



Surface charge and dosage dependent potential developmental toxicity and biodistribution of iron oxide nanoparticles in pregnant CD-1 mice

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ABSTRACT

Iron oxide nanoparticles have attracted much attention because of their potential applications, such as drug delivery, biomedical imaging, and photocatalysis. Due to their small size and the potential to cross the placental barrier, the risk to pregnant women and the developing fetus from exposure to nanoparticles is of great concern. The developmental toxicity and biodistribution of a single dose versus multiple doses of iron oxide nanoparticles with positive or negative surface charges were investigated in vivo. Multiple doses of positively-charged nanoparticles given over several days resulted in significantly increased fetal deaths and accumulation of iron in the fetal liver and placenta. These results indicate both positively and negatively charged iron oxide nanoparticles have the ability to cross the placenta and accumulate in the fetus, though greater bioaccumulation and toxicity was observed with a positively-charged surface coating.

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1. Introduction

The term nanoparticles (NPs) generally refers to small particles (1–100 nm in diameter) which may exhibit unique size-dependent properties that are not present in bulk materials. These unique properties, small size, as well as the large surface area to size ratio, have generated ample interest in NP technology. By 2015, the world market for nanomaterial-containing products is anticipated to reach \$2.6 trillion [1] and 240 nano-enabled products are estimated to enter the pharmaceutical pipeline [2]. The increased use of NPs in consumer products and biomedicine has led to a significant increase in human exposure to engineered nanomaterials, which has raised serious concerns about the potential risk of nanomaterials, mainly NPs, to human health [3–6]. For example, it is difficult for consumers to avoid the titanium dioxide nanoparticles in sunscreen and silver nanoparticles in food packing. Another growing area of NP research has been pharmaceutical or biomedical research due in part to the small size and potentially increased

biodistribution of NPs. Some of the desirable properties of nanomaterials utilized for the biomedical field include the photothermal transduction of gold nanorods, as well as the paramagnetism of iron oxide NPs. A wide variety of NPs (gold, silver, platinum, iron, titanium dioxide, etc.) have been investigated for many biomedical uses such as carriers in drug delivery systems, imaging contrast agents, cancer treatments, contraceptives, and diagnostics [7,8]. The surface of NPs are often modified to tailor them to specific applications. For example, biomedical applications of iron oxide NPs require a hydrophilic surface coating to increase water solubility as well as prevent NP aggregation. Further studies are needed to examine how this surface modification may influence the toxicity of the NPs.

Iron oxide NPs have been widely explored in drug delivery [9,10], as contrast agents in magnetic resonance imaging (MRI) [11], for soil and groundwater remediation [12], and as photocatalysts [13,14]. In addition, iron oxide is a major potential product of zero-valence iron NPs, the most popular metallic NPs in environmental remediation applications [15–19]. This application has been successfully commercialized in the United States with more than 50 established sites [20]. All of these applications lead to increased production of iron oxide NPs, subsequently increasing their levels in the environment and human exposure to iron oxide NPs. Iron oxide

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NPs are generally believed to be safe [21] and can be potentially reabsorbed through normal iron metabolic pathways (biodegradable) [22,23]. In fact, iron oxide NPs have been in clinical use as MRI contrast agents [24]. However, concerns remain about the potential longterm [25] and developmental [26] effects of iron oxide NPs.

The risk to pregnant women and the possibility of NPs crossing the placenta and reaching the developing fetus are of particular concern [27,28], because fetuses are more sensitive to environmental toxins than adults [29]. Pregnant women may be at risk for single or multiple exposures to iron oxide NPs through multiple sources such as biomedical uses (e.g. MRIs, drug delivery) or environmental exposures due to NPs use in groundwater remediation. Several studies on various NPs using perfused human placenta produced mixed results; some NPs enter the placental tissue and fetal circulation while other NPs enter the placental tissue but do not enter fetal circulation. Gold NPs [30] were able to perfuse into the placental tissue but were not found in fetal circulation; however, various quantum dot NPs perfused into the placental tissue and then entered the fetal circulation [31,32]. The ability to enter fetal circulation appears to be dependent on factors such as NP size and length of perfusion time [31].

Animal studies have shown that NP exposure can cause adverse effects on pregnant mice and their offspring. Silica and titanium dioxide NPs were shown to cross the placenta and accumulate in fetuses in pregnant mice [33]. Titanium dioxide NPs were shown to cross the placenta, transferring from the pregnant mice to their offspring, resulting in brain damage, nerve system damage and reduced sperm production in male offspring [34,35]. In another study of pregnant mice exposed to platinum NPs [36], NPs did not produce fetal abnormalities, fetal death, or accumulation of NPs in maternal uterus, ovaries, or liver but post-natally an increase in pup mortality and a decrease in growth rate were observed. Very little information exists on the effects of iron oxide NPs on embryo-fetal development. In fact, only one published study has been found examining the in vivo developmental toxicity of iron oxide NPs in rodents [26,37]. Noori et al., using a 50 mg NP/kg body mass intraperitoneal dose, observed decreased infant growth as well as an alteration in testicular morphology in offspring who has been exposed to NPs in utero [26,37]. Therefore, further studies on the maternal and fetal effects of NPs are urgent and critical.

Here, spherical iron oxide NPs, approximately 28–30 nm in hydrodynamic diameter, were synthesized as reported previously [38–42] and the hydrophilic ligands polyethyleneimine (PEI) or poly(acrylic acid) (PAA) were attached to the surface of the NPs following reported procedures, yielding NPs with positive and negative surface charges, respectively [40,43]. The aim of this study was to determine whether the surface charge or chemistry of iron oxide NPs influences their ability to cross the placenta and whether they will induce any negative effects on pregnant dams and embryo-fetal development in CD-1 mice when given as a single, low dose or when given as eight consecutive low doses during pregnancy via intraperitoneal injection. In particular, the intent of the work described herein is to correlate the developmental toxicity and possible fetal biodistribution of NPs with surface charge and dosage. The results of these evaluations can be applied to other similar NPs as the risk to pregnant women from exposure to other NPs such as TiO₂, Au, and Ag NPs is also concerning due to the ubiquitous nature of these products in consumer products such as sunscreens and food additives.

2. Materials and methods

2.1. Animals and husbandry

Male and female CD-1 mice were obtained from Charles River Breeding Laboratories, Wilmington, MA. Animals were acclimated

for two weeks prior to mating. Individual animals were uniquely identified by earpunch and cage cards. The temperature was maintained at 22 ± 2 °C with 40–60% relative humidity. The animals were maintained with a 12 h photoperiod, 12 h of light then 12 h of darkness. Untreated animals were bred naturally, two females with one male. Mating was confirmed with the observation of a copulation plug, which indicated Gestation Day (GD)0. Females were randomly assigned to treatment groups immediately after mating and individually housed in polycarbonate shoe-box style cages (29 cm × 19 cm × 13 cm) with hardwood bedding. Mice were provided Teklad LM-485 rodent diet (Harlan Teklad, Madison, WI) and tap water ad libitum throughout the study. All procedures performed on the animals were reviewed and approved by The University of Alabama's Institutional Animal Care and Use Committee (IACUC) and were in accordance with established guidelines. These guidelines include institutional guidelines, International Council of Harmonisation (ICH) guidelines, and the AVMA Guidelines for the Euthanasia of Animals [44,45].

2.2. Nanoparticle preparation and characterization

Iron oxide NPs were prepared by following a modified “heat-up” method, where trioctylphosphine oxide (TOPO) was added during synthesis as a weak binding co-surfactant [38–43]. In brief, the previously described iron oleate complex (2.5 g, 2.8 mmol) was heated up to 320 °C (2.5 h) with the surfactants oleic acid/TOPO (OA – 0.22 mL, 0.7 mmol, TOPO – 0.2 g, 0.5 mmol) in 1-octadecene (10 mL). After the reaction mixture cooled down (20 °C), the as-prepared NPs were separated from the solvent by centrifugation and dried under vacuum. To render NPs water soluble, the hydrophobic surfactants around NP surface were directly replaced by hydrophilic molecules (PAA and PEI) via a ligand-exchange method, as described previously [40]. Briefly, well-dried NP powder was redissolved into chloroform to achieve a stock solution (5 mg/mL). One mL of stock solution was then mixed well with PAA or PEI into 49 mL of dimethyl sulfate oxide (DMSO) by sonication. The NP surface iron atoms to exchange ligands molar ratio was set roughly at 1:5. After 48 h mixing, the water soluble NPs were precipitated out by centrifugation, washed with and redispersed into nanopure H₂O (18 mΩ) (1 mg/mL). NPs were then examined by transmission electron microscopy (TEM) to confirm uniformity of size and distribution in water. Zeta potential was measured using a Zetasizer nano series dynamic light scattering (DLS) instrument to ensure the stability and charge of the NPs. No precipitation was observed after months of storage for these water soluble NPs.

2.3. Treatments

Mated female CD-1 mice were randomly assigned into one of the following treatment groups: (1) a control group, 8 doses distilled H₂O (*n* = 14), (2) 1 dose (10 mg NPs/kg body mass) PEI-NP (*n* = 18); (3) 1 dose (10 mg NPs/kg body mass) PAA-NP (*n* = 16); (4) 8 doses (10 mg NPs/kg body mass) PEI-NP (*n* = 16), and (5) 8 doses (10 mg NPs/kg body mass) PAA-NP (*n* = 16). The concentration of the exposure solutions were 1 mg NPs/mL in nanopure water. All doses of NPs were 10 mg NPs/kg body mass which equates to 2.5 mg Fe/kg body mass. Ferumoxtran-10 (Combidex) is an intravenous iron oxide NP MRI contrast agent which has been used in many studies and clinical trials [46,47]. The dose of 10 mg NP/kg body mass (2.5 mg Fe/kg body mass) was chosen to represent the approximate dose of iron oxide NPs one would receive due to undergoing MRI. Male CD-1 mice were euthanized at the completion of the mating period. Clinical observations were recorded daily and females were weighed on GD 0, as well as before each dosing. Treatments were delivered by intraperitoneal injection(s) during gestation. Animals in groups (2) and (3) were administered a single dose of the test

Table 1
Treatment groups and number of animals per group (n).

	Treatments	n
(1) Controlx8	8 doses of DI H ₂ O given on GD 9 through GD 16	14
(2) PEIx1+	1 dose (10 mg NPs/kg body mass) PEI-NP given on GD 9	18
(3) PAAx1–	1 dose (10 mg NPs/kg body mass) PAA-NP given on GD 9	16
(4) PEIx8+	8 doses (10 mg NPs/kg body mass) PEI-NP given on GD 9 through GD 16	16
(5) PAAx8–	8 doses (10 mg NPs/kg body mass) PAA-NP given on GD 9 through GD 16	16

material on GD 9, while animals in groups (4) and (5) were administered the test material once daily from GD 9 through GD 16. The dosage volume was 0.01 mL/g body weight. The control group received an equivalent volume of the vehicle (H₂O) (Table 1).

2.4. Data collection

Throughout the gestation period, pregnant females were monitored daily for signs of morbidity, behavioral changes, changes in general appearance, and mortality. For treatment groups (2) and (3) maternal body weights were measured on GD 0, GD 9, and GD 17 (without the gravid uterus). For treatment groups receiving 8 doses, groups (1), (4), and (5), maternal body weights were measured on GD 0, GD 9 through GD 16, and on GD 17 after the fetuses were removed. Dams were sacrificed on GD 17, one day prior to parturition which occurs on GD 18. Animals were euthanized by CO₂ inhalation in accordance with institutional guidelines and the AVMA Guidelines for the Euthanasia of Animals [45]. Presumed pregnant females were euthanized by CO₂ asphyxiation, their uteri were exposed, and the uterine contents were examined for the numbers of live and dead fetuses, early or late resorptions, and total implantation sites. If no implantation sites were observed, the female was considered not to have been pregnant. Live fetuses were removed from the uterus, weighed individually, and examined changes in external morphology. Maternal body weight, minus the gravid uterine weight, was then obtained. Maternal body weight gain was calculated by subtracting the maternal body weight on GD 0 from the maternal body weight on GD 17 minus the gravid uterus.

Placenta, fetal liver, and fetal kidney were collected from each treatment group on GD 17 in order to measure the ability of the positively and negatively surface-charged coated NPs to cross the placenta and enter the fetus during pregnancy. In order to qualitatively observe changes in iron concentration, tissue samples were fixed in 4% paraformaldehyde prior to histological sectioning and stained with Prussian Blue, an iron selective stain. Increases in iron content were visualized by an increase in blue pigment when viewed with an optical microscope, indicating increased iron oxide

NPs. These visual results were quantified by assaying the samples for iron using an ultraviolet/visible spectrophotometer by the colorimetric ferrozine method [48].

Live fetuses were euthanized via intraperitoneal administration of Euthasol and fixed in 70% ethanol in compliance with IACUC standards. Fetuses were subsequently eviscerated, cleared with KOH, and stained with Alcian blue and Alizarin red (Sigma–Aldrich, St. Louis, MO) using the double-staining technique described by Webb and Byrd [49]. Bony structures and cartilage of all fetuses were examined for malformations and variations using a dissecting microscope.

2.5. Statistical analysis

The litter or the pregnant female were used as the experimental unit for statistical analysis. This study was performed in multiple replicates. The data from each replicate were calculated independently, tested for homogeneity of variance by means of the Levene statistic using SPSS (SPSS, Inc., Chicago, IL), and then pooled and analyzed to give the results reported. All tabular data are presented as the mean \pm standard error (SEM). Data were analyzed by one-way analysis of variance (ANOVA) or Kruskal–Wallis one-way ANOVA followed by a least significant difference (LSD) or Dunn's post hoc test, respectively, to determine specific significant differences ($p \leq 0.05$).

3. Results and discussion

3.1. Nanoparticle synthesis and characterization

Fig. 1 shows the transmission electron microscopy (TEM) images of the PAA and PEI coated iron oxide NPs. The TEM images show the NPs are spherical in shape with a uniform, narrow size distribution. The groups of NPs on the image were the result of NPs that fell on top of each other during sample preparation, not NP aggregates. The water dispersity of these NPs were previously determined by DLS analyses where the hydrodynamic sizes of the PAA and PEI coated NPs were about 28 and 30 nm, respectively [43]. Zeta potentials

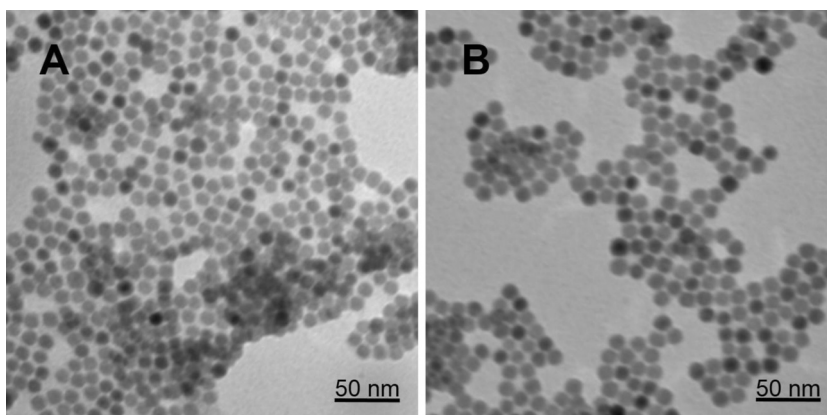


Fig. 1. TEM images of (A) PEI-NPs and (B) PAA-NPs in H₂O.

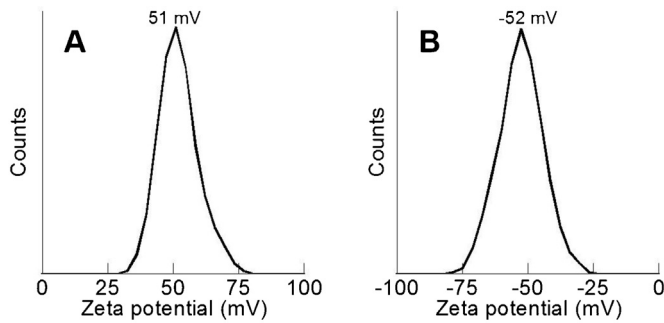


Fig. 2. Zeta potentials of (A) PEI-NPs and (B) PAA-NPs in H₂O.

were measured to determine surface charges of the polymer-coated NPs. Zeta potential values above 30 mV or below –30 mV indicate stability of a colloid system [43]. The measured zeta potential values (Fig. 2) of 51 mV for PEI-NPs and –52 mV for PAA-NPs indicate high stability of these NPs as their absolute values are well above 30 mV. This high stability of the colloid system should lead to a resistance toward aggregation, which was confirmed with TEM.

3.2. Effect of charged NPs on dams

Maternal body weight gain during gestation is an indicator of maternal health during pregnancy and can have long term effects on the developing fetus [50,51]. A single, low dose of either the positively or negatively coated NPs when given on GD 9 (treatments (2) and (3)) had no effect on maternal weight gain. Maternal body weight gain significantly decreased about 40% ($p \leq 0.05$) during gestation when the animals received the positively charged PEI-NPs for eight consecutive days (4) when compared to the control group (1), indicating an apparent treatment effect (Fig. 3). This effect was not observed in animals receiving the negatively charged PAA-NPs for eight consecutive days (5), indicating a difference in toxicity with different charged polymeric coatings (Table 2).

No evidence of morbidity, mortality, changes in behavior, or changes in general appearance was observed for any treatment group. Decreased maternal weight gain observed in treatment (4) with multiple maternal exposures across several days to positively charged iron oxide NPs indicates that these NPs may be accumulating in the mother, negatively affecting maternal health. These results were not observed in dams dosed with negatively charged

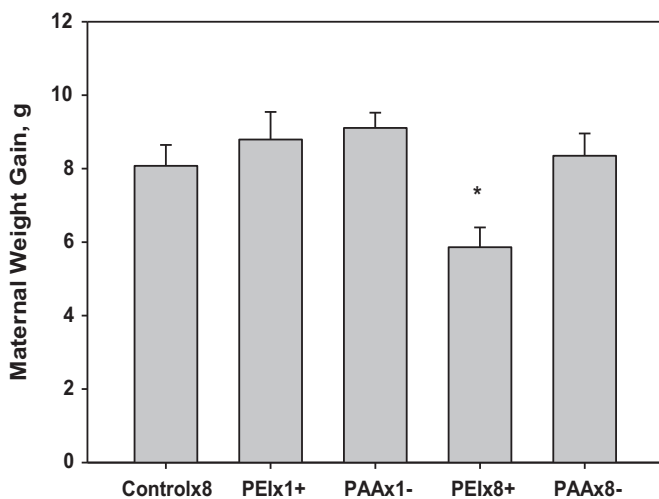


Fig. 3. Maternal weight gain assessed by subtracting the female body mass measured on GD 0 from the final body mass minus gravid uteri on GD 17, $n = 14–18$, *significant differences compared to all other groups ($p < 0.05$).

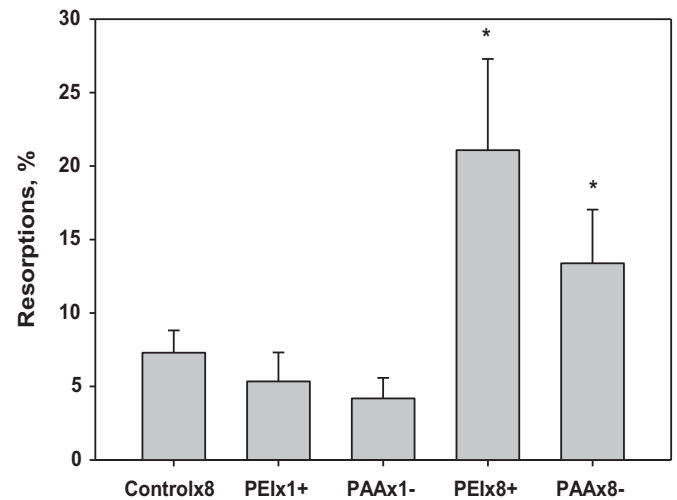


Fig. 4. Percent resorbed fetuses, $n = 14–18$, *significant difference versus control and single dosed treatment groups ($p < 0.05$).

NPs (5), indicating a difference in toxicity based on surface charges. As the health of the mother has a direct influence on the health of the fetus, maternotoxicity could translate into adverse effects on fetal development such as decreased fetal weight, skeletal anomalies and post-implantation loss [52].

3.3. Effects of charged NPs on litter values

The number of implantations did not differ significantly between treatment groups. The percentages of resorbed or dead fetuses was significantly higher in the PEI-NP and PAA-NP treatment groups ((4) and (5)) that were treated with 10 mg/kg body mass daily for eight consecutive days (GD 9–16) when compared to the control ((1), H₂O only) (Fig. 4). The animals dosed only once with NPs ((2) and (3)) did not show increased resorptions or fetal death and were comparable to the control group. There was no effect on litter size or fetal weight among all treatment groups. Few changes in external morphology were observed in treatment groups exposed to 8 doses of NPs ((4) and (5)). One fetus in treatment (5), PAAx8–, exhibited signs of talipes (club foot) in combination with a shortened, bent tail. In treatment (4), PEIx8+, four mice from two litters exhibited altered external morphology. In the first litter, one fetus was observed to have a bent tail. The second litter contained three abnormal fetuses, one exhibited talipes (club foot), a second exhibited talipes with a short tail, while a third exhibited exencephaly and a curly tail. The dam which gave rise to three offspring with external malformations also gave birth prematurely. No changes in external malformation were observed in mice in the control group, or either treatment given a single dose of either NP (treatment (1), (2), or (3)). A slight increase was observed in the number of skeletal variations such as supernumerary ribs in treated litters as shown in Table 3, but these increases were not statistically significant. Incidence of supernumerary ribs appeared highest in offspring of females treated with positively charged PEI-NPs (single (2) or multiple dose (4)).

Perhaps the most troubling result is that multiple low-dose exposures of either charged NP ((4) or (5)) lead to significant increases in post-implantation loss, specifically resorptions (both early and late depending on surface coating, Table 4). The average resorption incidence for the controls (1) and single-dosed groups ((2) and (3)) ranged from 4 to $7.3 \pm 1.6\%$ resorptions, and it is not uncommon to see a small number of resorptions in control groups. To compare, the average resorption incidence for multiple doses of positively charged PEI-NPs (4) and negatively charged PAA-NP

Table 2Maternal weight gain (g \pm SEM) for treatment groups as follows (1) Controlx8, (2) PEIx1+, (3) PAAx1–, (4) PEIx8+, and (5) PAAx8–, $n = 14$ –18.

	Controlx8	PEIx1+	PAAx1–	PEIx8+	PAAx8–
Maternal weight gain, g	8.1 \pm 0.6	8.8 \pm 0.8	9.1 \pm 0.4	5.9 \pm 0.5*	8.4 \pm 0.7

* Significant differences compared to all other groups ($p < 0.05$).**Table 3**Litter values for treatment groups as follows (1) Controlx8, (2) PEIx1+, (3) PAAx1–, (4) PEIx8+, and (5) PAAx8–, $n = 14$ –18.

	Controlx8	PEIx1+	PAAx1–	PEIx8+	PAAx8–
Litter size	15.6 \pm 0.7	14.3 \pm 0.4	14.1 \pm 0.6	14.1 \pm 0.6	14.1 \pm 0.5
Fetal body mass GD 17, g \pm SEM	0.99 \pm 0.03	0.98 \pm 0.02	1.06 \pm 0.03	1.02 \pm 0.02	1.00 \pm 0.05
Resorptions, % \pm SEM	7.3 \pm 1.4	5.8 \pm 2.2	4.2 \pm 1.4	21.5 \pm 6.2*	13.5 \pm 3.7*
Supernumerary ribs, % \pm SEM	12.4 \pm 3.5	18.8 \pm 5.1	14.8 \pm 4.1	21.8 \pm 5.5	16.4 \pm 4.8

* Significant difference versus control and single dosed treatment groups ($p < 0.05$).**Table 4**

Resorptions and dead fetus distribution. Total resorptions and dead fetuses are expressed as the average percentage in each litter.

	Controlx8	PEIx1+	PAAx1–	PEIx8+	PAAx8–
Total resorptions, % \pm SEM	7.3 \pm 1.4	5.8 \pm 2.2	4.2 \pm 1.4	21.5 \pm 6.2*	13.5 \pm 3.7*
Early resorptions, %	40.0	57.1	100	75.6	25.9
Late resorptions, %	60.0	42.9	0	24.4	74.1
Dead fetuses, %	0.5	0.5	0	0.9	0.5

Early and late resorptions are presented as a percentage of the total resorptions. $n = 14$ –18.* Significant difference versus control and single dosed treatment groups ($p < 0.05$).

(5) were $21.5 \pm 6.2\%$ and $14.8 \pm 4.1\%$, respectively. The increase in the percentage of resorptions for both the negatively and positively charged NPs when given a small dose for eight consecutive days indicates that the NPs are negatively effecting embryo-fetal survival. The observed increase in resorption incidence may be a result of a single small dose of NPs on a specific gestation day or an accumulation effect from multiple exposures to NPs. More studies are needed in order to ascertain which is occurring. The resorption incidence appeared higher in the positively charged PEI-NPs (4) compared to the negatively charged PAA-NPs (5) given for eight consecutive days, but they were not found to be significantly different from each other, though they are both significantly different from the control (1). Though the percentage of resorptions between females given both the positively (4) and negatively (5) charged NPs 8 times were comparable, the stage in pregnancy in which the resorptions occurred varied. Approximately 76% of the resorptions observed in treatment (4), PEI-NPx8+, occurred as early resorptions while approximately 74% of the resorptions observed in treatment (5), PAA-NPx8–, occurred late during pregnancy. This difference in the occurrence of resorptions indicates that the mechanism of toxicity of the positively and negatively charged NPs may be affecting the fetus at different stages of gestation.

3.4. Biodistribution of charged NPs in fetal tissues

Iron concentrations were measured in samples taken from the mother and fetus to determine if the NPs were able to cross the placenta into the fetus. No differences were observed in the level of iron in the kidneys of fetuses from dams given PEI-NPs ((2) and (4)) or PAA-NPs ((3) and (5)) on GD 9 or GD 9 through GD 16 compared to controls (1). In addition, when mice received only one dose of NPs with either coating ((2) or (3)), no differences were observed in the level of iron in the fetal liver or placental samples compared to controls (1). Significant increases in iron content were observed in the fetal liver and placenta in the animals treated with positively charged PEI-NPs for eight consecutive doses (4), but not in other treatment groups (Figs. 5 and 6). This sharp increase in iron indicates an increased concentration of iron oxide NPs. The observation of increased iron concentration in the mice dosed with the positively charged PEI-NPs (4), but not in the negatively charged PAA-NPs (5) indicates that the surface charge of the NPs may play a role in bioaccumulation in the developing fetus. Increased iron concentrations in the liver and placenta of fetuses dosed with NPs were only observed in the treatment group receiving multiple doses of NPs (4). These particular NPs (iron oxide) are used as contrast

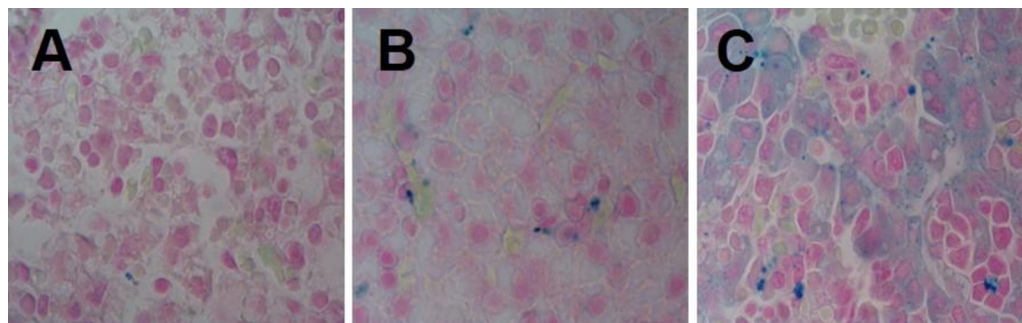


Fig. 5. Fetal livers stained for iron content using Prussian Blue (blue indicates presence of iron) in (A) Control (H_2O treated) (1), (B) 1 dose of PEI NPs (2), and (C) 8 doses of PEI coated NPs (4).

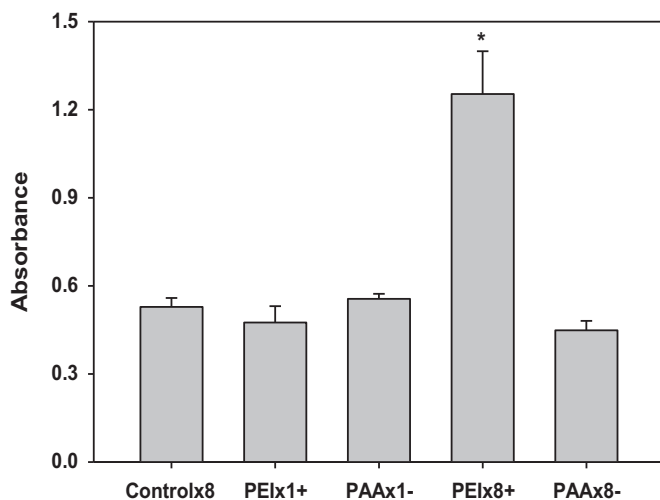


Fig. 6. Fetal liver iron content, $n=9$, *significant differences compared to all other groups ($p < 0.05$).

agents for MRI due to their ability to deposit in organs such as the liver, spleen, lymph nodes, and bone marrow. Biodistribution studies of similar NPs (Ferumoxytol) with a similar size and polymeric coating uptake primarily in the reticuloendothelial cells of the liver [53]. Though it cannot be determined whether the iron observed in the fetal liver are NPs (Fe_2O_3) or Fe^{3+} , the sharp increase in iron concentration is only observed in the treatment receiving multiple doses of positively-charged PEI-NPs, it can be ascertained that the increase in iron in the fetal liver is a result of NP exposure.

The results observed throughout this study exhibit similarities to other studies of metal oxide NPs as well as differences. Similarly to the study herein, investigations into the developmental toxicity of another metal oxide, TiO_2 , observed an increase in fetal resorptions as well NPs present in the fetal livers and placentae with exposure to TiO_2 NPs on GD 16 and GD 17 [33]. The TiO_2 particles were uncoated and the negative developmental effects observed were ameliorated with addition of a charged surface coating ($-\text{COOH}$ or $-\text{NH}_4$). The TiO_2 NPs were several times larger than the iron oxide NPs being discussed herein, and size is a very important factor in NP toxicity and biodistribution. A 2011 study into the developmental toxicity of anionic dimercaptosuccinic acid (DMSA) coated Fe_3O_4 NPs monitored pups after prenatal exposure to a single, intraperitoneal dose of NPs on GD 8 [26]. Fetuses were examined at GD 13 for iron accumulation in the liver using Prussian Blue staining. Aggregates of iron oxide NPs were observed in the placentae as well as the sinusoids and hepatocytes of the fetal liver as in this study [26]. Noori et al. went on to observe pup weights and testes development, indicating abnormal development of the seminiferous tubules when given at higher doses (>50 mg NP/kg body mass). The observations presented throughout this study as well as other studies of metal oxide NPs support the data that NPs have the ability to cross the placenta, accumulate in the fetus, and cause detrimental effects on development in a dose and surface coating dependent manner [26,33].

4. Conclusions

Due to their small size, customizability, and unique properties, NPs may be beneficial in many fields, including biomedicine, but particular care is needed to evaluate their toxicity. Increased toxicity due to exposure and charge were observed here. Following 8 consecutive days of dosing, PEI-NPs (4) reduced maternal body weight gain and increased the level of iron in placentae and fetal livers. These observations were not observed in groups that

were given 8 consecutive doses of PAA-NPs (5), a single dose of either PEI-NPs (2) or PAA-NPs (3), or the control (1). Increased postimplantation loss was observed in treatment groups receiving 8 consecutive doses of either PAA-NPs (5) or PEI-NPs (4). Though the NPs are composed of the same core material, when their surface charge is changed by varying the polymer coating they interact and accumulate in the mother and fetus differently. Through multiple exposures, positively charged NPs (4) appear to accumulate in the fetal liver, while accumulation of the negatively charged NPs (5) was not observed. Overall, the positively charged PEI-NPs (4) induced greater toxic effects when given multiple times; increasing postimplantation loss significantly ($21.5 \pm 6.2\%$ versus $7.3 \pm 1.4\%$ of controls), significantly decreasing maternal weight gain, and crossing the placenta to accumulate in the fetal liver. Though these differences were observed between charged NPs, multiple exposures of either charged NPs ((4) or (5)) induced significantly increased fetal death.

No negative developmental effects were observed when dams were given a single, low-dose of iron oxide NPs with either charged coating ((2) or (3)), but when given multiple doses ((4) and (5)), increased fetal death and decreased maternal weight gain was observed dependent on the polymeric coating. Thus, pregnant women and their offspring exposed to such NPs may be at risk with multiple exposures.

These results bring up a more pressing issue which is the regulation and toxicity of NPs. Though the core material (iron oxide) is consistent, the functionalization of the surface with different polymers with different charges induces different developmental toxicity. Surface charge should be considered when evaluating new NPs, especially for consumer or biomedical applications. These preliminary studies indicate an increased risk of maternotoxicity and fetotoxicity with multiple exposures to positively charged NPs compared to negatively charged NPs. More in depth studies are needed to elucidate the role of surface charge in the developmental toxicity of NPs.

Conflict of interest

The authors report no conflicts of interest.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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