Estimating Combined Health Risks of Nanomaterials and Antibiotics From Natural Water: a Proposed Framework

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Research Article

Keywords: Antibiotics, nanoparticles, interaction, water, health risk, oral exposure

Posted Date: June 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-541720/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on October 1st, 2021. See the published version at https://doi.org/10.1007/s11356-021-16795-x.

Estimating combined health risks of nanoparticles and antibiotics from natural water: A proposed framework

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Abbreviations

ABs	antibiotics
ADD	average daily dose
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	bio-accessibility fraction
CDI	chronic daily intake
CuO	copper oxide
Fe_3O_4	iron oxide
HQ	hazard quotient
IRIS	Integrated Risk Information System
NPs	nanoparticles
PNEC	Predicted No-Effect Concentration
RfD	reference dose
TiO_2	titanium dioxide

USEPA	United States Environmental Protection Agency
WoE	Weight of Evidence
ZnO	zinc oxide

Abstract

Nanoparticles are the major class of emerging contaminant detected at relatively high concentrations in aquatic environment. They are likely to co-exist with other chemical pollutants such as antibiotics in natural water systems. There are chances that if they are taken up orally, might pose adverse effects to human health. To address this issue, a risk framework is developed to study the combined exposure of nanoparticles and antibiotics in natural waters for the first time. The framework was applied to a hypothetical exposure of nanoparticles (CuO, ZnO, Fe_3O_4 and TiO_2) and antibiotics (ciprofloxacin, CIP; ofloxacin, OFX; norfloxacin, NOR; levofloxacin, LEVO) to estimate human risks in a six-step approach for two different exposure scenarios i.e. availability adsorption isotherm data and vice versa. Risk was also estimated for the released fragments of antibiotics, nanoparticles and metal ions in the human digestive system. Mixture toxicity risk assessment was conducted for pairs (i) antibiotics and metal ions, (ii) antibiotics and nanoparticles, and (iii) nanoparticles and metals ions. Though the estimated risk values were observed to be less than 1 (both hazard quotients and hazard interactions less than 1) for all the conditions and assumptions made but it requires through monitoring of the studied contaminants in water to protect humans from their adverse effects, if any. Maximum allowable concentrations at which no risk occurs to humans was found to be (maximum values): antibiotics (233.8 µg/L, NOR); metal ions (1.02×10^9 mg/L, Ti^{2+} ions), and nanoparticles (6.68×10^5 mg/L, TiO_2), respectively.

Keywords: Antibiotics; nanoparticles; interaction; water; health risk; oral exposure

1 Introduction

In the past few years, increased concerns have been raised due to occurrence of a wide variety of emerging contaminants including nanomaterials, pharmaceutical drugs etc., in natural water systems. Studies suggest that the concentration of these contaminants in water ranged from µg/L to ng/L (Chen et al., 2016; Ebele et al., 2017) and even high concentration values has also been reported. Nanoparticles usually display unique physical and chemical properties, and because of their inherent reactivity with other pollutants, nanoparticles may act as a carrier and co-occur with other pollutants producing long-term environmental and health risks (Azizi et al., 2016; Wang et al., 2016).

Upon release and emission, contaminants like nanoparticles may interact with chemicals (antibiotics) in the environment, potentially leading to a co-exposure of organisms and the occurrence of mixture effects (Naasz et al., 2018). That co-exposure to nanoparticles and antibiotics may occur is a valid assumption. Both substances are likely to be present and co-exist in the aquatic environment (Lammel et al., 2019). Although studies have been conducted to identify the occurrence of these contaminants but very little or restricted information is available about their environmental exposure (Coll et al., 2016; Holden et al., 2014). In real life scenarios, they might differ in their toxicity or can undergo transformation to produce products which under certain circumstances might show harmful effects and thus needs to be addressed.

Organisms are usually exposed to multiple mixtures of contaminants instead of single compounds (Uwizeyimana et al., 2017). During the process of passage, it is possible that nanoparticles or pharmaceutical drugs can form nanoparticle-toxin complexes or antibiotics

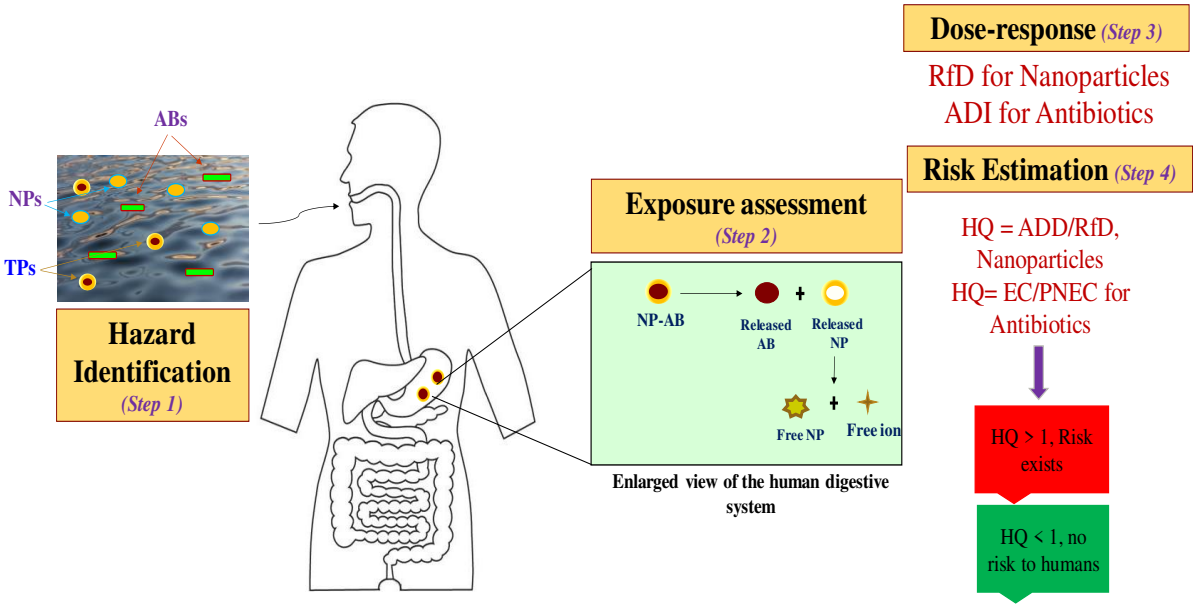
complexes or even produce inter-category complexes of two contaminants due to nanoparticles high surface area and large aggregates (Zhu et al., 2011) thus, there are ongoing concerns on evaluating the environmental risk for the mixtures containing NPs. However, studies on the interaction of two different types of emerging contaminants have so far not been reported. In recent years the scientific community has undertaken enormous efforts to assess the eco-toxic potential of nanomaterial (Menard et al., 2011), but there are comparatively few studies that have investigated their interaction and combined toxicological effects with co-existing "traditional" environmental pollutants (Canesi et al., 2015; Hartmann and Baun, 2010; Naasz et al., 2018).

Looking into the potential adverse effects of these contaminants on human health, risk assessment studies have been conducted for some classes, for example, nanoparticles (Parsai and Kumar, 2020), pharmaceutical drugs (antibiotics) (Kumari and Kumar, 2020) etc., (Supplementary Table S1) but none of the reported studies (as per authors best knowledge) have tried to capture the interaction aspect linked with nanoparticles and antibiotics in natural water systems. Lack of available guidelines and regulations adds to the ongoing problem and makes it even more difficult, if not properly taken care of. Therefore, it becomes imperative to study and identify the interaction of these contaminants in water so that guideline values can be formulated for exercising appropriate control measures.

This study aimed at proposing a framework to determine the risk exposure effects of nanoparticles and antibiotics to the human digestive media or GI-tract followed by oral ingestion. Widely detected nanoparticles, NPs (ZnO, CuO, Fe_3O_4, and TiO_2) and pharmaceutical drugs, antibiotics (Ciprofloxacin, Ofloxacin, Norfloxacin, and Levofloxacin) in natural water were selected.

2 Methodology

Figure 1 shows the flow diagram of the proposed framework for determining health risk estimates due to the interaction of nanoparticles and antibiotics in natural water followed by oral ingestion. The study used a six-step risk assessment approach to determine risk exposure effects of nanoparticles and antibiotics to human health (Kumari and Kumar, 2020a; Kumar et al., 2014). Briefly, this framework assumes that when these contaminants enters into the human body via oral route they might get disintegrate into respective antibiotics and nanoparticles with due course of time in the human digestive system. The released nanoparticles might get further dissolved into their respective ions, releasing free nanoparticles. These released metals can cause harmful health effects in spite of the fact that zinc, copper, and iron are essential elements for all living organisms if present in excessive quantities (Evangelou et al., 2007; Twining et al., 2005). Therefore, this approach assumes the probability of risk exposure effects due to released antibiotics and released nanoparticles in the human digestive system. Risk assessment study was also conducted for free nanoparticles and free metals ions after the dissolution of nanoparticles in human digestive liquid to check whether they pose any possible health risks or not. This study does not consider the size, shape, charge, and surface area of nanoparticles as they might show large variation after their dissolution in human digestive system as it is reasonably tough to capture these aspects in real life scenarios.



123

124 **Fig. 1** Hypothetical schematic diagram showing the uptake of nanoparticles-antibiotics
125 transformed products and their dissolution in the human digestive system (NPs =
126 nanoparticles, ABs = antibiotics, TPs = transformed products, HQ = hazard quotient, EC =
127 Environmental concentration, PNEC = Predicted no-effect concentration; RfD = Reference
128 dose; ADI = Acceptable daily intake)

129

130 2.1 Hazard Identification

131 Hazard identification involves identifying the material of interest and collecting information
132 for which risk evaluation needs to be done. To determine the suitability of the suggested
133 framework, this study considered the hypothetical exposures of nanoparticles and antibiotics
134 for illustrative purpose. Nanoparticles such as CuO, ZnO, Fe_3O_4 and TiO_2 were considered as
135 they are used as antimicrobial agents and additives in consumer and health-care products. The
136 risk assessment of selected nanoparticles is essential as several research investigations have
137 shown their adverse effects to human health (Croteau et al., 2014; Ye et al., 2018). Amongst
138 the nanoparticles selected iron oxide nanoparticles (Fe_3O_4, γ-Fe_2O_3 and superparamagnetic

IONPs) have been extensively used for pharmaceutical applications (Ding & Guo, 2013; Namvar et al., 2014). CuO NPs might induce oxidative stress resulting in destruction of human liver cell (Shukla et al., 2013). ZnO is one of the most frequently detected NPs in surface water (Kurlanda-Witek et al., 2014) and has been reported be cytotoxic and harmful compared to other metallic nanoparticles (Li et al., 2020).

To study the interaction of nanoparticles with antibiotics, the most widely detected fluoroquinolones (FQs) antibiotics [ciprofloxacin (CIP), ofloxacin (OFX), norfloxacin (NOR), and levofloxacin (LEVO)] in the environmental media were selected owing to their use in the treating pulmonary, urinary, and digestive infections. The selected antibiotics are also effective in treating wide range of pathogenic bacteria (gram-negative and gram-positive) and mycoplasmas (Hooper and Wolfson, 1993; MacGowan and Andersson, 2003). CIP, NOR, and OFX common antibiotics administered to humans (Alder et al. 2001; Choma 2003). Amongst these, CIP is one of the most extensively used drug in the world (Sukul and Spiteller, 2007) and LEVO has been listed as one of the most essential medicine of human use by the World Health Organization (Hooper and Rubinstein, 2003). The environmentally occurring concentration (EC) of antibiotics and nanoparticles is taken from published scientific literature. The information is provided as supplementary text Table S2 and S3.

2.2. Exposure assessment

This research study evaluated health risks due to the exposure of inter category emerging contaminants (nanoparticles and antibiotics) to children as they have been recognised to be the most sensitive sub-population compared to adults (Preston, 2004). To determine exposure effects, health risks was estimated considering two different scenarios as mentioned above in Fig. 1a. Detailed information is provided below.

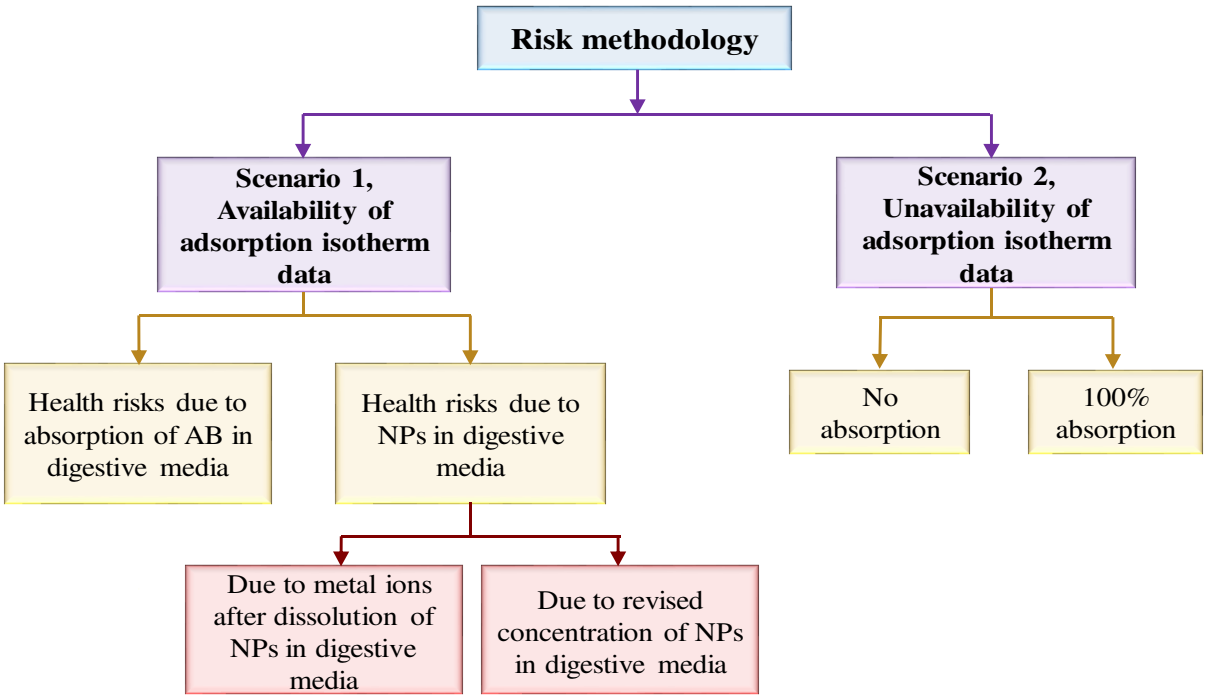


Fig. 1a Scenarios considered for determining risk exposure to humans

Under scenario 1, the study considered nanoparticles for which the adsorption isotherm data was available in literature. Scenario 2 indicates the condition where the adsorption isotherm data for the selected nanoparticles was not available in published literature. For scenario 1, risk was estimated for two different exposure routes (1) Risks due to absorption of antibiotics in the digestive media only, and (2) Risk due to absorption of nanoparticles in digestive media. Under exposure route 2, two sub-routes were considered: (a) Route 2a: risk due to exposure to metal ions after the dissolution of nanoparticles in digestive liquid, (b) Route 2b: risk due to the revised concentration of nanoparticles in digestive media. For scenario 2, two cases (i) no absorption, and (ii) 100% absorption were considered. The detailed information is provided in the sections to follow.

179 *2.2.1 Risk estimation of NPs in the digestive media followed by GI-tract absorption when the*
180 *isotherm data is available*

181 It is believed that oral ingestion serves as the main and primary route for the probable uptake
182 of NPs to target sites or structures followed by their dissolution in the human digestive
183 system (Fig. 1). In risk assessment, dissolution of NPs in the human digestive system gives a
184 realistic and accurate quantity of nanoparticle loading to the digestive system. During risk
185 estimation process, bio accessibility fraction of nanoparticles (BAF_{NP}) was considered to
186 show the influence of parameters governing the interaction of NPs with digestive liquid, and
187 also to calculate the realistic exposure dose. BAF_{NP} serves an important parameter as it
188 considers the dissolution of nanoparticle to metals ions in the human digestive system. This
189 study used the BAF values of nanoparticles available in literature for risk assessment purpose
190 (Zhong et al., 2017). It is observed that the dissolution of nanoparticles led to the release of
191 metal ions in the digestive system. Therefore, it is also important to determine exposure doses
192 for the residual concentrations of released metal ions and nanoparticles in the digestive
193 system to get the overall risk exposures of NPs absorption in the digestive media.

194 Risk estimation of metal ions released after bio-assimilation of NPs in the digestive
195 media was carried out in terms of the average daily dose of metal ions ($ADD_{Mi,\ DM}$) to the
196 human digestive system using the BAF_{NP} as mentioned above. $ADD_{Mi,\ DM}$ (mg/kg/day) for
197 individual metal ions in the digestive system was estimated using Eq. (1). Similarly, risk of
198 revised concentration of NPs in the digestive media followed by GI-Tract absorption was
199 calculated as $ADD_{R\_NP,\ DM}$. $ADD_{R\_NP,\ DM}$ of individual NP was determined using Eq. (2).

200

201 $$ADD_{M_i,DM} = \frac{C_{Mi} \times BAF_{NP} \times IR_w \times EF \times ED}{BW \times AT} \qquad (1)$$

202

$$ADD_{R_{NP},DM} = \frac{C_{NP,DM} \times BAF_{NP} \times IR_w \times EF \times ED}{BW \times AT} \quad (2)$$

Where, C_{Mi} is the concentration of released metal ions in the digestive media; BAF_{NP} is the bio-assimilation potential of NPs; $C_{NP,\ DM}$ is the revised concentration of NPs in the human digestive system.

2.2.2 Risk estimation of ABs in the digestive media followed by oral administration when the isotherm data is available

In this case, concentration of nanoparticles was assumed to be 1 mg/L. Concentration of ABs after adsorption by nanoparticles (mg/g) is taken from published literature, provided as supplementary information, Table S2. To determine the concentration of antibiotics in adsorbed form on nanoparticles in mg/L, the concentration of nanoparticles (mg/L) was multiplied by concentration of antibiotics after absorption on NPs (mg/g) and divided by 1000. 1000 is the conversion factor. To calculate the concentration of antibiotics in the human digestive media followed by GI-tract absorption ($Conc_{AB\_DM}$) after oral ingestion, dissolution rate of antibiotics in the digestive media and the rate at which antibiotics is getting absorbed by the GI-tract is taken into account. Dissolution rate and absorption rate of data of antibiotics is taken from those reported in literature and is provided as supplementary Table S4. $Conc_{AB\_DM}$ is calculated using Eq. (3).

$$Conc._{AB\_DM} = Conc.\ of\ AB\ in\ absorbed\ form\ of\ NPs \times Dissolution\ rate\ of\ AB \times Absorption\ rate \quad (3)$$

To determine the risk exposure effects, chronic daily intake (CDI) values (mg/kg-bw/day) were estimated using Eq. (4).

$$CDI_{AB\_DM} = \frac{C_{AB\_DM} \times IR \times ET \times ED}{BW \times AT} \quad (4)$$

Where, C_{AB_DM} is the concentration of antibiotics in the digestive media followed by GI-tract absorption in GI-tract in mg/L, BW is the body weight (Kg); IR is the intake rate of water, EF is exposure frequency (365 days/year), and ED is the exposure duration (70 Yrs.).

2.2.3 Risks estimation when the adsorption isotherm data were not available

This study also tried to determine risk exposure effects for human health for a hypothetical situation where the adsorption isotherm data of ABs on adsorption by nanoparticles is not available. Under this scenario, two different cases (i) no absorption, and (ii) 100% absorption were considered to determine risk estimates.

(i) No absorption: Under this case, it is assumed that no absorption of NPs takes place in the GI-tract, and the ABs ingested remains as free ABs in the digestive media. Risk exposure of ABs was estimated using the surface water concentration of ABs as mentioned in the hazard identification section. Acceptable daily intake (ADI) values of individual antibiotics were used to calculate the Predicted no-effect concentration (PNEC) values. ADI values specifies the level of daily intake that should not result in any damaging effects to human health from direct exposure (Cunningham et al., 2009) whereas PNECs represent the lowest concentration values at which no harmful effects are anticipated. Input parameters used to estimate PNEC values and ADI values of the antibiotics is given in Table 1. PNEC values were estimated using Eq. (5) as given below.

$$PNEC = \frac{ADI \times BW}{IR_w \times GI_{AF}} \quad (5)$$

Where, ADI is Acceptable Daily Intake (µg kg-day^{-1}); BW is body weight of children in Kg; IR_w is the intake rate of water in L day^{-1}; and GI_{AF} is the gastrointestinal absorption factor of antibiotics, assumed to be 1 in this study.

(ii) 100% absorption: Under this case, it is assumed that the amount of NPs and ABs is getting fully absorbed in the GI-tract. Risk was estimated similar to the approach mentioned in section 2.2.1.

2.3 Dose-response assessment

Limited available data on the reference doses (RfDs) of nanoparticles, for instance, TiO_2 and Fe_3O_4 in the human digestive system makes it difficult to determine possible risks to human health. To fill this knowledge gap, this study used the recommended reference dose values of ions to denote the reference dose values of nanoparticles, where the RfD values of nanoparticles were considered equal to their corresponding ions. As mentioned earlier, since the RfD values for a majority of nanoparticles considered in this study is not available in the published literature hence, toxicity values of metal ions were used to represent the toxicity of NPs. A reference dose value for iron is neither available in the Integrated Risk Information System (IRIS) (U.S. EPA, 2006a) nor the Drinking Water Standards and Health Advisories list (U.S. EPA, 2005). Therefore, in this case, the provisional RfD (p-RfD) values for iron, derived using the lowest-observed-adverse-effect level (LOAEL) and uncertainty factor (UF) was considered (U.S. EPA, 2006b). RfD values of other nanoparticles i.e., ZnO NPs and CuO NPs is taken from recently published work (Parsai and Kumar, 2020). ADI values of individual antibiotics used to estimate the PNEC values is taken from published literature. Table 1 lists information about the parameters used for risk estimation.

Table 1 Information about parameters used to determine risk estimates

Parameters	Units	Values	References
Body weight, BW	Kg	16.7	Argall et al., 2003
Average time, AT	Year	365 × 70	ATSDR, 2005
Exposure Duration, ED	Year	70	
Exposure Frequency, ED	Days	365	
Reference dose values, RfD			
ZnO NPs	mg $kg^{-1}day^{-1}$	0.0315	Parsai and Kumar, 2020
CuO NPs	mg $kg^{-1}day^{-1}$	0.0262	
TiO_2 NPs as Ti^{2+} ion	mg kg-$bw^{-1}day^{-1}$	3	Ramoju et al., 2020
Fe_3O_4 NPs as Fe^{3+} ion	mg kg-day	0.7	U.S. EPA, 2006b
Zn^{2+}	mg $kg^{-1}day^{-1}$	0.3	IRIS, 2005
Cu^{2+}	mg $kg^{-1}day^{-1}$	0.04	

2.4 Risk estimation and characterization

Hazard quotient (HQ) values were calculated to determine risk to children. HQ is the ratio of the possible exposure to a contaminant and the extent to which no adverse effects are expected to occur. If the HQ is observed to be less than 1, then no adverse health consequence is anticipated as a result of exposure (Kumari et al., 2015). HQ values were calculated using the CDI and ADD values estimated in the exposure assessment section and RfD values taken from table 1 as given in Eq. (6-9). Estimated HQ values were used the calculate the hazard index (HI) values for interactions of (i) antibiotics with metal ions, (ii) antibiotics with nanoparticles, and (iii) metal ions with nanoparticles in the human digestive

system as mentioned ins Eq. (10-12). Limited information is available in published literature on how antibiotics interact with metal ions or nanoparticles. Urbaniak et al. (2007) used '*k*' values to provide information on the strength of interaction between fluoroquinolones and metals and observed strong interaction between them. Few studies reported synergistic effects for the interactions of antibiotics with nanoparticles (Abo-Shama et al., 2020) or metal ions (Nazari et al., 2012) however antagonistic effects do occur as well. Turel (2002) in his study showed that the activity of fluoroquinolones reduces in the presence of metal ions. Complexation with metal ions is one of the primary reason for the reduced activity of fluoroquinoloes (Seedher and Agarwal, 2010), and also modifies their solubility and binding capacity (Djurdjevic et al., 2007). This study assumed that synergistic effects occurs for the interaction of antibiotics with metal ions or nanoparticles. For case (i) and (ii), HI values were estimated using the modified USEPA Weight of Evidence (WoE) approach adopted from Kumari and Kumar (2020a) study. The detailed information and formula is provided as supplementary Text information, T1. Under WoE approach, M_{ij} which indicates the magnitude of interaction i.e. the influence of j^{th} compound on the toxicity of i^{th} compound was taken as 5, the default value as per USEPA recommendations, and B_{ij} denotes the strength of evidence for which scores were assigned as per the UEPA classification scheme (USEPA 2009a, b). For case (iii) i.e., HI values for the interaction of metal ions with NPs, it was assumed that they do not interfere with each other and therefore does not pose any toxic effects to human health. Dose-addition method was used to estimate HI values as mentioned in Eq. (12).

$$HQ_{AB\_DM} = \frac{CDI_{AB\_DM}}{ADI} \quad (6)$$

$$HQ_{Mi\_DM} = \frac{ADD_{Mi\_DM}}{RfD_{Mi}} \quad (7)$$

310 $$HQ_{R_{NP\_DM}} = \frac{ADD_{R_{NP\_DM}}}{RfD} \quad (8)$$

311 $$HQ = \frac{EC}{PNEC} \quad (9)$$

312 $$HI = HQ_{antibiotics} + HQ_{metal\ ions} \quad (10)$$

313 $$HI = HQ_{antibiotics} + HQ_{NPs} \quad (11)$$

314 $$HI = HQ_{metal\ ions} + HQ_{NPs} \quad (12)$$

315

316 2.5 Risk management

317 Maximum allowable concentration (C_{max}) can be defined as the concentration beyond which
318 no adverse effects or risk exposure can occur. C_{max} specifies the upper limit values of
319 substance under study and can provide a helping hand to regulatory agencies for managing
320 the risk. To calculate the C_{max} values, the HQ values in Eq. (6-9) was set as 1, and the
321 concentration was calculated.

322

323 **3 Result and discussion**

324 3.1 Estimation of nanoparticles and antibiotics loading in the digestive media

325 The results revealed that Fe_3O_4 NPs showed high bio-accumulation in the digestive system
326 with a value of 2.58×10^{-3} mg/L. The comparative analysis indicated that Fe_3O_4 NPs has the
327 highest accumulation in the digestive media which was followed by ZnO NPs (5.77×10^{-8}
328 mg/L), CuO NPs (2.40×10^{-9} mg/L) and TiO_2 NPs (7.19×10^{-10} mg/L). Overall,
329 concentration of Fe_3O_4 NPs was found to be highest amongst all the nanoparticles considered
330 for this study. High accumulation of nanoparticles like ZnO and CuO NPs in the digestive
331 system can be related to their size. Larger the size of nanoparticles higher is the accumulation

potential in the human digestive system (Bergin and Witzmann, 2013). The observed sequence of nanoparticles in the human digestive system is similar to those observed by Parsai and Kumar (2020).

Similar to nanoparticles, the concentration of antibiotics in the human digestive media (C_{AB_DM}) after nanoparticles dissolution was also calculated to determine the accumulation potential of antibiotics in the human digestive system. The concentration of levofloxacin cannot be determined due to the lack of adsorption isotherm data. Hence, as a result further risk assessment studies were not carried out although, it can be done only if the data is available. Amongst the antibiotics considered, ofloxacin (1.8×10^{-3} mg/L) showed highest accumulation potential in the human digestive system next to ciprofloxacin (3.87×10^{-7} mg/L) and norfloxacin (4.44×10^{-7} mg/L). Wingender et al. (1985) in his study reported rapid absorption of ciprofloxacin in the upper GI-tract however comprehensive information on the absorption of ciprofloxacin or any other FQ in different parts of the human GI-tract does not exists (Harder et al., 1990). Furneri et al. (2000) suggested that accumulation of FQs antimicrobial agents is reduced by lowered pH and, under some conditions, by divalent cations as well.

3.2. Risk estimation

3.2.1 Risk estimation of NPs in the digestive media followed by GI-tract absorption when the isotherm data is available

3.2.1.1 Risk estimation of metal ions released in the digestive media after dissolution of nanoparticles

354 The results revealed that the HQ values for all types of metals ions released in the human
355 digestive system after nanoparticle dissolution were observed to be less than 1 under the
356 conditions assumed in this study. This indicated that the metal ions released from the
357 nanoparticles in the human digestive system does not show any significant health risks to
358 human health [HQ values ranged from 1.44×10^{-11} (for Ti^{2+} ions) to 2.21×10^{-4} (for Fe^{3+}
359 ions)]. HQ values more than 1 shows possible health concerns.

360

361 3.2.1.2 Estimation of risks due to the revised concentration of nanoparticles in the digestive
362 media after dissolution of nanoparticles

363 HQ values calculated for the revised concentration of nanoparticles in the human digestive
364 system were in the sequence of (low to high): ZnO NPs, 3.43×10^{-8}; TiO_2 NPs, 3.19×10^{-7};
365 CuO NPs, 2.28×10^{-5}; Fe_3O_4 NPs, 2.85×10^{-3} (Table 2). The results showed that the
366 estimated HQ values for the revised concentration of nanoparticles were smaller than 1, the
367 acceptable risk level, indicating no significant risks to human health. Amongst the
368 nanoparticles considered, Fe_3O_4 NPs showed comparatively high HQ values than other
369 nanoparticles. It is important here to mention that till the time of writing no such studies (as
370 per the author's best knowledge) has been reported in literature to determine risks for the
371 released concentration of metal ions as well as nanoparticles in the human digestive system.
372 It is also not known how the released metal ions behave after their dissolution from
373 nanoparticles in the digestive media. Moreover, no guidelines or recommendations is
374 available on the use of nanomaterials as a coating agent for drug delivery. In spite of being
375 used in numerous biomedical and other industrial uses, the safety, toxicity, and its interaction
376 with and within the biological systems are still unclear (Snyder-Talkington et al. 2012; Zhong
377 et al., 2017).

Table 2 Summary of the estimated risk of nanoparticles released in the digestive media

Metal ions released in the digestive media	Risk of metal ions in the digestive media (HQ_{Mi})	Nanoparticles	Risk of revised concentration of nanoparticles in the digestive media (HQ_{R_NP})
Zn^{2+}	1.15×10^{-8}	ZnO	3.43×10^{-8}
Ti^{2+}	1.44×10^{-11}	TiO_2	3.19×10^{-7}
Fe^{3+}	2.21×10^{-4}	Fe_3O_4	2.85×10^{-3}
Cu^{2+}	3.59×10^{-9}	CuO	2.28×10^{-5}

3.2.1.3. Risk of revised concentration of antibiotics in the digestive system after release from nanoparticles and absorption in GI-tract

HQ values estimated using the revised concentration of antibiotics in the human digestive system ranged from 1.90×10^{-12} (NOR) to 3.37×10^{-8} (OFX). The observed values indicate that no possible risks exist to human health as the obtained HQ values were less than the acceptable risk level. Amongst all, it was observed that CIP concentration may pose risks to human health due to low values. Overall, the results of risk evaluations disclosed that there exist no risks to human health for the consumption of nanoparticles coated antibiotics though oral ingestion of water under the conditions assumed in this study and for the scenario's considered. Although the estimated risk values were below the acceptable risk level but still

391 the amount of nanoparticles to be used as a coating material for antibiotics needs to be
392 regulated prior to its use for human.

393

394 *3.3. Risk estimation when the adsorption isotherm data is available*

395 3.3.1 No absorption

396 The term "No absorption" implies that under the hypothetical scenario considered no
397 adsorption of antibiotics on nanoparticles takes place. As mentioned in the exposure
398 assessment section, in this case, risk was estimated only for the oral ingestion of surface
399 water contaminated with antibiotics. PNEC values were estimated to determine risk exposure
400 effects. PNECs provides more accurate estimates and can be refined using detailed
401 information considering different assessment factors (Bopp et al., 2019). The calculated
402 PNEC values were observed to be (high to low): norfloxacin (233.80 µg/L) > ofloxacin
403 (53.44 µg/L) > ciprofloxacin (26.72 µg/L) > levofloxacin (2.505 µg/L). HQ values ranged
404 from 1.68×10^{-3} (norfloxacin) to 4.3×10^{-2} (levofloxacin) and are observed to less than the
405 acceptable risk level (HQ < 1), therefore does not pose any significant risks to human health.
406 An inverse relation was observed between PNEC and HQ values. Lower the PNEC values,
407 higher the risk will be. Moreover, the PNEC values is directly dependent on the concentration
408 of antibiotics in the environmental media. The results presented in this study is somehow
409 similar to those reported by the authors (Kumari and Kumar, 2020a) in their previous work
410 on sulfamethoxazole, ampillicin, and amoxicillin. Even though the presence of antibiotics in
411 the water environment pose insignificant risks to human health, the risk management of these
412 substances is required so as to protect human beings from their detrimental effects, if any. A
413 thorough monitoring of these antibiotics in water bodies is required to protect the human
414 health.

3.3.2 100% absorption

The term "100% absorption" implies that under the hypothetical scenario, it was assumed that total adsorption of antibiotics by nanoparticles occurs. Figure 2 shows the HQ values of individual antibiotics for different nanoparticles. As can been seen, the HQ values for all the antibiotics were observed to be less than 1, the acceptable risk, indicating no significant risks to human health. Amongst the NPs studied, high risk values were observed for ZnO NPs compared to others. Similar to the other cases, here also, absorption of ZnO NPs in the human digestive system was found to be the highest. Parsai and Kumar (2020) found high HQ values for ZnO NPs thorough fish consumption exposure. The toxicity of ZnO nanoparticles might be due to their solubility. It is reported that dissolution of ZnO nanoparticles takes place in the extracellular region, which in turn increase the level of intracellular Zn^{2+}. However, the mechanism behind the increased level of intracellular Zn^{2+} ions and dissolution of ZnO nanoparticles in the medium is still unclear (Pandurangan and Kim, 2015).

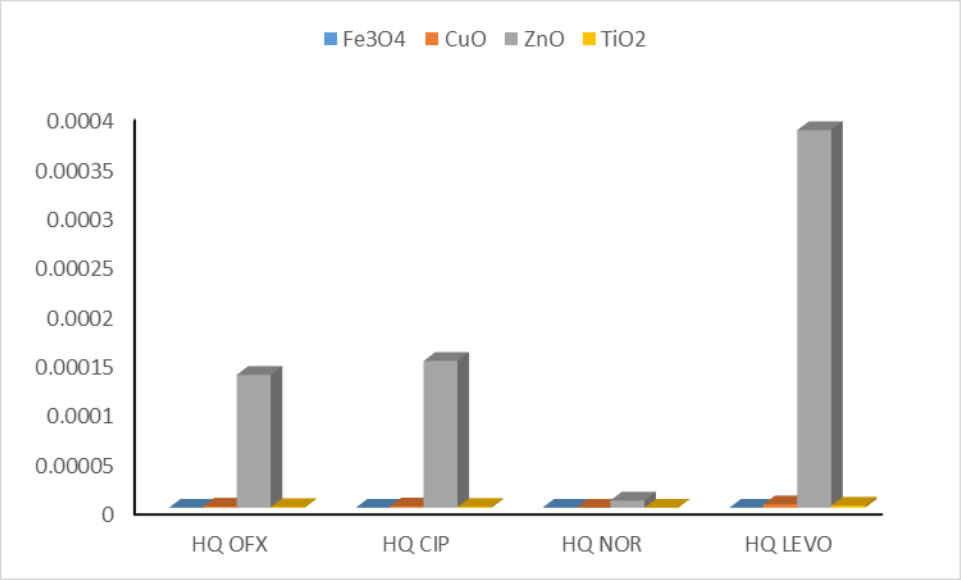


Fig. 2 HQ values of nanoparticles for nanoparticles

This study estimated risks due to the exposure of single type of nanoparticle at-a-time. The chance assembly of more than one type of nanoparticles or antibiotics was not considered due to lack of information on the BAF values of mixture of NPs, dissolution and absorption rate of mixture of antibiotics in the GI-tract, dissolution of nanoparticles to their corresponding ions in the digestive system and RfD values. The present study showed an example of determining risk estimates in digestive system after including all the important parameters and highlighted its significance for human health. This type of risk assessment studies has not been conducted and this is the first time an attempt has been made to predict risk of contaminants within the human digestive system. The developed framework will help in assessing risk effects of the exposure of NP coated ABs to human in the digestive system. Though, few studies have estimated risks due the exposure of nanoparticles (Pizzol et al., 2019; Yang et al., 2017) and antibiotics (Kumari and Kumar, 2020a; 2020b) but none of them have considered the effect of human digestive media on the fate of nanoparticles and antibiotics including the reference dose values of NPs. The proposed framework included the effects of dissolution of nanoparticles to their corresponding ions in digestive system and estimated the revised concentration of antibiotics in the digestive media so as to provide more realistic values of both nanoparticles and antibiotics in the human digestive system. The suggested framework provides a step-wise approach to determine the risks exposure effects of NPs and ABs-complexes in the human digestive system.

3.4 HI interactions

Under the conditions assumed in this study, the NPs coated ABs after oral ingestion undergoes absorption in the GI-tract and dissolved to produce different fractions as mentioned in the exposure assessment section, therefore, it is essential to calculate the risk of these released components in the human digestive system to provide more realistic risk estimates. For case (i) HI interaction of antibiotics with metal ions, the results revealed that the interactions of antibiotics with metal ions in the human digestive system does not pose any risks to human health as the estimated HI values were less than 1 (HI_{int} for antibiotics-metal ion pairs < 1) for all the conditions assumed in this study. In case (ii) HI interaction of antibiotics with nanoparticles, the calculated HI_{int} values were also observed to be less than 1, the acceptable risk level and therefore, does not pose any risk to human health, if present together. For case (iii) HI interaction of antibiotics with metal ions, similar to the results obtained for the above two cases, here also the interaction results were smaller than 1, indicating no significant health risks to human health. Overall, it was observed that no significant health was observed for the three mixture pairs as mentioned above. Although the HI interaction values were observed to be less than the acceptable risk level, still guideline values needs to be developed so as to regulate the amount and use of nanoparticles in targeted drug delivery systems as even a nano-gram increase in their concentrations might show adverse effects and can be detrimental to human health. Supplementary Table S5 shows information about the results obtained for all the cases and combinations mentioned above.

3.5 Maximum allowable concentration of metal ions, nanoparticles, and antibiotics in water

Table 3 provides the calculated maximum allowable concentration for antibiotics, metal ions, and nanoparticles. C_{max} values of metal ions at which no risks occur ranged from (high to low) 6.68×10^5 mg/L (for Ti^{2+} ions) to 6.27 mg/L (for Zn^{2+} ions). The estimated C_{max} values

of nanoparticles at which no health risks effects were observed ranged from 0.658 mg/L (ZnO NPs) to 6.68×10^5 mg/L (TiO_2 NPs). Amongst the NPs studied, ZnO NPs showed highest risk, and therefore, the C_{max} values for ZnO NPs was found to be lowest followed by Fe_3O_4, CuO, and TiO_2 NPs, respectively. If we compare the C_{max} values of metal ions with that of nanoparticles, it can be seen that the Zn^{2+} ions (0.658 mg/L) showed comparatively high C_{max} values than ZnO NPs (6.27 mg/L). Previous studies also reported high C_{max} values for ZnO NPs in water bodies for the inadvertent ingestion of NPs through fish consumption exposure (Parsai and Kumar, 2020). Similar C_{max} values for Ti^{2+} ions and TiO_2 NPs, and Fe^{3+} ions and Fe_3O_4 NPs as can be seen from Table 3 is due to the use of similar RfD values for metal ions and NPs (due to unavailability of RfD values for these NPs) during risk estimation. Therefore, there is a need for conducting *in vivo* and *in vitro* studies for determining RfD of nanoparticles to get accurate risk estimates. The observed results demonstrated that stern actions and control measures must be taken to reduce the risk exposure effects of metal ions and nanoparticles.

The C_{max} values of antibiotics were also calculated to determine their allowable concentration in water systems. The study observed that C_{max} of antibiotics beyond which no risk effects can occur was observed to be 2.5 µg/L (for levofloxacin), 53.44 µg/L (for ofloxacin), 26.72 µg/L (for ciprofloxacin), and 233.80 µg/L (for norfloxacin). On the basis of C_{max} values, it can be said that levofloxacin pose maximum risk to children whereas norfloxacin shows minimum risk. Different C_{max} values of antibiotics have been reported by researchers (Lubasch et al., 2000; Owen et al., 1997) which might be related to the administered dose of antibiotics and age of the population considered. It was observed that an inverse relationship exists between the maximum allowable concentration and hazard quotient values i.e., the lower the C_{max} values the higher the risk will be and vice versa. The results obtained in this study can be used by the regulatory bodies like USEPA, OECED and

504 WHO for setting up the guidelines values for metal ions, nanoparticles, and antibiotics in
505 water.

506 **Table 3** Maximum allowable values (C_{max}) of metal ions, nanoparticles and antibiotics
507 assuming HI values as 1; lowest values are shown in bold text and are italicised

	Metal ions (mg/L)			
Maximum allowable concentrations, C_{max}	***Zn^{2+}, 6.27***	Cu^{2+}, 2.23×10^{3}	Fe^{3+}, $4.79 \times 10^{1*}$	Ti^{2+}, $6.68 \times 10^{5\#}$
	Nanoparticles (mg/L)			
Maximum allowable concentrations, C_{max}	***ZnO, 0.658***	CuO, 1.46×10^{3}	Fe_3O_4, $4.79 \times 10^{1*}$	TiO_2, $6.68 \times 10^{5\#}$
	Antibiotics (µg/L)			
Maximum allowable concentrations, C_{max}	***Levofloxacin, 2.51***	Ofloxacin, 53.44	Ciprofloxacin, 26.72	Norfloxacin, 233.80

508

509 **4 Effect of assumptions used on risk values**

Due to lack of information on parameters used to determine risk estimates due to exposure of nanoparticles and antibiotics from natural waters, a lot of assumptions were made to fill the data gaps which includes (i) concentration of NPs: to calculate risk estimates of due to the interaction of nanoparticles with antibiotics, this study assumed the nanoparticle concentration to be 1 mg (NPs) per mg of antibiotics (data taken from literature) to determine the concentration of antibiotics adsorbed on nanoparticles (mg/L). Under this assumption, it was observed that the estimated risk values for all the conditions studied does not pose any concerns to human health. However, risk estimates might vary as it directly depends on the concentration of substances used for the study (Kumari and Gupta, 2018) (ii) RfD values: Due to unavailability of reference dose values for nanoparticles considered in this study (as mentioned in the exposure assessment section), the RfD values of metal ions were taken (assuming that the values are equivalent and similar to that of nanoparticles) to estimate the risk values. At the assumed RfD values, no exists to human health for the conditions studied. The observed risk estimates might show different results, if estimated using accurate RfD values of nanoparticles (iii) BAF values: it determines the behavior of nanoparticles in solution. Transformation and actions of nanoparticles within the human digestive system is hard to anticipate (iv) ADI values of antibiotics: the values are taken from literature. Different values of the selected antibiotics have been reported by Wang et al. (2018) and Hanna et al. (2018) which creates a dilemma on which values to take for determining risk estimates, and thereby creating uncertainty in the overall process (v) B_{ij} values: The study assumed the B_{ij} values as 1 due to lack of information on the interaction of antibiotics with nanoparticles or metal ions. The risk estimates presented in this study indicate the point estimate values and can vary depending on variability of these parameters, creating uncertainty in risk estimates. Uncertainty analysis using Monte Carlo simulations needs to performed to overcome these issues in risk estimates. Moreover, it is essential to recognize

those parameters which adds high variability in HQ estimation so that efforts could be taken for reducing their variability in risk estimation process.

5 Implications of the proposed framework

This study proposed a framework using six-step risk assessment approach to determine the risk exposure effects of nanoparticles and antibiotics followed by oral ingestion and their possible interaction within the human digestive system. This study provides a systematic information on risk assessment involving the fate of nanoparticles and antibiotics within the human digestive system. Agencies like USEPA and FDA have suggested use of alternative testing strategies and data requirement for nanoparticles (Aschberger et al., 2016), however a systematic approach in dealing with nanoparticles and antibiotics does not exists. The proposed framework can be used by regulatory bodies such as USEPA, EU agencies and OECD for monitoring of nanoparticles and antibiotics in water. The outcome of the study will help in identifying the possible concentration of released compounds (antibiotics, nanoparticles, metal ions) in the human digestive system and the data generated can used for formulating guideline limits of both antibiotics and nanoparticles. Studies by Parsai and Kumar (2021) on nanoparticles and Kumari and Kumar (2020) has developed risk assessment framework for determining risk exposure effects alone and in mixture combination however none of the reported study analysed the interaction between these two contaminants. In this regard, this study will be helpful in understanding the interaction as well as the fate and behaviour two contaminants. Besides, the maximum allowable concentration values of contaminants derived in the study can be used by the regulatory bodies to regulate the concentration of antibiotics, nanoparticles and metal ions in natural water systems.

559 **7 Summary and Conclusions**

560 The major findings of the study are presented below:

561 • The present study proposed a framework for assessing health risk exposure effects caused
562 due to the interaction of nanoparticles and antibiotics followed by oral administration.
563 The developed framework was applied to a hypothetical scenario where environmentally
564 occurring concentration of nanoparticles (Fe_3O_4, ZnO, CuO, and TiO2) and antibiotics
565 (levofloxacin, ofloxacin, ciprofloxacin, norfloxacin) were taken for illustrative purpose.
566 • The study estimated the loading of antibiotics and nanoparticles in the human digestive
567 system after their release. Amongst the nanoparticles, Fe_3O_4 NPs (2.58×10^{-3} mg/L)
568 presented maximum accumulation in the digestive media whereas TiO_2 NPs the minimum
569 (7.19×10^{-10} mg/L). Similarly, for antibiotics, ofloxacin has the highest accumulation rate
570 in the human digestive system (1.8×10^{-3} mg/L).
571 • The risk estimated for two different scenarios showed hazard quotient values less than 1
572 under the conditions and assumptions made in this study. Therefore, on the basis of
573 results obtained it can be said that the interaction of two contaminants does not pose any
574 risks to human health followed by their release and dissolution in the human digestive
575 system.
576 • Mixture toxicity (HI interactions) studies was conducted for three different binary
577 combinations (i) antibiotics with metal ions, (ii) antibiotics with nanoparticles, and (iii)
578 metal ions with nanoparticles. The estimated HI values for all the mixture combinations
579 was observed to be less than 1, the acceptable limit, and therefore indicated no significant
580 risks to human health. However, more detailed studies on the interactions antibiotics with
581 metal ions and nanoparticles is required (B_{ij} and M*ij* values in the WoE approach) for
582 accurate risk predictions.

- Overall, this work significantly increases our understanding on the fate of nanoparticles as well as antibiotics in the human digestive system and provides the knowledge base for better assessment of risk estimates of the studied contaminants in natural water systems. Efforts are required for conducting proper *in vitro* and *in vivo* eco-toxicity studies so that better understanding can be made on the fate and behaviour of released fragments of nanoparticles and antibiotics in the human digestive system.

Data availability

All the data supporting the results reported in the article are included in the manuscript and can be found in supplementary file. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for Publication: Both the authors have their consent for publishing the manuscript

Availability of data and material: Not applicable

Competing interests: Not applicable

Funding: Not applicable

Authors' contributions: Minashree Kumari (Draft writing, analysis, Data interpretation, editing), Arun Kumar (Supervision, conceptualization and editing).

Acknowledgements: This research work was conducted by author Dr. Minashree Kumari as a part of her Institute Post-Doctoral Fellowship in the Department of Civil Engineering at Indian Institute of Technology, Delhi (IIT Delhi), India. The authors wish to acknowledge IIT Delhi for supporting this research.

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Figures

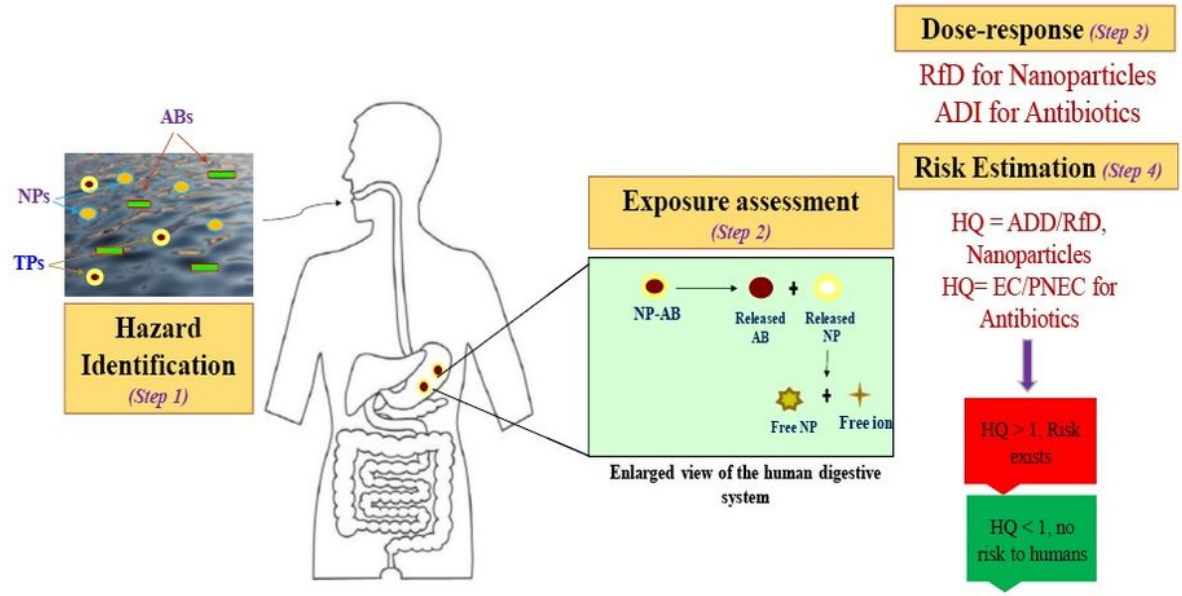

Fig. 1

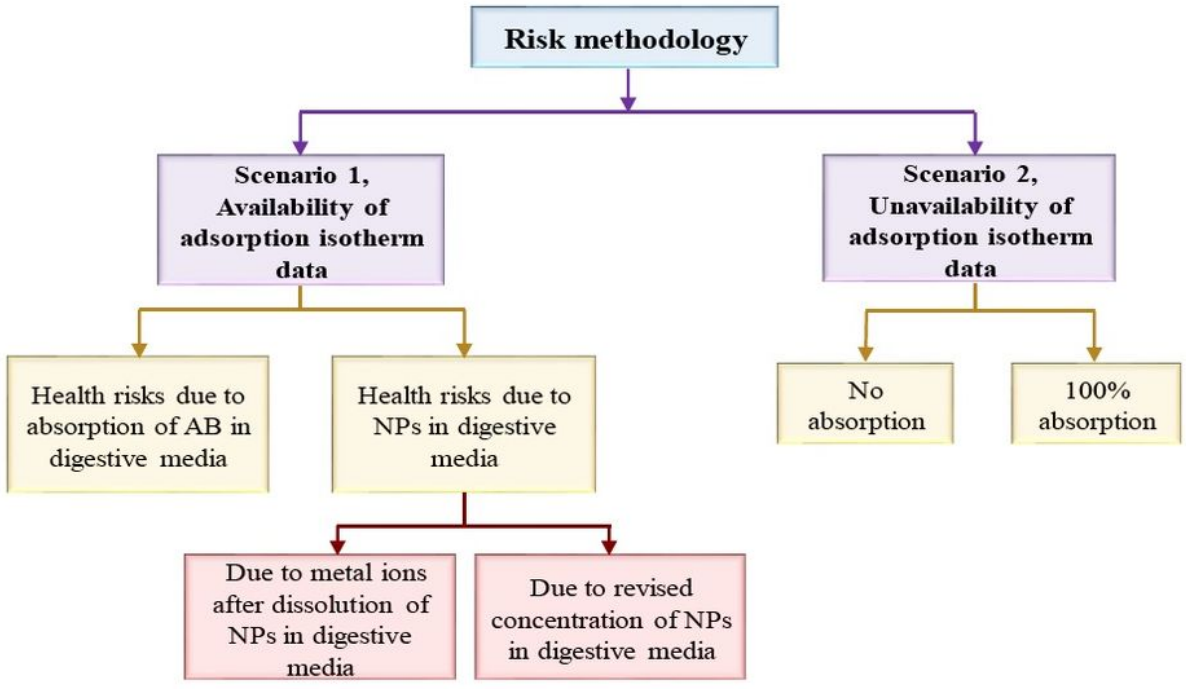

Fig. 1a

Figure 1

Hypothetical schematic diagram showing the uptake of nanoparticles-antibiotics transformed products and their dissolution in the human digestive system (NPs = nanoparticles, ABs = antibiotics, TPs = transformed products, HQ = hazard quotient, EC = Environmental concentration, PNEC = Predicted no-

effect concentration; RfD = Reference dose; ADI = Acceptable daily intake). 1a Scenarios considered for determining risk exposure to humans

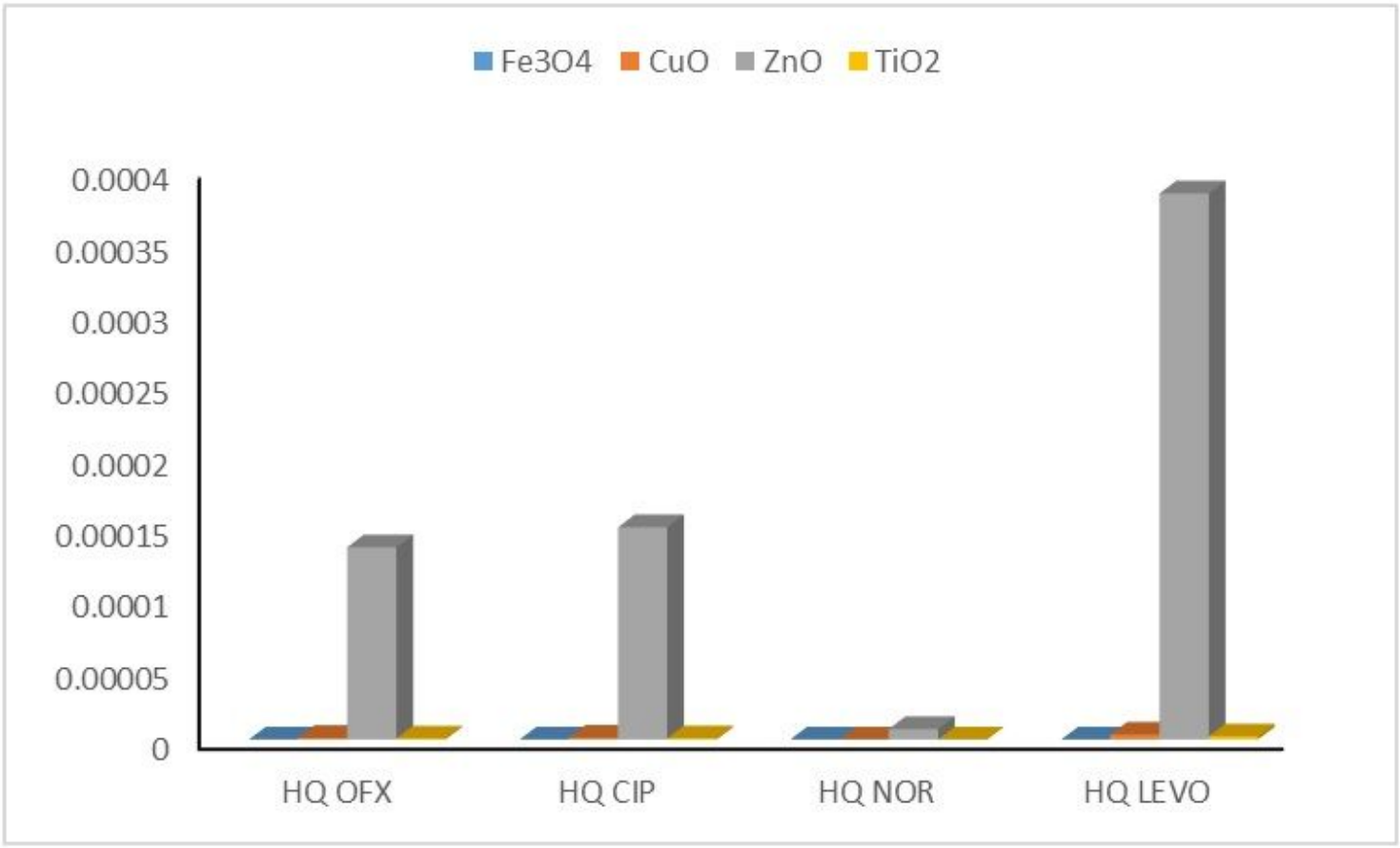

Figure 2

HQ values of nanoparticles for nanoparticles

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.doc