development, while others may cause birth defects. We found several defects in chicken after normal periods of development: chicks with poor development; CNS alterations or even anencephaly; lack of limb motor coordination; spasticity; and a lack of abdominal wall closure. Some limited data from animal reproductive studies and nanomaterials suggest a potentially increased risk of early miscarriages, impaired growth and birth defects [42, 43]. A particular birth defect may be caused by several mechanisms, including folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor or enzyme-mediated teratogenesis. In addition, some drugs may be involved in multiple mechanisms leading to birth defects [44].

Somitogenesis, in particular, is a reiterated process occurring over time with strict periodicity. A pair of somites is formed every 90 min in the trunk of the chicken embryo from the anterior tip of the presomitic mesoderm (PSM). As development proceeds, ventral somitic cells migrate around the axial organs, giving rise to segmented structures such as vertebrae, intervertebral disks, and trunk and limb developments, whereas more dorsal somitic cells give rise to the dermis and all of the striated muscles of the adult body [45]. Somitogenesis can be achieved only by the integration of multiple signaling pathways involving intricate molecular machinery, cell proliferation and the genetic clock [46]. It has been reported that the use of transcription factor chemical inhibitors or genes induces severe defects in somite formation due to segmentation clock desynchronization [47]. Recent reports have shown evidence that the presence of reactive oxygen species (ROS) may affect the metabolic regulation of the ultradian biological oscillator with important pathophysiological implications for somitogenesis [48].

In our study, although higher doses were used in the embryotoxicity study, the lack of development and birth defects was clear, and even with the same doses there were variations in embryo development, which could be explained by individual susceptibility. Mortality was high, and the malformations and stages of development were not compatible with life. However, the presence of defects in limb neuromotor coordination, the lack of abdominal wall closure and neuronal alterations suggest that CdS-maltodextrin QDs might be affecting the early stages of somitogenesis. We suggest that CdS-maltodextrin QDs primarily affect the number of cells that segment together to form individual somites in chicken embryos and that this is responsible for the mortality and all the observed effects on the fetuses. It has been shown that CdS-maltodextrin QDs are able to induce cytotoxicity and cell death, alter cell proliferation and induce the production of radical oxygen species (ROS) in a dose-dependent manner [14]. QD-induced perturbations of cellular mechanisms may cause different pathophysiological processes depending on concentration and duration of exposure [49–51].

In conclusion, our data indicate that CdS-maltodextrin QDs induce embryotoxic and teratogenic effects with all doses used. QDs induced abnormalities associated with structures derived from somites in embryos and fetuses. Therefore, according to the results, there is a high probability that the prolonged accumulation of CdS-maltodextrin QDs in the maternal

organism may be potentially harmful to embryo and fetus development. However, further studies using mammalian species are needed in order to discard more toxic effects.

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References

- [1] Ruenraroengsak P, Cook JM, Florence AT. Nanosystem drug targeting: facing up to complex realities. *J Control Release*. 2010; 141(3):265–76. doi:10.1016/j.jconrel.2009.10.032.
- [2] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, Zhang J. The role of surface chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. *Biomaterials*. 2013; 34(34):8741–55. doi:10.1016/j.biomaterials.2013.07.087.
- [3] Hassan S, Singh AV. Biophysicochemical perspective of nanoparticle compatibility: a critically ignored parameter in nanomedicine. *J Nanosci Nanotechnol*. 2014; 14(1):402–14. doi:10.1166/jnn.2014.8747.
- [4] Brendel K, Duhamel RC, Shepard TH. Embryotoxic drugs. *Biol Res Pregnancy Perinatol*. 1985; 6(1):1–54. doi:10.1007/s11010-011-0733-5.
- [5] Thompson BL, Levitt P, Stanwood GD. Prenatal exposure to drugs: effects on brain development and implications for policy and education. *Nat Rev Neurosci*. 2009; 10(4):303–12. doi:10.1038/nrn2598.
- [6] Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. *Nano Today*. 2014; 9(2):223–43. doi:10.1016/j.nantod.2014.04.008.
- [7] Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, Zou Y, Xu S, Xu K, Ji A, Sheng L. Transfer of quantum dots from pregnant mice to pups across the placental barrier. *Small*. 2010; 6(5):670–8. doi:10.1002/sml.200902049.

- [8] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, Diener PA, Zisch A, Krug HF, von Mandach U. Barrier capacity of human placenta for nanosized materials. *Environ Health Perspect.* 2010; 118(3):432–6. doi:10.1289/ehp.0901200.
- [9] Zalgevičiene V, Kulvietis V, Bulotiene D, Didžiapetriene J, Rotomskis R. The effect of nanoparticles in rats during critical periods of pregnancy. *Medicina (Kaunas).* 2012; 48(5):256–64. doi:10.2147/IJN.S70092.
- [10] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima H, Shishido K, Kawai Y, Mayumi T, Tsunoda S, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat Nanotechnol.* 2011; 6(5):321–8. doi:10.1038/nnano.2011.41.
- [11] Bayne K. Revised guide for the care and use of laboratory animals available. American Physiological Society. *Physiologist.* 1996; 39(4):199, 208–11. PMID: 8854724.
- [12] Zhu X, Tian S, Cai Z. Toxicity assessment of iron oxide nanoparticles in zebrafish (*Danio rerio*) early life stages. *PLoS One.* 2012; 7(9):e46286. doi:10.1371/journal.pone.0046286.
- [13] Bonfanti P, Moschini E, Saibene M, Bacchetta R, Rettighieri L, Calabri L, Colombo A, Mantecchia P. Do nanoparticle physico-chemical properties and developmental exposure window influence nano ZnO embryotoxicity in *Xenopus laevis*? *Int J Environ Res Public Health.* 2015; 12(8):8828–48. doi:10.3390/ijerph120808828.
- [14] Rodríguez-Fragoso P, Reyes-Esparza J, León-Buitimea A, Rodríguez-Fragoso L. Synthesis, characterization and toxicological evaluation of maltodextrin capped cadmium sulfide nanoparticles in human cell lines and chicken embryos. *J Nanobiotechnol.* 2012; 10(47):1–11. doi:10.1186/1477-3155-10-47.
- [15] Gutiérrez-Sancha I, Reyes-Esparza J, Rodríguez-Fragoso P, García-Vázquez F, Rodríguez-Fragoso L. Bright green emitting maltodextrin coated cadmium sulfide quantum dots as contrast agents for bioimaging: a biocompatibility study. *Int J Nanomed Nanosurg.* 2015; 1(2). doi:10.16966/2470–3206.107.
- [16] Gutiérrez-Sancha I, Reyes-Esparza J, Rodríguez-Fragoso P, Rodríguez-Fragoso L. Pharmacokinetic of maltodextrin coated cadmium sulfide quantum dots in rats. *J Nanomed Biotherap Discov.* 2016; 6:139. doi:10.4172/2155-983X.
- [17] Jelínek R, Marhan O. Validation of the chick embryotoxicity screening test (CHEST). A comparative study. *Funct Dev Morphol.* 1994; 4(4):317–23. PMID: 7612953.
- [18] Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *J Morphol.* 1951; 88(1):49–92. PMID: 24539719.
- [19] Wegner KD, Hildebrandt N. Quantum dots: bright and versatile *in vitro* and *in vivo* fluorescence imaging biosensors. *Chem Soc Rev.* 2015; 44(14):4792–834. doi:10.1039/c4cs00532e.

- [20] Ding C, Zhu A, Tian Y. Functional surface engineering of C-dots for fluorescent biosensing and *in vivo* bioimaging. *Acc Chem Res.* 2014; 47(1):20–30. doi:10.1021/ar400023s.
- [21] El-Ansary A, Al-Daihan S, Bacha AB, Kotb M. Toxicity of novel nanosized formulations used in medicine. *Methods Mol Biol.* 2013; 1028:47–74. doi:10.1007/978-1-62703-475-3_4.
- [22] Landsiedel R, Fabian E, Ma-Hock L, van Ravenzwaay B, Wohlleben W, Wiench K, Oesch F. Toxicokinetics of nanomaterials. *Arch Toxicol.* 2012; 86(7):1021–60. doi:10.1007/s00204-012-0858-7.
- [23] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, Zhang J. The role of surface chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. *Biomaterials.* 2013; 34(34):8741–55. doi:10.1016/j.biomaterials.2013.07.087.
- [24] Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. *Nano Today.* 2014; 9(2):223–43. doi:10.1016/j.nantod.2014.04.008.
- [25] Holden PA, Nisbet RM, Lenihan HS, Miller RJ, Cherr GN, Schimel JP, Gardea-Torresdey JL. Ecological nanotoxicology: integrating nanomaterial hazard considerations across the subcellular, population, community, and ecosystems levels. *Acc Chem Res.* 2013; 46(3):813–22. doi:10.1021/ar300069t.
- [26] Maldiney T, Richard C, Seguin J, Wattier N, Bessodes M, Scherman D. Effect of core diameter, surface coating, and PEG chain length on the biodistribution of persistent luminescence nanoparticles in mice. *ACS Nano.* 2011; 5(2):854–62. doi:10.1021/nn101937h.
- [27] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, Zhang J. The role of surface chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. *Biomaterials.* 2013; 34(34):8741–55. doi:10.1016/j.biomaterials.2013.07.087.
- [28] Lee YK, Jeong JM, Hoigebazar L, Yang BY, Lee YS, Lee BC, Youn H, Lee DS, Chung JK, Lee MC. Nanoparticles modified by encapsulation of ligands with a long alkyl chain to affect multispecific and multimodal imaging. *J Nucl Med.* 2012; 53(9):1462–70. doi:10.2967/jnumed.111.092759.
- [29] King-Heiden TC, Wicinski PN, Mangham AN, Metz KM, Nesbit D, Pedersen JA, Hamers RJ, Heideman W, Peterson RE. Quantum dot nanotoxicity assessment using the zebrafish embryo. *Environ Sci Technol.* 2009; 43(5):1605–11. doi:10.1021/es801925c.
- [30] Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, Zou Y, Xu S, Xu K, Ji A, Sheng L. Transfer of quantum dots from pregnant mice to pups across the placental barrier. *Small.* 2010; 6(5):670–8. doi:10.1002/sml.200902049.
- [31] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima

- H, Shishido K, Kawai Y, Mayumi T, Tsunoda S, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat Nanotechnol.* 2011; 6(5):321–8. doi:10.1038/nnano.2011.41.
- [32] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, Diener PA, Zisch A, Krug HF, von Mandach U. Barrier capacity of human placenta for nanosized materials. *Environ Health Perspect.* 2010; 118(3):432–6. doi:10.1289/ehp.0901200.
- [33] Zalgevičiene V, Kulvietis V, Bulotiene D, Didžiapetriene J, Rotomskis R. The effect of nanoparticles in rats during critical periods of pregnancy. *Medicina (Kaunas).* 2012;48(5):256–64. doi:10.2147/IJN.S70092.
- [34] Agarwal R, Domowicz MS, Schwartz NB, Henry J, Medintz I, Delehanty JB, Stewart MH, Susumu K, Huston AL, Deschamps JR, Dawson PE, Palomo V, Dawson G. Delivery and tracking of quantum dot peptide bioconjugates in an intact developing avian brain. *ACS Chem Neurosci.* 2015; 6(3):494–504. doi:10.1021/acscchemneuro.5b00022.
- [35] Libralato G, Minetto D, Totaro S, Mičetić I, Pigozzo A, Sabbioni E, Marcomini A, Volpi Ghirardini A. Embryotoxicity of TiO₂ nanoparticles to *Mytilus galloprovincialis* (Lmk). *Mar Environ Res.* 2013; 92:71–8. doi:10.1016/j.marenvres.2013.08.015.
- [36] Chan WH, Shiao NH. Cytotoxic effect of CdSe quantum dots on mouse embryonic development. *Acta Pharmacol Sin.* 2008; 29(2):259–66. doi:10.1111/j.1745-7254.2008.00743.x.
- [37] Di Guglielmo C, López DR, De Lapuente J, Mallafre JM, Suárez MB. Embryotoxicity of cobalt ferrite and gold nanoparticles: a first *in vitro* approach. *Reprod Toxicol.* 2010; 30(2):271–6. doi:10.1016/j.reprotox.2010.05.001.
- [38] Maisano M, Cappello T, Catanese E, Vitale V, Natalotto A, Giannetto A, Barreca D, Brunelli E, Mauceri A, Fasulo S. Developmental abnormalities and neurotoxicological effects of CuO NPs on the black sea urchin *Arbacia lixula* by embryotoxicity assay. *Mar Environ Res.* 2015; 111:121–7. doi:10.1016/j.marenvres.2015.05.010.
- [39] Yoo MH, Rah YC, Choi J, Park S, Park HC, Oh KH, Lee SH, Kwon SY. Embryotoxicity and hair cell toxicity of silver nanoparticles in zebrafish embryos. *Int J Pediatr Otorhinolaryngol.* 2016; 83:168–74. doi:10.1016/j.ijporl.2016.02.013.
- [40] Agarwal R, Domowicz MS, Schwartz NB, Henry J, Medintz I, Delehanty JB, Stewart MH, Susumu K, Huston AL, Deschamps JR, Dawson PE, Palomo V, Dawson G. Delivery and tracking of quantum dot peptide bioconjugates in an intact developing avian brain. *ACS Chem Neurosci.* 2015; 6(3):494–504. doi:10.1021/acscchemneuro.5b00022.
- [41] Cupaioli FA, Zucca FA, Boraschi D, Zecca L. Engineered nanoparticles. How brain friendly is this new guest? *Prog Neurobiol.* 2014; 119–120:20–38. doi:10.1016/j.pneurobio.2014.05.002.
- [42] Wu T, Zhang T, Chen Y, Tang M. Research advances on potential neurotoxicity of quantum dots. *J Appl Toxicol.* 2016; 36(3):345–51. doi:10.1002/jat.3229.

- [43] Sun Y, Zhang G, He Z, Wang Y, Cui J, Li Y. Effects of copper oxide nanoparticles on developing zebrafish embryos and larvae. *Int J Nanomed*. 2016; 11:905–18. doi:10.2147/IJN.S100350.
- [44] van Gelder MM, van Rooij IA, Miller RK, Zielhuis GA, de Jong-van den Berg LT, Roeleveld N. Teratogenic mechanisms of medical drugs. *Hum Reprod Update*. 2010; 16(4):378–94. doi:10.1093/humupd/dmp052.
- [45] Resende TP, Ferreira M, Teillet MA, Tavares AT, Andrade RP, Palmeirim I. Sonic hedgehog in temporal control of somite formation. *Proc Natl Acad Sci U S A*. 2010; 107(29):12907–12. doi:10.1073/pnas.1000979107.
- [46] Ozbudak EM, Lewis J. Notch signalling synchronizes the zebrafish segmentation clock but is not needed to create somite boundaries. *PLoS Genet*. 2008; 4(2):e15. doi:10.1371/journal.pgen.0040015.
- [47] Yabe T, Takada S. Molecular mechanism for cyclic generation of somites: lessons from mice and zebrafish. *Dev Growth Differ*. 2016; 58(1):31–42. doi:10.1111/dgd.12249.
- [48] Ventre S, Indrieri A, Fracassi C, Franco B, Conte I, Cardone L, di Bernardo D. Metabolic regulation of the ultradian oscillator *Hes1* by reactive oxygen species. *J Mol Biol*. 2015; 427(10):1887–902. doi:10.1016/j.jmb.2015.03.007.
- [49] Liu XQ, Zhang HF, Zhang WD, Zhang PF, Hao YN, Song R, Li L, Feng YN, Hao ZH, Shen W, Min LJ, Yang HD, Zhao Y. Regulation of neuroendocrine cells and neuron factors in the ovary by zinc oxide nanoparticles. *Toxicol Lett*. 2016; 256:19–32. doi:10.1016/j.toxlet.2016.05.007.
- [50] Schiavo S, Oliviero M, Miglietta M, Rametta G, Manzo S. Genotoxic and cytotoxic effects of ZnO nanoparticles for *Dunaliella tertiolecta* and comparison with SiO₂ and TiO₂ effects at population growth inhibition levels. *Sci Total Environ*. 2016; 550:619–27. doi:10.1016/j.scitotenv.2016.01.135.
- [51] Boran H, Ulutas G. Genotoxic effects and gene expression changes in larval zebrafish after exposure to ZnCl₂ and ZnO nanoparticles. *Dis Aquat Organ*. 2016; 117(3):205–14. doi:10.3354/dao02943.