External Peer Review of EPA's Draft Report, Nanomaterial Case Studies:Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen

Contract No.: EP-C-07-024
Task Order 91

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July 2, 2010

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QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by EPA. EPA confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled EPA's selection criteria. Reviewers conducted the review according to a charge prepared by EPA and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to EPA. ERG did, however, format reviewers' comments as needed for consistency in this final peer review summary report.

Contents

Responses to Charge Questions

1. Chapter 1 provides introductory material regarding the approach used in these case studies, definitions of conventional and nanoscale TiO2, the use of nano-TiO2 in drinking water treatment and sunscreens, and analytical methods for characterizing nano-TiO2. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. How might this chapter be improved?

Reviewer	Comments	Responses
Hansen	• **Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document.** In general I find the information provided very clearly presented and very useful for the remainder of the report. Minor comments include: a) Section 1.2, page 1-3, Line 20: Not clear what is meant by "…such materials…" Is references being made to all kinds of nanomaterials (first-, second-, third-, fourth-generation of nanotechnology) or is reference being made specifically to nano-metaloxides? b) Section 1.5, page 1-7, Line 6-7: Would be good to have a reference for that P25 and Aeroxide T805 have the same particle size and surface areas, but differ in reactivity and it would furthermore be good to know how reactivity was measured? c) Section 1.6.2 The work by Tiede et al. and Hasselov et al. should be consulted: **1. Imaging of engineered nanoparticles and their aggregates under fully liquid conditions in environmental matrices.** Tiede K, Tear SP, David H, Boxall AB. Water Res. 2009 Jul;43(13):3335-43. Epub 2009 May 13. 2. **Considerations for environmental fate and ecotoxicity testing to support environmental risk assessments for engineered**	• **A) Revised sentence to clarify (Tom)** • **B) Citation is to Degussa pamphlet; pamphlet not available in HERO. Find as time permits; reactivity statement not a major finding. (Tom)** • **C) these references are included now in a new paragraph regarding environmental detection of ENMs, found at the end of section 1.6.2, p.1-19 lines 22-27**

	nanoparticles. Tiede K, Hassellöv M, Breitbarth E, Chaudhry Q, Boxall AB. J Chromatogr A. 2009 Jan 16;1216(3):503-9. Epub 2008 Sep 7. 3. **Detection and characterization of engineered nanoparticles in food and the environment.** Tiede K, Boxall AB, Tear SP, Lewis J, David H, Hassellov M. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008 Jul;25(7):795-821. 4. **Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles.** Hassellöv M, Readman JW, Ranville JF, Tiede K. Ecotoxicology. 2008 Jul;17(5):344-61. 5. **Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health?** Boxall AB, Tiede K, Chaudhry Q. Nanomedicine (Lond). 2007 Dec;2(6):919-27 **6.** **Nanomaterials for environmental studies: classification, reference material issues, and strategies for physico-chemical characterisation.** Stone V, Nowack B, Baun A, van den Brink N, Kammer F, Dusinska M, Handy R, Hankin S, Hassellöv M, Joner E, Fernandes TF. Sci Total Environ. 2010 Mar 1;408(7):1745-54. • **How might this chapter be improved?** It could be made clearer why CEA was chosen as the approach to study and organize the available information in the two case studies. What others approaches are out there and what are their pros and cons compared to CEA and why was CEA selected? It would furthermore be good to know why nano-TiO2	• Added paragraphs from nAg document describing selection of case study applications by EPA workgroup. (Tom)

	was chosen as a example of a nanomaterial and why the two specific applications (sunscreens and water treatment) were chosen to be subject to more in-depth analysis. Providing such arguments would increase transparency. It is not quite clear how primary and secondary information has identified and it might be good to have a methodology section where is clearly stated which literature databases (e.g. pubmed, ISI web of science, ICON's nanoEHS literature database) were searched including which kinds of search terms were used. Sources of secondary information might be TiO2 and sunscreen manufacturers and it might be good to know whether, for instance, US TiO2 manufacturers has been systematically contacted and how this contact came about (questionnaires, personal contact and alike).	Added sentences from nAg at the end of Section 1.2 addressing this point. (Tom)
Hristovski	This chapter represents a well conceived introduction with overall accurately and clearly presented information. The goal of this report is clearly emphasized on several occasions in addition to providing supplementary information about potential goal-related misconceptions that may misdirect a reader. This approach represents organizational strength of this chapter allowing the potential reader to remain focused on the goal. The information follows a clear line of thoughts and provides a good introduction, background and support for the next chapters. Several suggestions for improving the quality of this chapter are given below. On Page 1-15 Ln 5-7, a paper by Hewit is cited with a statement that "Different methods for measuring the same parameter may yield different results for the same material…and therefore stating the testing method is important." This statement is correct and often neglected when presenting and discussing results.	

	On page 1-9, Ln 15-23, the aggregation of nanoparticles is discussed, yet only information about 10 min sonication is provided. Key elements that can determine the size and size distribution of the nanoparticles in a dispersion are related to the power of a sonication (e.g W/L, type…); the initial concentration of a dispersion; the pH of a dispersion; the analytical tool used; and reported distribution (e.g. is it an effective radius reported; number or volume distribution; monomodal or multimodal…). These information are important to obtain a better understanding of the complexity of the issue. Addition of similar information in Table 1-2 would be suitable. For example, what is the method used to determine the particle size? What is the water matrix for the information about the median particle size (is it deionized, buffered…)? What is the concentration of the PBS? Although this table is a mere example, it would prove to be a much stronger illustration if such information is to be included.	Added sentences to end of this paragraph making this point. (Tom) Added size method (DLS) to text; added footnote to table on water matrix. (Tom)
	Page 1-12, Ln 21-29, discusses the formation of inner-sphere complexation of arsenic species onto nano-TiO_2. Here it is stated that only DMA forms monodentate ligands. In reality, the other arsenic species mentioned here can also form monodentate ligands in addition to the bidentate. The bidentate ligands are more thermodynamically stable and as such they can be the dominating mode of sorption when compared to monodentate ligands. However, where sorbate-to-sorbent ratio is high (e.g. in presence of high concentrations of competing ions), arsenic species may be forced to form monodentate ligands rather than bidentate ligands.	Added language clarifying ligand formation (Tom)
	On Page 1-13, Ln 15-19 scattering of nanoparticles is discussed and the reader is redirected to Appendix A. Although statements are made from a reference (Fairhyrst and Mitchnik, 1997, 196248) that the optimal scattering is thought to occur when the	

	particle diameter is approximately half the wavelength of the light to be scattered, the important phenomenon of Rayleigh scattering that relates to nanoparticles is clearly neglected. Discussion related to this scattering and how applies to nanoparticles is essential especially considering the fact that it is valid for particles that are much smaller than the wavelength of electromagnetic radiation in question here (UV-A; UV-B; and UV-C). On Page 1-16, Ln 7 a statement is made that "Currently technologies are unavailable to measure the total amount of nanomaterials in tissue." This statement may be a bit misleading considering the fact that studies have shown that metal content in tissues originating from nanomaterials (including nano-TiO_2) can be easily measured. So if the intent of this statement is to minimize or eliminate the above mentioned approach, then this statement may not accurately depict the reality and may require rephrasing. Consistency when using acronyms is very beneficial in context of document readability. For example, on page 1-18, Ln 12, the acronym for Field Flow Fractionation is FIFFF, yet on the next page and in other places it is FFF. Improving the consistency of the acronyms where applicable may result in better readability.	Rayleigh scattering beyond scope – this is not a technical discussion of light scattering, just a rationale for the use of nTiO2 in sunscreen. (Tom) Revised to clarify statement (Tom). Changed to "flow FFF" and requested doc prod'n do the global change. (Tom)
Klaine	The introduction chapter is reasonable but could benefit from a few modifications. Page 1-1. Readers unfamiliar with Comprehensive Environmental Assessment (CEA) may have difficulty understanding where it differs from traditional risk assessment (RA). It might be useful to begin with the EPA risk paradigm figure and show where CEA elaborates and expands on this method. Currently, if I did not	Clarified later in section (Tom)

	know a bit about RA and CEA I would find Figure 1 confusing. Page 1-3. I recommend adding a couple more sentences that expands on the concept stated in lines 8 – 11. Page 1-4. Lines 16-18 help with CEA. Unfortunately, the reader has to wait for a couple pages before that happens. I suggest your include concepts like this in the CEA section even at the risk of reiterating them here. Section 1.5 is well done. Page 1-11. The point made in lines 9-11 that early studies neglected to address adequate particle characterization could also be made for many current studies. I suggest inclusion of a statement that encourages the reader to evaluate particle characterization and reinforces the concept of lines 19-22 on Page 1-15. Page 1-15. Line 3 states a conclusion about what was addressed in Section 1.3. I have read and reread Section 1.3 and do not agree with this statement. If you want this statement to be true then I recommend you insert a paragraph in Section 1.3 that discusses characterization needs for the use of nanomaterials in research. Page 1-17. Did you purposely leave out any discussion of the measurement of surface charge or pH_{pzc} in Table 1-3 or Table 1-4?	Done. (Tom) Left alone to avoid redundancy (Tom) Text added. (Tom) Incorrect section reference (1.3 changed to 1.5). (Tom) Text added. (Tom)
Nowack	1. Remark: A lot of the points listed under this question actually apply to the whole document but are listed here because this chapter contains the overview of the whole document. 2. The document is pretending to make "case studies", however, it is rather using	Regarding comment #5, the Kaegi reference is already present in section 4.3 about aggregate pathways; this seemed appropriate so I left it where it is.

	two applications of nano-TiO_2 to organize to some extent the information extracted from the scientific literature and to guide the questions. However, these are not real case studies. From a case study I would expect that only this information is summarized that is pertinent to the case studies. My expectation was that the document contains some general chapters that are then followed by two "case studies" where the information that is relevant for these cases is listed based on the life cycle aspects of the two applications. At the moment just all information about nano-TiO_2 is listed and the case study information gets completely lost. In principle this document is a normal review of nano-TiO_2 with some additional focus on sunscreens. But it's not really a case study. 3. This chapter should also contain a justification why the two case studies were chosen. Why these and not two of the many other uses of nano-TiO_2? 4. Footnote 5 on page 1-5 should be covered in the text. It makes the important distinction between aggregation and agglomeration and this should not just be a footnote but deserves at least a whole paragraph if not more. 5. Chapter 1.4 on conventional TiO_2 needs to be expanded considerably and much more information and details should be given. The distinction between conventional TiO_2 and nano- TiO_2 is often not clear and thus the traditional uses of TiO_2 need to be described in much more details. At this place also a new chapter on natural TiO_2 should be added. This information is needed to establish the baseline of natural TiO_2 exposure. And it is very important because also from conventional TiO_2 use nanoparticulate TiO_2 can be released to the environment. For example the work by Kaegi et al (2008) have shown that from façade paints nano-TiO_2 is released in natural waters. This is a very important information	Leave as is – no major reorganization (Tom). Text added from nAg. (Tom) Text adequate; revised footnote to clarify. (Tom)

	because it means that even when banning all pure nano-TiO_2 uses there is still nano-TiO_2 reaching the environment from traditional TiO_2-uses. 6. Chapter 1.5: This chapter describes the coating of nano-TiO_2 with silica, alumna or other compounds. One aspect that is never mentioned in the whole document is whether a alumina-coated nano-TiO_2 should be considered an alumina or TiO_2 particle? What the environment and organisms see is alumina and if this coating is not degraded then it will remain an alumina particle. Do we need to assess its risk by using data from alumina? And if coated with silica use data from pure silica rather than from pure TiO_2? It is correct to evaluate this particle together with pure TiO_2? This is an issue that will come up on almost every page of the document and needs to be discussed somewhere. It is absolutely central for he risk assessment of nano-TiO_2. The document contains a lot of information on the actual nano-TiO_2 used in products and his information needs to be used to steer the discussion of the data. That's not really done in the current document and is also due to the fact that not really case studies are discussed (see second remark above). If the whole document is really organized in the two case studies then information on the actual use of certain nano-TiO_2 forms should be used to extract this information form the literature that is relevant for this case study. If no uncoated nano- TiO_2 are used in sunscreens then it means that for this case study all fate and behavior and ecotox data with uncoated TiO_2 should not be used. 7. Table 1-3 and 1-4: nanoparticle tracking analysis (NTA) is missing in both tables.	Added text discussing this issue. (Tom)
Santamaria	**a. Is this information accurately and clearly presented?** Yes, I the introductory Chapter 1 is accurately and clearly presented.	Regarding the comment on p. 1-9, line 16 that Klaessig needs a better source: A better source

	b. Please comment on the utility of the chapter in providing background and support for the remainder of the document.	does not exist so I changed the language of the sentence to more clearly indicate that this is a claim made by the company, which then makes the reference more appropriate.
	This chapter provides a succinct overview of the purpose and organization of the case studies. However, it may be useful to include more detail explaining why the two case studies were chosen as examples. In particular, why the choice of nano TiO_2 as a water treatment agent, since it is not currently used in this manner? This does not seem to be the most appropriate case study to select since it is primarily hypothetical. Perhaps a more appropriate case study would have been the use of nano iron oxide for cleaning hazardous waste sites.	Added text from nAg case study (Tom).
	The chapter should end right after the section with the heading "Sunscreen" and not include the subsequent section entitled, "Analytical Methods" This last section seems out of place in the introduction and would be better as a separate chapter or incorporated into a different chapter. In addition, the section entitled, "Methods of Instrumentation to Assess Workplace Exposure" does not seem to be necessary or relevant for this document (or perhaps move it to Chapter 4, Exposure-Dose Characterization).	No major reorganization at this point. (Tom)
	c. How might this chapter be improved? A few specific comments and edits that may improve the chapter are listed below. • p. 1-1, paragraph 1: There should be more discussion of the types of products that nanomaterials are/will be used in to provide the reader with more background about why nanomaterials are being developed and used. The paragraph should also include what is meant by nanomaterials offering the	• Added citation to list of products from Project on Emerging Nanotechnologies. (Tom)

	"potential for benefits and risks" (brief description of what are the benefits, risks?) • p. 1-5, line 24: can more information be provided about AEROXIDE P25 such as form of TiO_2 (rutile, anatase) and size distribution/mean of the nanoparticles? • p. 1-6, line 25: change "bulletin" to "Current Intelligence Bulletin" • p. 1-6, footnote: change "increase" to "increases" • p. 1-7, line 27: explain what is meant by "the levels of effects" – what are the effects of external surface area? Without further explanation, the purpose of this paragraph describing surface area and its significance is unclear. • p. 1-9, line 1: should porosity be defined in a footnote? • p. 1-9, line 16: find a more appropriate reference for the particle diameters than the Klaessig 2006 phone call • p. 1-9, line 36: add a space between "to" and "150" • p. 1-15, line 30: explain what is meant by "presence and location" – doesn't TEM also provide information on shape, size, and whether particles are agglomerated/aggregated? • p. 1-17, Table 1-3: move information in column to directly beneath the "Liquid" header; explain what the dashes (-) mean with a footnote. • P. 1-20, section 1.6.4: this paragraph uses the term "nanomaterial" rather than "nanomaterials" that had been used throughout the chapter.	• This info is found at various locations in document. (Tom) • Done • Done • Clarified • P. 1-9, line 1: No. • Done • Clarified • Revised table to clarify • Changed to plural
Sellers	**1.1 Is this information accurately and clearly presented?** In general, this introduction clearly and accurately introduces the case studies. Some specific comments follow:	Regarding the Hoyt book chapter, this now appears in section 1.6, p. 1-16 lines 16-17.

	• Page 1-1. Consider adding explanation of why these two case studies were chosen, i.e., why are these two specific uses singled out for examination? • Page 1-2, line 1. Define "secondary contaminant". • Page 1-1, line 24 and page 1-2, Figure 1-1. Add "sediment" to environmental pathways. See related comment in Section 3.1 below. • Page 1-5, footnote 5. The footnote indicates that the terms aggregation and agglomeration have not been standardized. Consider citing and using the definitions developed by ASTM International [1], which distinguishes between agglomeration and aggregation of nanoparticles as follows. An agglomerate is a group of particles held together by relatively weak forces (such as Van der Waals force) that can be broken apart. An aggregate is a discrete group of particles composed of individual components that are tightly bonded together and not easily broken apart. • Page 1-7, lines 25-26. Readers not familiar with the behavior of nanomaterials will need additional explanation to understand the statement "Humic acid-coated nano-TiO_2 had lower zeta potential…" Please see related comment below on Chapter 3 (Section 3.3 of these comments). • Page 1-11, lines 17-19. Provide a reference for the statement "Further, some particle characterization techniques can affect measurement accuracy…"	• Done • Done • Done in text • Added ASTM ref; clarified footnote (Tom). • Not necessary to know zeta potential to understand sentence; leave as is. (Tom) • Revised statement to clarify. (Tom)

[1] ASTM International. 2006. Designation: E 2456 – 06. Standard Terminology Relating to Nanotechnology**.**

	and/or explanation. • Page 1-15, lines 11-15. For another excellent overview of analytical methods for nanomaterials, see Chapter 5: Analyses of Nanoparticles in the Environment, by Marilyn Hoyt, in *Nanotechnology and the Environment*[2]. • Page 1-18, line 17. The meaning of the following statement is unclear "…were also the two techniques that appear to be most prone to artifacts." **1.2 Comment on the utility of the chapter in providing background and support for the remainder of the document. How might this chapter be improved?** This chapter provides substantial background and support for the remainder of the document. No improvements are suggested other than as described above.	• Revised to clarify (Tom)

[2] Sellers, K., et al., 2009. *Nanotechnology and the Environment*. Taylor & Francis Press. Boca Raton, FL.

2. Chapter 2 presents information on the lifecycle of nano-TiO2, including potential releases to the environment. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO2 as used for arsenic removal in water treatment systems? To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO2 as used in sunscreens? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Reviewer	Comments	Responses
Hansen	• **To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems?** I believe that the chapter is both accurate and sufficient in the manner in which the various stages of the lifecycle of nano-TiO2 is described in regard to its use for arsenic removal. • **To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens?** I believe that the chapter is both accurate and sufficient in the manner in which the various stages of the lifecycle of nano-TiO2 is described in regard to its use in sunscreens. • **To what extent is the material effectively organized and sufficiently informative to support planning for future research?** The material is presented very clearly in regard to what is known, but it is less clear in this chapter what is unknown. This is however mentioned repetitively in the subsequent sections • **How might this chapter be improved?**	

	It might be a good idea to have a section in each chapter of the report clearly stating the research needs identified in the chapter making a clear distinction between 1) information that exists, but not is not publicly available at the point in time and 2) gaps in our scientific and technical knowledge.	• Chapter 6 should cover this information-GD
Hristovski	According to the title and the introduction of this chapter, the life cycle stages of nano-TiO_2 are discussed. Although the cradle-to-grave of nano-TiO_2 (with respect to arsenic treatment and sunscreens applications) approach appears to be considered, it appears that emphasis on several stages is either minimized or neglected. To the knowledge of this reviewer, there are two commercially available nano-TiO_2 based sorbents for arsenic as suggested by the manufacturers. DOW Chemicals produces Adsobsia GTO, while Hydroglobe produces MetsorbG. These sorbents are designed for removal of arsenic in packed bed configurations. The packed bed configurations are the types of systems that are predominantly used in water arsenic treatment for arsenic. It is possible that information on the preparation, distribution and storage of these sorbents can be obtained from the manufacturers or by searching the patent literature. These information can be introduced in Sections 2.2.1 and 2.3.1. In Section 2.5.1., two scenarios are discussed about disposal of nano-TiO_2 containing sludge. The first discusses physical/chemical water treatment (flocculation) and the second discusses accumulation of nanoparticles in sludge from biological wastewater treatment plants. However, the second scenario does not emphasize this difference. This may be confusing for a reader who does not have a technical proficiency in the field. There are several studies that discuss the interaction and fate and transport of nano-TiO_2 in the environment.	• Out of scope, not introducing new data into the document-GD • Changes to this section are being made to distinguish between fate of nano-TiO2 in by-products of drinking water treatment and waste water treatment. Paragraph added on p. 3-1, lines 9-19 (2nd paragraph). CH

	1. Battin TJ, Kammer FVD, Weilhartner A, et al. 2009. Nanostructured TiO_2: Transport Behavior and Effects on Aquatic Microbial Communities under Environmental Conditions; ENVIRONMENTAL SCIENCE & TECHNOLOGY 42, 8098-8104 2. Gottschalk F, Sonderer T, Scholz RW, et al. 2009. Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions. ENVIRONMENTAL SCIENCE & TECHNOLOGY 43, 9216-9222. 3. Kiser MA, Westerhoff P, Benn T, et al. 2009. Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants. ENVIRONMENTAL SCIENCE & TECHNOLOGY 43, 6757-6763. There is evidence that nano-TiO_2 is accumulated in biomass which is then used in agricultural application. This has to be emphasized. On Page 2-3 Ln 3-6, Klaessig 2006 is cited. According to this citation, Flame hydrolysis is used for manufacturing of P25 and yields agglomerated particles with mean diameters about 3.6 µm, with the smallest 4% of the particles having average diameter of 160 nm. Here, it is not stated in what environmental conditions these measurements were conducted (see comments in Charge Question 1 Section). There are number of studies showing that discrete P25 nanoparticles can exist in water matrices. These particles have size of 30-50 nm. This contradicts the above cited statement. On Page 2-4 Ln 7-9, a statement is made that photostability can be increased by doping nano-TiO_2 with metals including iron. Introduction of Fe in nano-TiO_2 may also cause increased photocatalytic activity as a result of creating intermediate energy levels between the valence bands and the conduction band in TiO_2, which can facilitate	• All three references are added to section 3.1, p 3-3, lines 23-32, • Kiser and Battin are also added in 3-4, lines 20-22 • This is a telephone conversation. Not sure how the references (un-specified) contradict Klaessig. No change. GD

	formation of free radicals by electromagnetic radiation with higher wavelengths. It is prudent to state that doping with some metals can decrease the photostability and facilitate creation of ROS. On several occasions waste water instead of wastewater term (e.g. Page 2-3 Ln 23) is used; and ground water instead of groundwater term (e.g. Page 2-6 Ln 3) is used. A paragraph/subsection summarizing the entire chapter would be beneficial and would provide a good ending to this section.	• People are trying to move TiO_2's photocatalytic activity into the visible spectrum by doping. However, I think the sentence can stay as is because it just says this technique <u>can</u> be used to increase photostability (not that it's always used that way). Tom • Changed to "wastewater" and "groundwater" throughout doc for consistency. GD • S/b covered in Ch 6. No change. GD
Klaine	In general, this chapter points out that there is a lot more that we do not know about the various life stages of nano-TiO2 used in water treatment or sunscreen. The material is well organized and readable. I only have one comment. Page 2-3. Line 7 refers to the "chloride method" and I assume you mean the method discussed in the prior paragraph. I recommend using that term in the discussion so the reader readily knows which method is being discussed.	• Changed to "flame hydrolysis" method. GD
Nowack	1) On a qualitative level this chapter list what is already known about lifecycle of nano-TiO_2 and release to the environment. However, what is missing is a quantitative evaluation. I expected from this chapter to get numbers of release to the environment during the different life cycle stages. The whole chapter remains very vague and again, it is not really a case study but listing some qualitative data. From a case study I expect to see quantitative data, for example a material flow diagram showing the flows from TiO_2 production, formulation, use and disposal to the environment. Even	• Out of scope. No change. GD

	if a lot of data is missing, we can still make some estimations and best guesses. 2) Chapter 2.4.2.: Most nano-TiO_2 in sunscreen will be released during the use phase. Where is it released to? How much ends up directly in the environment (released during swimming in lakes or the sea), how is it washed off during showering, how much is adhering to clothes and washed off during washing? These aspects that are absolutely crucial are not mentioned at all. Again, this is of primary importance if the document should really be a "case study".	• Not introducing new data at this point. No change. GD • Also, these possibilities are mentioned in Ch 3, although no specific data were identified. (Tom)
Santamaria	**a. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems?** This chapter seems to accurately and sufficiently characterize what is known and unknown regarding the various stages of the lifecycle of nano-TiO_2 for arsenic removal in water treatment systems. Because this is a hypothetical/experimental use and is not being widely implemented, there are still a lot of unknowns and data gaps regarding the use of nano-TiO_2 for this purpose, and that is conveyed in this chapter. The section entitled "2.4.1 Water Treatment" is lacking in quantitative detail about the process involved with using nanoTiO_2 for removing arsenic; in addition, the section does not really explain the mechanics of how nanoTiO_2 will be used in water treatment systems, as it only provides hypothetical or experimental processes. This seems to be a significant omission, as it is difficult to develop an adequate case study without this fundamental process information. **b. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens?**	• There is discussion about nano-TiO2 in filters or slurry usage. Its use is still experimental or hypothetical, thus the nature of the section's discussion. No change. GD

	This chapter seems to accurately and sufficiently characterize what is known and unknown regarding the various stages of the lifecycle of nano-TiO_2 in sunscreens. One additional piece of information that should be added to section "2.3.2. Sunscreen" is the estimated distribution of sunscreen from internet sales. Also highly recommend obtaining data on the distribution of sunscreen that is more recent than 20 years ago (1990) in this section -- this section should be updated to more accurately reflect current distribution levels of sunscreen.	• Text states that the information may be dated. Not searching out and introducing new data at this point. No change. GD
	c. To what extent is the material effectively organized and sufficiently informative to support planning for future research? The information is logically organized, and includes the appropriate Life Cycle stages and what is known and unknown about each for both case studies. With respect to being informative to support planning for future research, it would be beneficial to include a summary paragraph that highlights what the most critical data gaps are regarding the life cycle stages for both case studies to highlight and direct the need for research to address those data gaps.	• Chapter 6 should cover this. No change. GD
	d. How might this chapter be improved? Below is a suggested comment to improve this chapter. p. 2-9, line 1-8: this paragraph doesn't seem to fit in here – it is about stability of the coating on a particular manufacturer's nano-TiO_2, and should be moved to a section that describes the chemistry of nano-TiO_2. The data appear to be experimental and it is not clear whether this type of nano-TiO_2 is actually used in sunscreen formulations and how relevant the results of this experiment are to the actual use of sunscreen.	• Moved para on coatings to Ch 1 section 1.5 with other text on coatings and agglomeration. Also moved preceding para on photostability to Ch 1, section 1.5 with other text on photostability. GD

Sellers	**2.1 To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems?** An explanation of the process for using nano-TiO_2 in drinking water treatment would provide a reader unfamiliar with water treatment some context for the discussion. First, consider including a process flow diagram for a conventional water treatment plant in Section 2.4.1 with a brief description of the processes so that readers not versed in water treatment will have a basis for understanding subsequent references to the processes of coagulation, flocculation, sedimentation, filtration, and disinfection. Second, additional description of the process(es) by which nano-TiO_2 could be used in treatment, to supplement the brief mention on page 2-6, lines 14-15, would provide further context for potential exposures. Reference 196092 contains some information on the scale of nano-TiO_2 use in the Photo-Cat system that would provide dimension to the discussion in lines 8-11 of page 2-7: the maximum use level is 75 grams (g) TiO_2 per reactor assembly certified for a minimum flow of 33,600 liters per day (L/day). Section 2.5.1, regarding the water treatment case study, could characterize the disposal stage of the life cycle more accurately as follows: • The first paragraph, which describes waste generated from sand filters, is only relevant to this case study if nano-TiO_2 were applied as the first treatment process in the plant. That might not be the case if nano-TiO_2 were applied in a polishing step in a reactor containing nano-TiO_2 fixed in a matrix. • The second paragraph suggests that contaminants in water treatment sludge placed in a landfill could leach into underlying groundwater. In fact, landfills	• Second request for more detail about how nano-TiO2 could be used for arsenic removal. No change. GD • No figure for drinking water treatment to be added. GD • Cannot find this piece of information on the website. Not added. GD • There are different definitions of a "polishing" step, one of which conceives of the sand filter as such a step. Too much detail to add in absence of a more detailed discussion of drinking water treatment processes (or

	are designed to collect and treat leachate. While no system is completely leak-proof, this discussion of possible releases from landfill should acknowledge that lined landfills are designed and maintained to minimize leaching into groundwater. • The third paragraph indicates that sludge from water treatment could be used for land application. It appears that this paragraph is incorrectly conflating the disposal of water treatment sludge and the disposal of wastewater treatment sludge. The sludge from potable water treatment contains primarily inorganic substances and water treatment chemicals and is not land applied. In contrast, sludge wasted from biological wastewater treatment processes is high in organic matter and is sometimes land applied after suitable treatment as described in this paragraph. • The fourth paragraph mentions the possibility that nano-TiO_2 residuals in drinking water would eventually reach the ambient environment or sewage treatment facilities. Can the information from bench-and pilot-scale testing be used to bound the possible maximum concentrations which would be released? **2.2 To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens?** The discussion of the life cycle of nano-TiO_2 in sunscreen neglects the release of nano-TiO_2 into surface water during recreation while using sunscreen and also neglects the "disposal" of nano-TiO_2 in sewage treatment plants after showering or bathing. The following references, which are not reflected in the draft report, contain information relevant to this discussion and to portions of Chapter 3:	diagram). No change. GD • Update on previous. I just added a simple sentence in section 2.5.1, page 2-9, lines 3-5.-CH • Added two sentences on leachate control in landfills on 2-9, lines 10-11. GD • Changed the references and the text regarding the disposal of sludge waste from drinking water treatment, in section 2.5.1, page 2-9, lines 6-16. CH • Fixed by distinguishing the two different scenarios for nano-TiO2 fate: its use in drinking water treatment and that process' byproducts, and its entry into wastewater from being wasted down the drain in use in sunscreen. CH • No introduction of new data at this point. No change. GD • CH added the sentence "Additional exposure pathways other than specific, purposeful

	• Johnson, A., et al., 2009. Should we be concerned about the discharge of sunscreen nano TiO_2 to the environment? Poster presented at SETAC Europe: 19th Annual Meeting, 31 May-4 June 2009. Goteborg, Sweden. (These researchers estimated the release of nano-TiO_2 to surface water after sunscreen use. An electronic copy of the poster is appended to these comments.) • Kiser et al., 2009. Titanium nanomaterial removal and release from wastewater treatment. Environmental Science and Technology. 43(17): 6757 – 6763. (The authors report on the occurrence, characterization, and removal of nano- and larger-sized Ti at wastewater treatment plants. Reference is apparently made to a preliminary report on this work in a conference proceeding abstract [157466], on page 3-3.) • Limbach et al., 2008. Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency. Environmental Science and Technology. 42(15): 5828-5833. (While this research primarily pertained to the removal of cerium oxide, the authors indicate the relevance of the work to nano-TiO_2. This paper is discussed on page 3-3 [reference 155628].) • Mueller and Nowack, 2008. Exposure modeling of engineered nanoparticles in the environment. Environmental Science and Technology. 42(12): 4447-4453. (The aim of this study was to use a life-cycle perspective to model the quantities of engineered nanoparticles released into the environment, including nano-TiO_2. This study is mentioned on page 4-12 of the draft document, but is also relevant to Sections 2 and 3.) **2.3 To what extent is the material effectively organized and sufficiently informative**	disposal of sunscreen containers are acknowledged as potentially important and will be addressed as part of the fate and transport discussion in Chapter 3" to the end of Chapter 2. • Added two sentences re: Johnson et al poster results on 2-8. GD • Kiser et al ref added to Ch 3 (substituting for Westherhoff conf abs). -CH • Limbach et al study discussed in Ch 3 Water Fate and Transport, Already included on page 3.3 lines 29-30 GD • Mueller and Nowack now mentioned in section 3 intro, on page 3-3. But not in detail -CH

	to support planning for future research? With the exception of the comments and recommendations above and in Section 2.4 below, this material is effectively organized and sufficiently informative to support planning for further research. **2.4 How might this chapter be improved?** Consider reorganizing the chapter so that the life cycle is presented for each case study in turn (rather than by reviewing each stage for both case studies).	• No change. GD

3. Information on the fate and transport of nano-TiO_2 in air, water, and soil is discussed in Chapter 3. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO_2 in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Reviewer	Comments	
Hansen	• **Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO_2 in the environment.** In general, I do believe that chapter 3 accurately characterizes what is known and anticipated behavior of nano-TiO2 in the environment. Minor comments include: a) Section 3.1.2, page 3-5, Line 30-32: was any information found on how the use of chlorine in pools might affect dis-/agglomeration of nano-TiO2? b) Section 3.2 A clear distinction should be made between soil and sediment c) Section 3.2, page 3-7, Line 2: what is meant by "… results being specific to the experimental proctocol…"? Didn't Lecoanet et al. follow the same proctocal in the nanomaterials tested? d) Section 3.2.2: It should be noted that TiO2 is expected to precipitate out of the water column and into the sediment • **To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health?** The information is presented in a consistent and thorough manner and all the main exposure routes relevant to biota and human health are presented • **To what extent is the material effectively organized and sufficiently informative to**	• Not that I could find. No change. GD • Text added re: sediment as an important medium in Section 3.1 CH • With regard to the Lecoanet experiment, I just changed the wording to make it clear that the controlled lab results can tell us something about real environmental behavior – the previous wording could sound like something was strange about her protocol.p.3-7 lines 21-23-CH • Don't have a reference for this. No change. GD

	support planning for future research? Gaps in our knowledge in regard to the subject of chapter 3 are profound and are mentioned consistently throughout the chapter by the authors of the report and in that sense the chapter is organized in a clear manner to support planning for future research • **How might this chapter be improved?** It might be a good idea to have a section in each chapter of the report clearly stating the research needs identified in the chapter making a clear distinction between gaps in our scientific and technical knowledge that can be addressed through more research and which cannot.	• Covered in Ch 6. No change. GD
Hristovski	This is a short but well organized chapter focusing on the fate, transport and behavior of nano-TiO_2 in the environment. The exposure routes are clearly defined, outlined and easy to follow. Much of the information presented here represents a good base for developing a clear understanding of the future research needs and directions. However, although most of the information presented here are accurate and represent good overview of existing knowledge of fate and transport of TiO_2 nanomaterials, it is evident that many studies published in the last 12 months (approximately) are not accounted. For example, there are 3 studies cited above that focus on environmental implications of nano-TiO_2 in the environment. It would be very beneficial if studies from the last 12 months are to be incorporated into this report. There is evidence in Kiser et al. (2009) that nano-TiO_2 exists in real world water matrices. These new findings mandate that some of the statements (e.g. page 3-2 Ln 1-3) be changed or restated. One of the main comments in this chapter is related to the study of Schmidt and Vogelsberger	• Kiser et al 2009 study summary added to page 3-4. Sentence updated to say that limited studies were found. GD • Update on above point –Kiser and Battin are added on page 3-4,

	(2006) which focuses on dissolution of TiO_2 nanoparticles. The main concern, here, is related to presentation of the level of dissolution. In their study, Schmidt and Vogelsberger discuss dissolution of TiO_2, however, this dissolution is in the range of µmol/L or nmol/L when saturated suspensions in the range of mg/L (or higher) are considered. The dissolved Ti is negligible when compared to the initial TiO_2 concentrations. The question here is "how relevant this dissolution is to the overall scheme of fate and transport of nano-TiO_2?" The manner in which the information from this paper is presented in this report conveys to the reader that dissolution of nano-TiO_2 is one of the main fate and transport mechanisms. A statement indicating that this dissolution is actually is negligible may contribute to better understanding of almost non-existent dissolution of TiO_2 which is also stated in number of other places in this report. On Page 3-3 Ln 24-25, the report suggest that most nanoparticles were removed via agglomeration with microorganisms. The proper term here to use is biosorption. There is evidence that the nanoparticle-microorganism interactions are more complex than aggregation, and include sorption and even endocytosis. For example references see: 1. Kiser et al., 2010, Biosorption of nanoparticles to heterotrophic wastewater biomass, Water Research, In press. 2. Asharani et al. 2009, Anti-proliferative activity of silver nanoparticles, BMC Cell Biology, 10, 65	lines 20-22, sentence also changed; now says "limited studies" rather than "no studies" are available on the subject of TiO2 behavior in environmental conditions. –CH • Dissolved Ti is thought to be poorly soluble. Added information on starting and ending concentrations of TiO2 and text to suggest that the Schmidt and Vogelsberger results indicate negligible Ti dissolution. 3-3, L13-17 GD • Authors use a variety of terms e.g., "nanoparticles agglomerate with the bacteria", "stick to", or "bacteria-bound adsorption". No change. GD • Added short summary of Kiser et al 2010 results to Section 3.1 Water. GD • Also added mention of Kiser to intro of ch. 3, changing from "no studies" to "limited studies" regarding TiO2 behavior in natural environments. P 3-4, lines 19-21. CH • Did not think this ref was particularly relevant as it seems to be about human tissue experiments and Ag treatments. Not added. GD – Fine with me, this one is a stretch and the

		biosorption point is already made with Kiser 2010 CH
Klaine	This chapter could best be described as a collection of "if…then" statements resulting from the paucity of definitive research on the fate and transport of TiO2. The chapter is an excellent discussion of potential fate and transport pathways and appears to be exhaustive. Further, this chapter accurately discusses the large number of research inadequacies and the discussion facilitates the prioritization of research needs. The chapter can be frustrating to read as in many cases the lack of conclusive data requires the discussion to be speculative at best. However, in the absence of more research it is difficult to recommend improvements to the chapter.	
Nowack	1) The following reference definitely needs to be considered because it is the first to investigate the behavior of nano-TiO_2 under natural conditions in rivers: Nanostructured TiO2: Transport Behavior and Effects on Aquatic Microbial Communities under Environmental Conditions. TOM J. BATTIN, FRANK V.D. KAMMER, ANDREAS WEILHARTNER, STEPHANIE OTTOFUELLING, AND THILO HOFMANN, Environ. Sci. Technol. 2009, 43, 8098–8104. This reference should also be used in chapter 5 because it also contain data on the effects of nano-TiO_2. 2) Chapter 3.1. This chapter discusses fate in water but a lot of the references are for biological growth media. These studies cannot give any clue about the behavior under natural conditions and need to be covered separately, preferably in a sub-chapter in the tox-part of the document. Biological growth media have a composition that is very different from natural waters with very high salt concentrations or organic compounds	• Battin et al. is added and discussed briefly on page 3-4, lines 21-25. Was hesitant to draw this out too much because this type of research question was ranked #3 at the workshop, and we don't have any way of knowing whether the hole in the research prior to including such references was part of what drove this to the top of the list. However, felt it was too important to omit completely. CH • Moved the biological media to section 4.6 on dose, p 4-13 lines 18-29. Added a brief intro to transition into this data.-CH

	that are know to have strong effects on the agglomeration behavior. 3) Chapter 3.1.2. In this sunscreen-specific chapter definitely the papers coming from the French group working with a nano-TiO_2 that is actually used in sunscreens need to be presented in detail: "Structural Degradation at the Surface of a TiO_2-Based Nanomaterial Used in Cosmetics", MELANIE AUFFAN, MAXIME PEDEUTOUR, JEROME ROSE, ARMAND MASION, FABIO ZIARELLI, DANIEL BORSCHNECK, CORINNE CHANEAC, CELINE BOTTA, PERRINE CHAURAND, JEROME LABILLE, AND JEAN-YVES BOTTERO, Environ. Sci. Technol. 2010, 44, 2689–2694, 4) Chapter 3.3. This chapter does not present data on fate and behavior in air but only concentration measurements. This does not belong to this section but to a chapter on analysis in the environment (preferably within chapter 4). Also the study mentioned in the first paragraph of section 3.1. (Wigginton et al.) belongs to this new chapter on analysis in the environment. This belongs to the chapter on exposure.	• Auffan reference is included and described in Section 3.1.2, page 3-6, lines 21-29. Also changed the beginning of the next paragraph to take out "parallels" because I could not make any sense of that.-CH • Not introducing a new chapter into the document. Concentration measurements have a legitimate presence in this chapter, as previous sections in Ch 3 also report measurements, depending on the media. No change. GD
Santamaria	**a. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health?** The information on fate and transport presented in Chapter 3 provides a useful overview of the possible exposure pathways to be considered when evaluating the use of nano-TiO_2 in waste water treatment and in sunscreens. The section on "Air" does not appear to be very informative to the possible fate and transport of nano-TiO_2 in air resulting from its use in water treatment plants or sunscreen. The information in this section is very limited and much more relevant to a facility that would be manufacturing nano-TiO_2 rather than the two case studies in this report. Further, although there are several data gaps where extrapolation of some experimental results may be appropriate, in several	• Added a sentence on 3-10, L12-13 indicating that nano-TiO2 is not expected to enter air via sunscreen application or drinking water treatment processes GD • Reviewer did not indicate where the speculative spots are. Perhaps s/he means 3-6, L6-14. No change made. GD

	instances in this chapter, there are speculations about the possible behavior of nano-TiO_2 in the environment that are not scientifically supported by the information included in the chapter.	
	b. To what extent is the material effectively organized and sufficiently informative to support planning for future research? Suggest having a summary paragraph highlighting the most important data gaps that research could be conducted in the future to fulfill the gaps and reduce uncertainties and speculation about fate and transport of nano-TiO_2.	• Should be covered in Ch 6. GD
	c. How might this chapter be improved? Below are a few suggested comments for improving this chapter. • p. 3-3, line11-17: these two sentences refer to a conference proceedings abstract that reported the occurrence of nano-TiO_2 at a waste water treatment plant; however, there is not enough information about the plant, what the levels of TiO_2 were, how the nano-TiO_2 measured, etc. to provide context and understanding about the significance of this reported finding. Also, because this is a non-peer reviewed conference abstract, I suggest removing this from the document. Further, lines 15-17 state what the authors “predicted” regarding nanomaterial concentrations – this appears to be speculation and should be removed from this report.	• CH deleted bit from conference proceedings and summarized the Kiser et al 2009 study and Battin et al study.
	• p. 3-3, lines 26-31: these sentences are pure speculation from Limbach et al. 2008 about the behavior of nano-TiO_2 based on their experimental results for cerium oxide in waste water and how the high nanoparticle concentration used in the study favors aggregation, and that at more realistic concentration, “a greater percentage of nanoparticles are likely to break through.” Because this is pure speculation, it does not belong in this case study document.	• It is clearly noted that this is the authors’ speculation, not ours. No change. GD
		• Inserted text on the mechanism of H2O2

	• p. 3-3, lines 32-35: There should be more detail about the Harbour et al. 1985 study so that the statement about the behavior of conventional TiO_2 photogenerating long-lived reactive oxygen species in aqueous environments can be put into context; that is, what were the conditions under which TiO_2 generated ROS and how relevant are those conditions to nano-TiO_2 that may be present in water as a result of its use in waste water treatment plants or in sunscreen? There are many variables that may affect the ability to generate ROS (e.g., coatings) that should be considered when extrapolating results from an experimental study to real world situations. Further, that statement, "Similar behavior would be anticipated for nano-TiO_2 is speculation, and should be removed since there are no data to support such a statement. • p. 3-4, lines 27-31: this sentence includes speculation from Zhang et a. 2008 about the "removal efficiencies would be lower for small aggregates than large aggregates at the same alum concentration"; suggest removing speculation. • p. 3-8, lines 19-23: a study by Berges et al. 2007 was mentioned in this section as reporting airborne TiO_2 levels "outside the plant"; however, it is impossible to put this information into context and understand its relevance to these case studies, as there is no information about what type of plant was studied in the Berger et al. 2007 study, duration of the measurement, or how it was obtained. Suggest adding more detail about this study or removing it from this section. • p. 3-9, lines 4-7: The study by Murr et a. 2004 is cited as evidence that nano-TiO_2 may associate with other airborne nanoparticles; however, there is no detail about the Murr et al. 2004 study, making it impossible to determine how relevant the experimental results are to actual ambient conditions.	formation and that Harbour conducted these experiments in the laboratory, not in "the environment". Also clarified that it is not clear how relevant this study's results are for anticipating nano-TiO2's behavior in drinking water or wastewater treatment plants. On 3-4 L22-26. GD • It is clearly noted that this is the authors' speculation, not ours. No change. GD • Added a clause in 3.3 on the fact that it is a European facility that manufactures TiO2 for sunscreens and cosmetics. Also added sentence stating that the duration or env conditions of measurements was not noted. GD • Added a few words about the fact that the TPs were located 1.5m above ground in a variety of outdoor areas in El Paso, TX and that the environmental conditions were not described. GD

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<tr><td>Sellers</td><td>3.1 Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO2 in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health?

Add a discussion of sediment, as a matrix which could be affected by the release of nanomaterials and could be an exposure route relevant to biota and human health. This would provide context for the discussion of exposure by sediment-dwelling organisms on page 4-3. Relevant references include but are not limited to:

• Boncagni, et al., 2009. Exchange of TiO_2 nanoparticles between streams and streambeds. Environmental Science and Technology. 43(20): 7699-7705. (The authors performed a series of experiments to examine the deposition of P25 and synthesized nano-TiO_2.)
• Gottschalk, et al., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for different regions. Environmental Science and Technology. 43(24): 9216-9222. (In this study, the authors predicted environmental concentrations of nano-TiO_2 based on a probabilistic material flow analysis. The paper discusses assumptions regarding partitioning to sediments.)

Specific comments on the draft chapter follow.

• Page 3-1, lines 20-22. The report indicates that the degree of aggregation generally</td><td>• Clarified paragraphs on 3-5 and 3-9 where “sediment” and “sludge” were used interchangeably. Sludge is the only material listed now. GD

• Sediment is now discussed in Section 3.1, page 3-4, lines 28-33, and the suggested references are included.

• Added a paper in HERO by Kim et al. 2009, rather than inserting the Hoon or Kennedy papers on nanotubes. This reference directly tested aggregation of</td></tr>
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	increases with increases in the amount of organic matter in water. However, some experimental work has shown that increasing the level of natural organic matter in water limits the agglomeration of some nanoparticles. See for example: o Hoon Hyung et al., 2006. Natural Organic Matter Stabilizes Carbon Nanotubes in the Aqueous Phase Environ. Sci. Technol. 41:179 -184. o Kennedy, A.J., et al., 2008. Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. Environmental Toxicology and Chemistry 27(9): 1932-1948. Further, on page 3-4 (lines 1-7), the report acknowledges that sorption to humic acid increases the stability of nano-TiO_2 suspensions, which would seem to contradict the assertion on page 3-1, lines 20-22.	nano-TiO2 as a function of NOM and other environmental factors.-CH • Changed the wording and added Kim reference in three places to address this issue: o Added a sentence in Section 3, p. 3-2, lines 3-6 to express the complexity of interactions between NOM and nano-TiO2 with respect to aggregation. o On p. 5-8, lines 29-31, added clause to change from inducing aggregation to affecting it. o On p. 5-25, lines 32-33 changed so that NOM no longer "induces" aggregation but rather "affects the extent of" aggregation. CH
	• Page 3-2, line 17. Suggest clarifying whether the concentrations cited are the initial concentrations added to the solution, or the "concentrations" of the P25 in suspension.	
	• Page 3-3, line 13. As noted above, reference is apparently made to a preliminary report on experimental work in a conference proceeding abstract [157466] that was subsequently published as: o Kiser et al., 2009. Titanium nanomaterial removal and release from wastewater treatment. Environmental Science and Technology. 43(17): 6757 – 6763.	• EPA sources show that it is. No change. GD
	• Page 3-5, line 3. As noted above, drinking water treatment sludge is generally not land applied.	• Added clause to 3-6, L17. GD
	• Page 3-5, line 15. Suggest inserting the following underlined phrase: "… other inorganic compounds are not readily broken down in that environment <u>and nano-TiO_2 is poorly soluble</u>; however…."	• EPA sources show that it is. No change GD

	• Page 3-8, lines 3-4 and 11-12. As noted above, drinking water treatment sludge is generally not land applied. • Page 3-8, lines 19-23. Since air emissions controls can be somewhat dependent upon the local regulations, suggest indicating the location of the facility where Berges et al. made their measurements. Berges et al., 2007 (http://www.dguv.de/ifa/de/fac/nanopartikeln/taipei.pdf; 157594) indicates the facility was in Taipei. **3.2 To what extent is the material effectively organized and sufficiently informative to support planning for future research?** Prioritizing research needs based on the potential impact to various media would require at least a first approximation of the magnitude of potential releases. Further, without some effort to rank-order or prioritize those potential releases the public may be unduly alarmed about some possible exposure routes. Consider adding a summary and conclusions section, which refers to existing life cycle model predictions and, to the extent possible, identifies the major apparent sinks for nano-TiO_2. Even though this summary would be limited by the relative paucity of quantitative information, it would add value. **3.3 How might this chapter be improved?** The chapter could be improved, with respect to the reader new to the science of nanotechnology, by adding a brief discussion of the factors which generally control the fate and transport of nanoparticles. That is, fate and transport discussions primarily refer to suspensions of nanoparticles rather than dissolved concentrations in aqueous solutions (or nonaqueous phase liquids) considered in more conventional fate and transport	• Included the fact that the facility was in Belgium. GD • This would be introducing new information and new analysis. Out of scope. Tom, please confirm no changes needed. GD [Tom: this is borderline, but I agree that it's too much at this point. I mentioned magnitude of releases in 6.2.3.] • Current language in intro to Ch 3 talks about particles in clusters and the factors that influence their agglomeration and dis-agglomeration. It does not seem necessary to add language about the fact that we do not talk about dissolved concentrations that are prevalent in other fate and transport discussions. GD

	assessments; the buoyancy of those particles and their tendency to agglomerate via Van der Waals forces dominates their fate and transport. Electrostatic repulsion, represented by zeta potential and affected by pH and high ionic strength, counters the tendency to agglomerate. Sorption to natural organic matter can enhance the stability of nanoparticle suspensions. Alternatively, such a discussion could be included in Chapter 1.	

4. Chapter 4 provides information on exposure, dose, and translocation of nano-TiO_2 in biota and humans. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Reviewer	Comments	
Hansen	• **Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2.** Again, in general the chapter is well written and provides a thorough review of the literature on the exposure and what is known about the sub-sequent translocation of nano-TiO2 in biota and humans. Minor comments include: a. A distinction is made between dose and exposure and in footnote 11 it is stated that this is consistent with risk assessment usage. References should be provided for this interpretation b. Section 4.2.1.2, page 4-5, Line 25-32 and page 4-6, Line 1-13: It should be noted that these dermal exposure estimates are in line with estimates made by Hansen, S.F., Michelson, E., Kamper, A., Borling, P., Stuer-Lauridsen, F. & Baun, A. 2008, Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products. Ecotoxicology 17 (5): 438-447. c. Section 4.4, page 4-13, Line 6-15: It should be noted that Mueller and Nowack (2008) assumes that 97% and 90% of the nanoparticles would be cleared in the realistic and the high exposure scenario, respectively. These assumptions have yet to be validated d. Section 4.4, page 4-13, Line 6-15: Not clear to me why PNEC estimates made by Mueller and Nowack (2008) is relevant to report here. I recommend that it is deleted or move to the chapter 5	• Added a ref to EPA's Guidelines for Exposure Assessment. Tom • Hansen's reference is now included in section 4.2.1.2-CH • It is already acknowledged that various assumptions are used by Mueller and Nowack, which are by definition not validated, no special note made.-CH • Determined that Mueller and Nowack are clearly relevant, they remain here.-CH • Boxall is now included in

	e. The work by Boxall et al. should be reviewed and cited as well (Boxall, A.B. A., Chaudhry, Q., Sinclair, C., Jones, A., Aitken, R., Jefferson, B., Watts, C. 2008. Current And Future Predicted Environmental Exposure To Engineered Nanoparticles. York: Central Science Laboratory). Based on available information about the applied concentration of nanoparticles in cosmetics, personal care products and paints, Boxall *et al.* (2008) used a long series of algorithms (for among other pesticides, medicinal products, and ultrafine particles) to estimate the predicted environmental concentrations of nanoparticles in soil and water. Although anticipating that 10% market penetration probably provides a conservative estimate (with the exception of sunscreens), Boxall *et al.* calculated the PEC for three scenarios assuming that 10%, 50% and 100% of the products on the market contained nanoparticles. The total predicted concentrations in water were found to be for titanium oxide (24.5-245 μg/L) used in among others paints and sunscreens. It might furthermore be a good idea to look into the work recently reported by Gottschalk, F., Sonderer, T., Scholz, R. W. & Nowack, B. (2010). Possibilities and Limitations of Modeling Environmental Exposure to Engineered Nanomaterials by Probabilistic Material Flow Analysis. Environmental Toxicology and Chemistry, 29, no. 5, pp. 1036-1048. f. Section 4.6.6, page 4-30, Line 30: It is stated that "Quantitative risk assessment relies on dose-response relationships." and I wonder to which extend discussion about dose-response relationships is relevant in a chapter on Exposure-dose characterization? • **To what extent is the material effectively organized and sufficiently informative to support planning for future research?** As in the case of the previous chapter, gaps in our knowledge in regard to the subject of chapter 4 are profound and are mentioned consistently throughout the chapter by the authors of the report and in that sense the chapter is organized in a clear manner to support planning	section 4.5, page 4-13, lines 15-26, along with Gottschalk 2009 and 2010.-CH • I think dose-response relationship is ok here. It contextualizes the discussion of using particle surface area as a dose metric. No change GD

	for future research • **How might this chapter be improved?** It might be a good idea to have a section in each chapter of the report clearly stating the research needs identified in the chapter making a clear distinction between gaps in our scientific and technical knowledge that can be addressed through more research and which cannot.	• Chapter 6 should cover this. GD
Hristovski	This chapter represents a well organized prospectus addressing the potential for nano-TiO_2 exposure and dose in biota and humans. The information in this chapter is clearly presented and, in many cases, well summarized in tables. The routes of exposure are transparently described and easy to understand, especially by readers without significant scientific or technical background. The overall knowledge summarized in this chapter offers a good starting point for future research planning in addition to understanding the future research needs in this area. Addition of schematics and illustrations, like Figure 4-1, could further improve the ease of apprehension of the presented information. Although the importance of exposure to co-contaminants sorbed onto nano-TiO_2 is well presented and emphasized, discussions related to synergistic effects of nano-TiO_2 and co-contaminants appear to be minimized or neglected. With respect to more specific comments, on Page 4-13 Ln 18, the manner in which the statement is written gives an initial impression to the reader that the section 4.6. summarizes only studies that focused on fish that have been exposed to nano-TiO_2. This, of course, is not true as summarized in the latter part of this paragraph. On Page 4-14 Ln 4, "stock solution" is used as a term which may confuse a reader of this text because no stock solutions are used in the description; but rather "stock suspension" is discussed. Consistent and correct terminology is important and contributes for improved understanding of	• No additional figures will be added at this time to the document. GD • Synergistic effects are extremely difficult to evaluate; I think they're handled appropriately here (e.g., TiO_2 and arsenic uptake).Tom • Modified the paragraph to better reflect the actual studies evaluated. 4-14 L10-13. GD • No change. GD

	the presented information. On Page 4-14 Ln 15-22, environmental factors were discussed that could explain the differences between the studies conducted by Zhang et al. (2006) and Federici et al. (2007). Here, it is important to note that the available surface area in the experimental tanks could significantly contribute to experimental differences. Many studies have reported that nano-TiO_2 (and other nanoparticles) likes to interact with existing surfaces in the local environment. For example, larger available surface area could allow for higher "loss" of the suspended nanoparticles in the water matrix. On Page 4-15 Ln 15-16, statement is made that bioaccumulation was not observed in the quantum dots study by Holbrook et al. (2008). Here it is not clear whether bioaccumulation of actual nanoparticles was examined or the quantum dots residual. Many quantum dots, for example CdTe or CdSe, once entering or interacting with biomass or other living organism can be "dissolved" to form ions. In this case, accumulation of the quantum dots into the living organism may be shown via increased concentrations of elements that comprise the quantum dots (e.g. Cd and Te in case of CdTe). This is not the case with nano-TiO_2 because these types nanoparticles do not dissolve. On Page 4-17 Ln 4, it is stated that adsorption equilibrium of Cd onto nano-TiO_2 occurred within 30 minutes. First, the proper term to use here is pseudo-equilibrium; and second, the time presented here period may be to short. Number of studies have shown that for sorption pseudo-equilibrium to occur minimum 2-6 hours are required. Furthermore, the porosity of the sorbent materials is one of the key factors that control the rate of sorption. In this study, two materials with completely different surface areas are compared (nano-TiO_2 and sediment particles). This comparison is inadequate and the derived conclusions may not be appropriate. Additionally, there are number of sediments that could have performed better than nano-TiO_2.	• The authors did not mention such factors, so any additions of this nature would be speculative on our part. No change. GD • Taking the reviewer's point that the study measured Cd2+ and assumed it was entirely in the form of quantum dots (with no dissolution), I added a sentence to this effect. 4-16 L12-14 Tom • Authors used "equilibrium", not "pseudo-equilibrium", so no change made. • We are presenting study results, not critiquing study design. No change made. GD

	For example, when dealing with sediment, sulfide (present in sediments) can strongly bind available Cd (probably Cd^{2+}), so one can clearly state that sediment with higher sulfide concentration may have higher adsorption capacity. Defining the term "instillation" in section 4.6.2. may contribute to improved understanding of this section. This term is explained in Chapter 5 and it is commonly used by the scientific community; however, a common reader may not be familiar with terminology. Table 4-4 provides an excellent overview of TiO_2 absorption/penetration studies; however, dividing this table into 2 separate tables may be beneficial. The first table could focus on sunscreen formulations containing nano-TiO_2; while the second table would could on other nono-TiO_2 formulations. It would be beneficial if a summary (summarizing the most important issues discussed in this chapter) is presented at the end of this chapter. The manner in which this chapter ends leaves the reader "hanging."	• Added a sentence to 4.6.2 defining "instillation". Left detailed discussion til Ch 5 GD • No change GD • Should be covered in Ch 6 GD
Klaine	This chapter benefits by the significantly more published research in this area as compared to that in chapter 3. In general, the chapter is well written and accurately reflects the state of the science (see specific comments below). While few real conclusions are made, there is less conjecture than in the previous chapter. Hence, the chapter does provide an excellent foundation for prioritizing research needs. Suggested edits and improvements are below. Page 4-3. Line 18-22 makes an excellent point but the statement should include floating aquatic plants. Page 4-4. I find it strange, although it is discussed later in this chapter, that section 4.2 does not	• No additional examples of affected organisms added. GD • Section 4.2 introduces detailed sections, one of which is all about sunscreen

	mention sunscreen as an exposure route for humans. Page 4-15. Line 15 and 16. This sentence is incorrect. Holbrook et al (2008) did show bioaccumulation (as discussed in the previous sentence). I suspect the sentence was supposed to read "**Biomagnification**, however, was not observed in the quantum dot study." Page 4-30. Section 4.6.6 is extremely well done and illustrates the complexity of this issue. The section might be best concluded by a caution to researchers to pay attention to multiple dose metrics and to regulators to interpret these studies with caution.	exposure in humans (4.2.1.2) • Added text clarifying that biomagnification was not found at the rotifer level. 4-16L 15-16. Tom added a statement about the applicability of this study to nano-TiO2.
Nowack	1) In a chapter on exposure I was expecting to read something about concentrations of nano-TiO_2 in different environmental media. However, section 4.1 is named "biota" with subsection on "aquatic species" and "terrestrial species". This is a very strange organization. In my point of view 4.1. should be about nano-TiO_2 concentrations in water (4.1.1.), sediments (4.1.2.) and soils (4.1.3.). In this part results from measurements and modeling about concentrations should be compiled. Mueller and Nowack (2008) and Gottschalk et al. (2009, 2010) have provided such data on concentrations (model results). Also Boxall et al (2008) have presented modeled data. Later in section 4.5. some modeled data are presented. On p. 4-13 the study by Mueller and Nowack (2008) is presented. However, the risk characterization part of that study does not belong to chapter 4 but to chapter 5 or 6. Only the modeled environmental concentrations belong to chapter 4 and the rest needs to be covered at another place in the document. 2) Chapter 4.3. is a very important one but again it remains qualitative. Based on data by Mueller and Nowack (2008) and Gottschalk et al. (2009, 2010) it is possible to relate the use of nano-TiO_2 in sunscreen to the total nano-TiO_2 use and thus evaluate the importance of sunscreen for total environmental exposure to TiO_2:	• Mueller and Nowack, Gottschalk (2009 and 2010 papers), and Boxall are all included in section 4.5, page 4-13, lines 15-26. • A reorg along these lines is out of scope. GD

	Gottschalk, F. Sonderer, T.; Scholz, R. W.; Nowack, B. (2009) Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ. Sci. Technol. 43: 9216-9222 Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B. (2010) Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. Environ. Toxicol. Chem. 29: 1036–1048.	
Santamaria	**a. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2.** The information included in this chapter does accurately and sufficiently characterize the information on exposure characterization. One suggestion is to change the title of this chapter to Exposure Characterization, as that is really the focus of this chapter, not dose characterization. Exposure characterization is also a more appropriate title for the specific case studies that are being developed. The organization of the information and the topics are appropriate with one suggested change. On page 4-7, section entitled, "Occupational": This section seems to be too long and out of place for these case studies, as most of the information focuses on facilities where TiO_2 is manufactured and much of the information is not relevant to the case studies. Suggest making this section shorter and more specific to the occupational settings involved in these case studies. **b. To what extent is the material effectively organized and sufficiently informative to support planning for future research?** It is not clear from reading this chapter what future research is necessary for characterizing exposure to TiO_2. Suggest including a summary paragraph highlighting the most important data gaps and what types of research needs there are regarding being able to characterize	Occupational exposure during manufacturing is a major exposure pathway to consider during a CEA, so the extended discussion is not out of scope. Little data are available for nano-TiO2, so conventional TiO2 results fill in. No change GD • Chapter 6 should cover this. GD

	exposure for these case studies. **c. How might this chapter be improved?** Below are a few specific suggested comments for improving this chapter: • p. 4-9, lines 1-11: The study by Berges 2007 appears to be a study of a facility that produces nano-TiO_2 for use in sunscreens, not a facility where the sunscreens are formulated. This distinction should be clearly stated. It is not clear how relevant the reported inhalable and respirable concentrations measured in this TiO_2 production factory would be to a sunscreen formulating facility. • p. 4-10, lines 7-13: this paragraph about the *in vitro* study by Liao et a. 2009 does not belong in this section – it should be in Chapter 5. Furthermore, the use of *in vitro* data to predict *in vivo* lung inflammatory responses is not appropriate, given that fact that there are several differences between single cell-type responses *in vitro* and *in vivo* whole tissue (lung) conditions. The statement that workers would have significant risk of cytotoxicity response is speculation and results from an *in vitro* study cannot support such a conclusion. • p. 4-11, Section entitled, "4.4 Cumulative Exposure to Nano-TiO_2 and Other Contaminants" appears to be primarily speculation with little scientific support. The "Trojan horse" effect is not something that has been observed with sunscreen, so this concept of exposure to other contaminants doesn't seem to apply to the sunscreen case study. Further, it is not clear whether there is any evidence to support this happening in waste water treatment plants either.	• Added a sentence with this caveat to 4-9 L 13-15. GD • The peer reviewed study's conclusion about risk is not something we can now say is untrue as it is already published in Liao et al. Including a sentence (page 4-10 lines 17-20) to clarify how the experiment was carried out using lung models rather than actual workers.CH • Deleted reference to "Trojan horse" effects as this seemed to be creating alarm. This is not a term that the study's authors use either, but they do make the case for co-contamination so to speak. GD • The text says that this concern applies to various stages of the life cycle, not just the finished product of sunscreen or drinking water treatment

	• p. 4-18, lines 4-12: the document should include a discussion about the differences between the nasal pathway and olfactory bulb in rodents versus humans (e.g., differences in the relative size of the rat olfactory mucosa and olfactory bulb likely predispose rodents, more so than humans, to nasal deposition and olfactory transport. • p. 4-20, Table 4.3: specify the duration of the study and treatment period for all of the studies • p. 4-24, Table4-4: add the following study: Bennat C. and Muller-Goymann CC. 2000. Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. Int. J. Cos. Sci. 22:271-283. Also, is it possible to add the names of the authors for the citations that are listed as "Refs in SCCNFP"? • p. 4-27, lines 13-18: sentence should only include information from the final study (Sadrieh et al. 2010) and not the 2008 meeting abstract.	compounds. No change GD • Sentence added to acknowledge that differing animal models could change results in section 4.6, pg. 4-14, lines 22-25. CH – also, this exact issue is covered on page 5-73 • I think Table 4.3 already does this to a good extent. No change. GD • Study added to Table 4-4 as it is about skin absorption CH • Re: SCCNFP refs, many of them could not be located, as they look to be internal company studies or reports in German, from the 1990s. I found #70 (Pflucker et al 1999). Deleted all SCCNFP study summaries from Table 4-4 that referred the reader to other refs, added the Pflucker et al study, and left overarching table entry for "various TiO2 studies" with the SCCNFP ref. Did not want to include refs that we cannot access or confirm ourselves. GD • Changed reference and updated findings. 4-28 L13-

	• p. 4-28, section 4.6.4: it should be mentioned that the gavage dose of 5g/kg is a high dose when discussing the results of this study. • p. 4-28, Section 4.6.5.: This section should include mention of the point that most of the studies were intravenous or intraperitoneal, routes of exposure that are not relevant for these case studies.	14. GD • Used wording from the study: "fixed large dose" and put as 5000 mg/kg in text and table 4-5 • Paragraph added to explain why unlikely routes of primary exposure are still important to internal exposure discussion – section 4.6 page 4.14 lines 27-31 - CH
Sellers	**4.1 Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2. To what extent is the material effectively organized and sufficiently informative to support planning for future research?** Specific comments on the draft chapter follow. • Page 4-2, lines 10-11. The draft report says that "Because typical wastewater treatment plants currently do not monitor for or specifically target nanomaterials, nano-TiO_2 might not be completely removed by sewage treatment." It would be more accurate to simply say that "Discharges of nano-TiO_2 from wastewater treatment plants are not currently regulated. Therefore they are not designed or operated to remove nano-TiO_2, although early research suggests that some removal can occur (Kiser et al., 2009)." • Page 4-4, lines 18-22. Consider adding the following information to this discussion of nano-TiO_2 in drinking water treatment. Currently, the levels of titanium are not regulated in public water supplies (http://www.epa.gov/safewater/contaminants/index.html). Therefore, water treatment facilities typically do not monitor the levels of Ti in potable water.	• Wording changed and Kiser reference now added in section 4, page 4-2, lines 9-12 • Christine's addition on previous page is sufficient to make the point that Ti is not currently regulated in drinking water. No change. GD • Because the case study was

	• Pages 4-5 and 4-6, discussion of dermal exposure. The estimated ranges of exposures are based on the assumption that sunscreen contains 5% nano-TiO_2, yet the report cites a maximum concentration of 15% nano-TiO_2 in sunscreen. Why was the maximum (15%) not used to develop upper-bound estimates? • Page 4-7, lines 14-15. Kiser et al., 2009 cite a second study by Lomer et al., 2000, which indicates that the daily human intake of TiO_2 (average size <200 nm) has been estimated to exceed 5.4 mg/day. They provide the following reference: o Lomer, et al., Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. *Analyst* **2000**, *125* (12), 2339–2343. • Page 4-8, lines 10-17. Suggest concluding the paragraph with a statement that occupational exposure can vary between facilities. • Page 4-10, lines 14-23. To put the observations of Li et al. into context, indicate that the facility was located in Shanghai. • Page 4-16, table 4-2. Suggest indicating in this table the means, if any, used to maintain nano-TiO_2 in suspension. • Page 4-27, lines 1-5. Mortensen et al. qualified their results by saying that under no circumstances is there evidence for massive quantum dot penetration and that quantum dots collected preferentially in the folds and defects in the stratum corneum, as well as in hair follicles. • Section 4.6.6. Please see the recent (June 2010) publication by OECD, i.e., Publication of the Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials. **4.2 How might this chapter be improved?**	not looking at an upper bound estimate of exposure. A mid-range concentration may be more widely applicable. GD • Lomer reference now included in section 4.2.1.2, page 4-7, lines 14-15 –CH • Added, 4-8, L18-19 GD • Inserted location and that this factory represented particularly substandard conditions. GD • No change, means not added. GD • Text added 4-28 L5-8 GD • Verbiage added about the OECD publication and added reference to.Section 4.6.6, page 4-30, lines 27-28-CH • No change. GD

	This chapter could be improved by moving the important discussion of dose metrics in Section 4.6.6 to the beginning of Section 4.6.	

5. Chapter 5 characterizes factors that influence ecological and health effects of nano-TiO_2 and discusses the currently available scientific evidence regarding these effects. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Reviewer	Comments	
Hansen	• **Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science.** In general, the chapter does accurately and sufficiently characterize that the state of the science in regard to what is known about the ecotoxcity of nano-TiO2. Minor comments include: a) Section 5.2.1.2 The study by Hartmann et al. should be reviewed and cited: N.B. Hartmanna, F. Von der Kammerb, T. Hofmannb, M. Baaloushac, S. Ottofuellingb, A. Baun. Algal testing of titanium dioxide nanoparticles—Testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology 269 (2010) 190–197. In regard to the Health effects of nano-TiO2 (Section 5.3) is outside the area of my expertise • **To what extent is the material effectively organized and sufficiently informative to support planning for future research?** I believe that the materiasl is effectively organized and sufficeniently information to support planning for future research, although I do think that it could be made more explicit what the key research gaps and research needs are, for instance in the form of a list of bullets in the end of section 5.2 and 5.3, respectively. • **How might this chapter be improved?** It could be made more explicit what the key research gaps and research needs are, for instance	• Hartmann now included with a new paragraph in section 5.2.1.2, page 5-21, lines 14-21-CH • Research questions captured at end – explicitly not identifying needs so this is not to be addressed as suggested – CH

	in the form of a list of bullets in the end of section 5.2 and 5.3, respectively.	• Same comment
Hristovski	This chapter appears to be the most organized chapter in this report. The approach to describing and outlining both the ecological and the health effects of nano-TiO_2 is well conceived and easy to understand. One of the strengths of this chapter is the clear emphasis on the factors that influence ecological and health effects of nano-TiO_2 at the beginning of this chapter. Additionally, probably the biggest plus of this chapter are the end of section summaries. End-of-chapter summary that summarizes the entire chapter would also be beneficial. Based on the summarized published literature, the existing research in this area needs to be augmented by employing stronger nano-TiO_2 characterization techniques and consequently correlating the characterization data with the toxicity data. One simple approach to do this and improve the quality of this report is to recalculate and introduce the surface area as a key parameter, in addition to mass, of P25. There are number of studies that show that P25 exhibits a specific surface area of ~ 50 m^2/g. Using this information, one can estimate the studied doses in m^2 rather than in grams. This new data for P25 can be introduced in the tables of this chapter. Another major recommendation with respect to improving the quality of this chapter is introduction of an appendix with explanation of specific medical terms. On many occasions, specific medical terms were used that are common knowledge among the scientist and medical personnel; however, readers who do not have specific knowledge in this area need a dictionary to understand the medical terminology. For example, medical personnel can easily understand what a pulmonary fibrosis is, but this term could be a puzzle for a reader who has limited medical knowledge. On Pages 5-6 and 5-7 tables 5-1 and 5-2 are presented summarizing recommendation for nanomaterial characterization. Here, it is not completely clear whether these tables are used as an	• Making a new recommendation that was not explicitly included in the questions reviewed by the workshop is outside the scope of this revision – characterization and surface area in particular are mentioned already in both characterization and dose chapters of the document - CH • Addition of medical appendix outside scope of this revision – CH • Good point - surface area is included as optional in the tables as recommended by

	illustration (i.e. example) or as recommendations of this document. If they are to be used as recommendations by this report, than surface area is one of the properties of nano-TiO_2 that must be characterized, especially for toxicity studies. In Section 5.1.2.1. dispersions of nano-TiO_2 are discussed with water and oils as the matrix. One important phenomenon that has to be addressed here is that the nanoparticles by themselves can act as a dispersant/surfactant, forming so called Pickering emulsions. In brief, nanoparticles can be the surfactant that helps make liposomes. On Page 5-8 Ln 20, the sentence starts with lowercase m instead of uppercase M. When discussing radical formation and scavenging inside the cell (on the same page), it is beneficial to make a statement that one of the reasons why in-cell ROS have not be verified is because of presence of radical scavengers that exist in cells. One of the statements which appear to exist in number of places in this document, and especially in this chapter is the statement related to organic matter and its interactions with nanoparticles. On number of occasions it is stated that the degree of aggregation generally increases with presence of organic matter. Considering that the most commonly found organic matter in water is the natural organic matter (NOM), it is safe to state that the discussion here is related to NOM rather than non-NOM organic matter in water. It appears that there is a misinterpretation of the NOM interaction with nanoparticles that possibly comes from the paper by Navarro et al. 2008 (cited in this chapter). In the majority of cases, NOM is a complex of organic compounds that contain one or more carboxylic groups and hydroxyl groups. In general, pKa of NOM is ~ 4.5, which makes it negatively charged in natural waters. The NOM has a tendency to sorb onto the nanoparticles (e.g. coat them); change their surface charge and stabilize them (in other words prevent them to aggregate). So unless the organic matter in question in this section is other than NOM (if so it needs	Oberdorster. Changed the titles of the tables to “published recommendations…” – also updated List of Tables TOC – CH • Added a sentence and requested Chen 2007 reference in HERO, which has to be dropped in.-CH • This is an area of research that is complex; some surmise that the lack of detection is due to presence of quenching, radicals but we would need many references and a new section, believe it is outside scope of revisions - CH • Added a sentence in Section 3, p. 3-2, lines 3-6, page 5-8, lines 28-30 and on 5-25, line 26, to express the complexity of interactions between NOM and nano-TiO2 with respect to aggregation. -CH

	to be specified), these statements are not completely true (e.g. Page 5-8 Ln 28-29). Also, addition of salts increases ionic strength, so statements, where "increase of salt concentrations" or "increase of ionic strength" are used together in the same sentence, are mere duplication. On Page 5-9 Ln 30-33, discussion about sonication and dispersion of nanoparticles is presented. It is essential to state that the particle size distribution during sonication is impacted by both the power input of the sonicator and the initial properties of the nanopowders (which in many cases may be comprised of microscopic particles that have nanocrystaliune structure). In brief, it needs to be emphasized that the dispersion method (e.g. power used; powder used; etc.) is very important. On Page 5-10 Ln 1-5, in the same paragraph as above mentioned comments, it is not clear what a secondary particle size is. Additionally, the study by Federici et al. (2007) uses electron microscopy to determine dispersion of P25 and from there extrapolates the statement of "good dispersion." This statement is copied into this report without clearly explaining what this "good dispersion" means. Splitting Table 5-3 into four different tables each focusing on different exposure or organism, may contribute for better readability and easier access to the desired information presented in this table. Page 5-24 Ln 4-6: This sentence is not clear. On Page 5-24 Ln 32-34, toxicity of ZnO to zebrafish eggs is discussed. It may be prudent to state	• Added reference Kim et al. 2009, rather than inserting the Hoon or Kennedy papers on nanotubes as suggested by Sellers. This reference directly tested aggregation of nano-TiO2 as a function of NOM and other environmental factors. Inserted now.- CH • Read paper – deleted "secondary" as unnecessarily confusing – changed "good" to "effective" – CH • Tables will not be split – CH • What the clarity issue was but I changed a couple of words in this area to help clarity- CH

	that ZnO has a tendency hydrolyze in water and release Zn^{2+} ions that may be toxic, in contrast to the stable nano-TiO_2 or Al_2O_3. Page 5-25 Ln 18-28: See the comment on NOM. In Section 5.2.1.4, it may be beneficial to mention that some of the interactions between nano-TiO_2 and contaminant may result in synergistic effects. Page 5-28 Ln 1: The sentence starts with lowercase m. Page 5-28 Ln 8: The sentence starts with lowercase m. In the summary of section 5.3.2.4., it may be beneficial to emphasize that persistent inflammation is the primary reason for the observed carcinogenic effects of nano-TiO_2.	• Without a reference for this phenomenon it seems unnecessary to add this tangential point. • NOM point answered • Added "and potentially synergistic" to opening sentence of paragraph – CH • The section directly above this summary section heavily emphasizes inflammation, and since it is a concise section I chose not to start the potentially complicated task of adding specifics such as this, when they are already covered nearby. –CH
Klaine	This is by far the most data-rich chapter of this document. It is well organized and I particularly appreciate the comments on page 5-1 lines 15 – 18 that discuss the need for material characterization and warn that many studies do not include this. Hence, a significant amount of data discussed in this chapter must be interpreted with caution. The discussion appears exhaustive and critical.	

	The discussion of the influence of physicochemical characteristics on TiO2 effects is much appreciated and certainly allows the reader to readily deduce the research needs in this area. The section ends with recommendations for nanomaterial characterization. While several publications are cited I would suggest inclusion of the material at www.characterizationmatters.org since is a compilation of an interdisciplinary workshop that not only included nanomaterial researchers but also representatives from regulatory agencies, funding agencies, and peer-reviewed journals. Section 5.1. The discussion of the influence of experimental conditions is very useful. The discussion in section 5.1.2.1 could be improved by adding a paragraph on the influence of suspension instability on actual exposure metrics and the resulting inaccuracies in dose-response relationships. The sentence at the bottom of page 5-9 and the top of page 5-10 is unclear and could be improved by deleting the last three words. Section 5.2. This section is a great compilation of the ecological effects research. It rightfully points out the lack of studies on TiO2 intended for sunscreen use. It could benefit from a discussion of the influence of suspension instability on the dose metric aquatic toxicity studies. This issue results in organism exposure to lower than expected nanoparticle concentrations. Hence, effects are actually attributed to higher concentrations than those actually causing effects. Section 5.3. This is a very well done section. My only suggestion is to add a discussion of i*n-vitro* studies since there are a large number of them. However, results from these must also be taken with caution because they have not been shown to be predictive of *in vivo* studies. This issue is still worthy of a discussion.	• Added a simple sentence including the website at the end of section 5.1.1 on page 5-3 lines 6 & 7.CH Waiting on HERO reference • Tom to add mention of suspension instability [done – Tom] • Clarified this sentence GD • See above on suspension instability • The text says For a review of nano-TiO_2 in vitro effects, see Fond and Meyer (2006, 196337). No change. GD
Nowack	1) In this chapter the point 6 mentioned in my comments to chapter 1 is crucial but is not really discussed: what does it mean that some nano-TiO_2 is coated with alumina or silica and what is the actual surface that an organism or cell sees? This issue is not explored but should receive a	• Inserted text that Tom added to Ch 1 regarding the unknown effects of coatings in 5.1.1.3. GD

	lot of attention. What if the ecotox and tox-data are grouped according to surface coating? Can we already see some trends? Are some coatings more or less toxic? How different are different TiO_2-formulations? 2) The first paragraph in section 5.1.2.2. has nothing to do with TiO_2 and should be deleted. 3) Chapter 5.2. is called "ecological effects". Under this title I would expect to read something about effects on ecosystems, however this section is about ecotoxicology and should thus also be named "ecotoxicology". 4) Chapter 5.2.1.3. There is a study available about nano-TiO_2 effects on willows that should be included: Seeger et al. (2009) J. Soils sediments 9(1), 46-53.	• C60 studies are used as an introductory illustration of how solution preparation can change a nanomaterial's effect. No change GD • Disagree. A number of different organisms are discussed, as well as food web effects. No change GD • Added summary of Seeger study to 5.2.1.3. and Table 5-3 GD
Santamaria	**a. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science.** The information included in this chapter seems to accurately and sufficiently characterize the state of the science very well. The discussion of studies in the "Health Effects" section was thorough and the shortcomings and strengths of some of the types of studies (e.g., inhalation studies) was clearly written. The level of detail for the summaries of the inhalation studies seems excessive, given that the inhalation route of exposure is limited to the occupational setting where the nano-TiO2 is added to the sunscreen. The fact that there have not been reports of workers at such facilities experiencing adverse respiratory health effects brings into question the relevance of the inhalation studies. **b. To what extent is the material effectively organized and sufficiently informative to support planning for future research?**	

	Suggest a summary paragraph highlighting the most important research needs. **c. How might this chapter be improved?** • p. 5-28, lines 3-7: Is there any scientific basis for the statement that coated photostable nano-TiO_2 in sunscreen could lose its coating? This appears to be speculation, and should be modified to accurately reflect what is known about TiO_2 coating. • p. 5-62, lines 4-13: suggest removing discussion of abstracts and posters in these case studies; they are not peer reviewed published studies. • p. 5-64, line 25-26: change lung cancer to lung tumors; should also include discussion of particle overload being a major contributing factor to the development of tumors in these studies. • For summaries of the various rodent carcinogenicity studies, the wording should be changed from "lung cancer" to "lung tumors" • p. 5-69, section entitled, "Intramuscular Injection": suggest removing this section; is not a relevant route of exposure. • p.5-72, lines 1-2: this statement is incorrect, the carcinogenicity of TiO_2 and nano TiO_2 has not been shown "repeatedly in inhalation and instillation studies" – recommend changing to "a few rat studies with TiO_2 have reported the development of lung tumors…."	• Should be covered in Ch 6 • Studies by Botta et al 2009, Barker and Branch 2008, and Auffan et al 2010 in the case study outline the scientific basis. GD • No change. GD • Changed to tumors; particle overload discussed on 5-72 GD • Looked at cited studies, and change to "tumor" where applicable. GD • Wording added • Sentence added mirroring Ch. 4 comment on this issue p5-66, lines 27-30.-CH • Deleted "repeatedly". GD
Sellers	**5.1 Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research?** This question is outside my primary area of expertise. With that caveat I offer the following	• Wording added in section 5.2, page 5-12, lines 24-26. References have been added, although that makes the line spacing really ugly. Perhaps

	comments.
	• Page 5-2, lines 28-30. Consider acknowledging the efforts underway through the OECD Sponsorship Programme. See http://www.oecd.org/document/47/0,3343,en_2649_37015404_41197295_1_1_1_1,00.html. And consider acknowledging the following databases:
	    o OECD Database on Research into Safety of Manufactured Nanomaterials, http://webnet.oecd.org/NanoMaterials/Pagelet/Front/Default.aspx?
	    o The Project on Emerging Nanotechnologies database on Environment, Health, and Safety Research http://www.nanotechproject.org/inventories/ehs/
	    o International Council on Nanotechnology, NanoEHS Database http://icon.rice.edu/virtualjournal.cfm
	• Section 5.1.1.4, recommended characterization of nanomaterial [sic] for ecological and toxicological studies. Suggest incorporating OECD guidelines, e.g., Publication of the Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme; First Revision.
	• Page 5-7, lines 4-6. Suggest editing to read as follows: "Experimental conditions, particularly the choice of medium/vehicle in which to disperse nano-TiO_2, preparation of testing solutions or suspensions, the formation of agglomerates, and/or measures taken to control the formation of agglomerates can influence the behavior and effects of nano-TiO_2 and other nanomaterials."
	
	5.2 How might this chapter be improved?
	
	This question is outside my primary area of expertise.
		Connie can work some magic.

6. Chapter 6 summarizes the information and research questions presented in the nano-TiO2 water treatment and sunscreen case studies, as well as discussing the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

Reviewer	Comments	
Hansen	• **We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments.** Chapter 6 is divided into two separate parts. One part provides a summary of the various chapters 1-5. This summary is good and I think that the list of important issues/research questions provided in Section 6.1.1, 6.1.2, etc. is good and very helpful in supporting future research strategies and assessments. The second part of chapter 6 is the suppose to be the core result of the whole report i.e. research recommendations and prioritizations and with that in mind section 6.2.1-6.2.3 stands out as rather weak. A prioritization list of research areas is cited to have been made by experts at a NCEA workshop, but it is not clear how the expert were, what the discussions about the various research gaps were and what the arguments for and against ranking one or the other research area higher or lower than another. The issue of prioritization might be the most important part of this report and hence this part should be expanded on. Minor comment: In section 6.2.2 and 6.2.3 the authors of the report use the term "…life cycle assessment…" whereas previously in the report the word "assessment" was not used. Instead the word "framework" or "stages" was used which seems more appropriate as life cycle assessment often includes issues not considered in this report e.g. energy	Pointed the reader back to the report for this information. (Tom). Changed to "stages". (tom)

	uses of different processes • **How might this chapter be improved?** It could be made more clear how the prioritization list of research areas was generated by experts at a NCEA workshop, what the discussions about the various research gaps were and what the arguments for and against ranking one or the other research area higher or lower than another. Furthermore, it would be interesting to discuss how knowing, for instance, whether or not the existing testing protocols are appropriate (as stated in section 6.2.1, page 6-16, Line 7) would change and impact the outcome of the CEA in the two case studies	This is identified as important information in the workshop summary, and the reader is referred there.
Hristovski	This chapter provides a well rounded summary of the information and research questions/needs presented in the previous four chapters. The integration of the evidence in order to provide a starting point for refinement of future research strategies is clear and well conducted. Overall, there are no major suggestions on how to improve the quality of this chapter. There are repeating comments from the previous chapters that are applicable to this section of the report which exist as a result of the process of putting the summary together. Addressing the comments from the previous chapters, and addressing the same comments in this section could improve the overall quality of this report. Bulleting may improve the readability of some sections in this chapter. For example, on Page 6-10 Ln 21-28, bulleting the important questions that deserving a further investigation may yield a text that is easier to read. Another example is Page 6-12 Ln13-22.	Added bullets (Tom).
Klaine	Section 6.1 is an accurate and very readable summary of the document. I don't see much	Not ready for this step. (Tom)

	integration (e.g. estimated exposures being compared with effects concentrations) although the state of this science may be premature for this to happen. Section 6.2.1 is an interesting report on the workshop and the method and outcome for setting research priorities. However, sections 6.2.2 and 6.2.3 fall short of discussing the next steps for this effort. I expected a more concrete and definitive discussion on how the efforts in the previous 5 chapters would be used to set research priorities within NNI or at least EPA. Instead of such a discussion the document close with a justification of the use of the CEA framework. The chapter could be improved by discussing the CEA framework, working through the existing data, and discussing uncertainty. The uncertainty discussion could be used to generate a prioritized list of research needs. Without this, the reader is left with an insurmountable mountain of data gaps with no real idea which data are most important to facilitate quantitative risk assessment	Beyond scope. (Tom) Workshop was used to prioritize; bullets may help orient reader to important issues. (Tom)
Nowack	1) Chapter 4 was on exposure, chapter 5 on the effect but what is missing is a chapter on the risk (risk = exposure x effect). This could be part of the integrative summary or could also be a stand-alone chapter. I would suggest to make a new chapter 6 on risk so that the old chapter 6 then becomes chapter 7. At least three studies are available that have already tried to perform such an environmental risk assessment: Mueller and Nowack (2008) and Gottschalk et al. (2009, 2010). The risk assessment result of these studies should be included and critically evaluated. This could also be performed for human risk assessment. Even if the data quality and quantity about exposure and effect is still sparse, it is possible to make some first conclusions about the risk, using established assessment factors to cope with the uncertainty. It is in my opinion a serious omission that nothing is	Not ready to evaluate risk in this manner; references added (Tom).

	said about risk assessment in the whole document, although it is described in chapter 1 that the CEA-approach is used.	
Santamaria	**a. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments.** This section clearly summarizes the main components of the two case studies and is well written. More information from section 6.2 about the role of the case studies may be useful to be included in the introduction of the document. The only suggested addition to this chapter is a more clear explanation of what the next course of action is regarding these case studies. **b. How might this chapter be improved?** Try to eliminate speculative statements (e.g., p. 6-6, lines 5-7 "the potential for removal of surface coatings…"	This general information is provided in Ch 1 and referred to in Ch 6; no specifics are available regarding the next course of action (Tom). Removed the word "potential", since this phenomenon has been observed. (Tom)
Sellers	This chapter provides an excellent and very well-written summary of the details presented in previous chapters. One specific comment follows. • Page 6-6, line 26. The report indicates here that the presence of organic matter in water increases the degree of agglomeration. As noted above in comments on Chapter 3, this is generally not the case.	Clarified that NOM and ionic strength "affect" agglomeration (Tom).

7. The case studies follow the comprehensive environmental assessment (CEA) approach, which combines a product life-cycle framework with the risk assessment paradigm. Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO_2 in particular and nanomaterials in general.

Reviewer	Comments	
Hansen	• **Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO_2 in particular and nanomaterials in general.** Before going into detail on how I think that the CEA approach could be improved, I would like to say that I missed some reflections from the authors of the report on what they have learned from trying to complete the CEA in the two cases and how they believe this approach could be improved. As I read through the report I noted only one or two references to the risk assessment paradigm (section 5.2, page 5-12, Line 21 and section 4.6.6, page 4-30, Line 18). I think that the CEA approach could be strengthen by making it more clear how the risk assessment paradigm is integrated into the lifecycle framework that is the backbone of the CEA approach. It is somewhat unclear why, how, when and which parts of risk assessment (i.e. hazard identification, dose-response assessment, exposure assessment and risk characterization) are used in the various elements of the CEA approach outlined in figure 1.1. I furthermore think that there is a need for some holistic reflections of the applicability of the CEA approach given the lack of information and scientific data. It would be good to have a section in the final chapter on the pros and cons of using CEA in the two cases and	

	some reflections on what has been learned by trying to complete a CEA. Is it even feasible to use the CEA approach considering that there is a wide range of nanomaterials and applications? To what extend can results be generalized? Finally, CEA approach needs an element on how to prioritize which knowledge gaps and research needs to address in order to complete the CEA and enable decisions without having to wait endlessly for more scientific research. In theory, completing CEAs and combining a life-cycle framework with a risk assessment paradigm is compelling, but I am struggling with the usefulness of this approach when "all it does" is to raise numerous additional questions without concrete guidelines on how to prioritize these.	
Hristovski	The comprehensive environmental assessment used in this report offers all-inclusive approach to understanding the life-cycle and the risks associated with using nano-TiO_2 in arsenic treatment and sunscreen products. Although the life-cycle stages of nano-TiO_2 are discussed in the second chapter, it appears that emphasis on several stages is either minimized or neglected (see comments for Charge Question 2); and these are included in the next chapter which addresses the fate and transport of nano-TiO_2 in the environment. Maybe a more compact life-cycle approach would be if nano-TiO_2 was evaluated from the cradle-to-grave perspective i.e. by examining the nano-TiO_2 interactions at each stage of its life. This approach coupled together with the risk assessment paradigm could minimize the risks associated with omission of important environmental implications of nano-TiO_2. This could allow for better assessment of the future research directions related not only to these two case studies, but nano-TiO_2 or other nanoparticles in general.	No change.
Klaine	I think the overall structure and approach of this document is very good. I like framing the document around CEA. The case studies provide a foundation for the development and	No change

	refinement of research directions. Unfortunately, no attempt is made to do this.	
Nowack	1) The environment is integrating over all releases of nano-TiO_2 from all its applications. In order to perform an environmental risk assessment of nano-TiO_2, it is thus imperative to have knowledge on the release during all life cycle stages of all nano-TiO_2 applications. It is thus not enough to look only at two case studies. However, these case studies can be used to identify important research questions but again only if a relevant case has been picked. This is definitely the case for sunscreens so the data provided in this document are useful. It is less clear how the drinking water treatment case (which is just a hypothetical one) should be used to guide further research. It would have been much more important to identify which other uses of nano-TiO_2 are important. A second case study with a completely different release scenario during the whole life cycle (e.g. use as UV-absorber in textiles or in paints) would have been much more informative. Both chosen cases are water-centered and thus have similar behavior of the released TiO_2. A use in polymers or in another matrix-bound form would have resulted in a much broader discussion of release. The choice of the second case study, the use in water treatment, is not really understandable and was clearly a bad choice because it resulted in a narrowing of the focus. The whole discussion about release of nanoparticles from materials was therefore not covered at all and this is a serious omission because a large percentage of nanoparticles is used in matrix-bound form. These aspects need to be critically discussed in this chapter 6. 2) As already mentioned in my comments to chapter 1 and in some other places, the most critical missing part of the document is the absence of quantitative information in many places. Even if there is still a large uncertainty regarding many aspects of fate and effects, we can still make some first evaluations of the data and perform a first risk assessment and	Added note that other uses may involve issues not considered here (Tom).

	this is completely missing.	
Santamaria	**a. Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO_2 in particular and nanomaterials in general.** This CEA approach seems to be a plausible and scientifically valid approach for evaluating products incorporating nanomaterials. It provides a framework that nanomaterial producers and users should consider when developing research programs for their materials. The organization , scope, and structure of the case studies seems to be appropriate and logical. The only suggestion for improvement is to more clearly state what the future research needs are and possibly rank the research priorities after each chapter. In addition, caution should be taken to not include too much speculation in the case studies; it is clear that there are many data gaps, so speculating about what may occur is not necessary. It would be more appropriate to identify data needs rather than speculate what may be the outcome.	No change
Sellers	The CEA approach provided an excellent framework for organizing and analyzing the literature to create case studies. The document will be a valuable reference. The two highest-priority topics for further research, i.e., the need to evaluate whether existing human and ecological toxicity test protocols are appropriate for use with nano-TiO_2 and the need to characterize the physicochemical properties of nano-TiO_2 at various stages, follow logically from the information presented. Ongoing work by OECD and other parties will be providing additional information on those topics.	

	As difficult as it might be considering the state of the science and the number of variables, the CEA approach could potentially be improved by including a rank-ordering of potential exposures. The outcome of the NCEA workshop, as described in the document, hints at this need ("priority topics included… evaluating exposure pathways and populations of greatest concern", page 6-16 line 12). Clearly, the criteria and nomenclature for rank ordering would need to be carefully considered. To illustrate the point qualitatively, in the case study regarding the use of nano-TiO_2 in water treatment the potential for worker exposures would be much greater than the potential for exposure to nano-TiO_2 from water treatment sludge that had leached from the sludge in a landfill and migrated beyond the leachate control system. This kind of prioritization might help to focus research efforts and would also provide valuable context for lay readers who might otherwise misunderstand the potential for exposure and consequent risk.	This is similar to the comment regarding evaluating the magnitude of releases; language was added to this effect. (Tom)

8. Please provide any additional comments you would like to make on the draft document.

Reviewer	Comments	
Hansen	I would like to acknowledge the pioneering work done here in regard to completing the CEA of two very comprehensive case studies and in regard to preparing an interesting and readable report on this highly relevant subject.	
Hristovski	The three appendices represent an excellent supplement to the two case studies. However, readers do not always read the appendices. In this case this may be important because there are interesting research questions (e.g. the penetration of hydroxyl radicals) that can help refine certain future research aspects. So inclusion of important information from the appendices into the main text could prove to be prudent and improve the overall quality of this document. **General Comments** This is a well written report that provides excellent starting point in outlining and organizing the research needs, priorities and directions related to nano-TiO_2. The organization of this report is clear and easy to understand both from the perspectives of personnel with expertise in this topic and average reader with solid science knowledge but limited knowledge in this topic. The presented information and summaries can easily be used not only to better understand the potential implications of nano-TiO_2 related to arsenic treatment and sunscreen applications, but also to provide context in which the environmental implications of other nanomaterials could be examined. Some comments and suggestions, however, are included in this review document which may help improve the quality of this report.	• Such movement and integration of text is out of scope of these revisions. No change. GD
Klaine	No additional comments provided.	

Nowack	No additional comments provided.	
Santamaria	No additional comments provided.	
Sellers	While the report is remarkably well written, some editorial suggestions (e.g., apparent typographical errors) follow: • Page 1-4, line 21. Spell out acronym "UV" at first use. • Page 1-11, line 31. Apparent missing word: "… removal in <u>drinking</u> water…" (to distinguish from wastewater treatment or treatment of contaminated groundwater, for example). • Page 1-12, line 31. Apparently missing radical symbol ("superoxide radical anions [•O_2^-}") • Page 1-12, line 35. Word choice unclear. Should "One generally accepted mechanism of nano-TiO2 antimicrobial <u>property</u> is the…" be "One generally accepted mechanism of nano-TiO2 antimicrobial <u>action</u> is the…" ? • Page 1-13, line 4. Apparently missing word: "… presence of UV <u>light</u>…" • Page 1-19, lines 1-2. Word choice unclear. Should "Workplace <u>exposure</u> thus far has focused on measuring nanoparticles in the air. Instruments that can be used for aerosol <u>sampling</u> are available, but…" be: "Workplace <u>monitoring</u> thus far has focused on measuring nanoparticles in the air. Instruments that can be used for aerosol <u>monitoring</u> are available, but…" • Page 2-1, lines 7-10. Consider moving the sentence "For the rutile-based manufacturing … " from the first paragraph of Section 2.1 to the discussion of manufacturing now in Section 2.2. • Page 2-2, lines 17-19. Missing words from the sentence "Nonetheless, nano-TiO_2	• Changed to ultraviolet radiation (UV) GD • Clarified. GD • Added radical symbol • Changed to "action". GD • Added word. GD • Changed "exposure" to "monitoring". GD • No change GD • Asked CH to fix. GD

	production based on *a predicted trend of graduate and* a theoretical upper bound…”? [emphasis added]	
	• Page 2-2, lines 24-26. Consider re-organizing this sentence to more closely parallel (and introduce) the paragraphs which follow, e.g., “Manufacturers and researchers report nano-TiO_2 synthesis by various techniques, including chemical vapor deposition (CVD), flame hydrolysis, the sulfate process, and other processes such as sol-gel, calcination, aerosol pyrolysis, and colloidal synthesis (Wahi et al., 2006, 090580).	• No change. GD
	• Page 2-3, lines 13-17. Consider moving this paragraph to the end of this subsection, as this paragraph describes post-manufacture processing and the following paragraph returns to the topic of production methods.	• Paragraph moved. GD
	• Page 2-4, lines 28-32. Consider moving the sentences “P25 presumably could be stored… good management practices.” to follow the first sentence of this paragraph, which also discusses P25.	• Text moved. GD
	• Page 2-5, line 13. Noun-verb agreement: “Industry data from the 1990s, although perhaps out of date, shed~~s~~ light on the distribution….”	• Changed. GD
	• Page 2-7, lines 16-26. Consider moving the discussion “Several studies have bench-tested nano-TiO_2 in slurry systems…” to follow the discussion of bench-scale testing which concludes with lines 1-2 on page 2-7.	• No change. GD
	• Page 2-8, line 22. Noun-verb agreement: “…survey data do~~es~~ not differentiate…”	• Changed. GD
	• Page 3-2, line 21. Apparent typographical error. “… can affect the surface charge~~ing~~ properties…”	• Changed. GD
	• Page 3-2, lines 25-30. Word choice unclear. Should “…solubility increased rapidly...” be “dissolved concentrations increased rapidly…”?	• Left as solubility because this is what they were concluding

		and it reads more simply than adding "dissolved concentrations of TiO2".-CH
	• Page 3-4, lines 32-33. Suggest clarifying meaning by rephrasing "Several different waste streams are generated from drinking water treatment facilities that could contain nano-TiO_2" as "Several different waste streams that could contain nano-TiO_2 could be generated from drinking water treatment facilities."	• Changed. GD
	• Page 3-6, line 30. Suggest clarifying, if appropriate, by adding underlined words: "… after three pore volumes of water passed through the column…"	• No change. GD
	• Page 4-9, line 15. Suggest adding underlined word: "… the estimated total airborne TiO_2…"	• No change. GD
	• Page 4-22, line 5. Instead of "A recent report using pig and hairless mice…" suggest "A recent study using pigs and hairless mice…"	• Changed. GD
	• Page 4-22, line 8. Instead of "…exposure of nano-TiO_2 to porcine skin in vitro…" suggest "…exposure of porcine skin to nano-TiO_2 in vitro…"	• Changed. GD
	• Page 4-22, line 13. Missing letter: "… muscle, hear**t**, liver…"	• Added letter. GD
	• Page 4-27, line 13. Missing word: "… into the dermis of minipigs…"	• Added word. GD
	• Page 5-3, line 22. Missing words: "… of nano-TiO_2 on the generation of reactive oxygen…"	• Changed. Tom
	• Page 5-8, line 20. Capitalize first word in sentence: "**M**ost rutile…"	• Changed (by CH)
	• Page 5-10, line 31. Missing word: "…UV light…" Similar comment on page 2-11, lines 6, 11, 12.	• Changed. Tom
	• Page 6-7, line 12. Apparent typographical error: "…photocatalytic generation of ~~generate~~ reactive oxygen species …"	• Changed. Tom

	Appendix: A copy of the poster by Johnson et al., 2009, referenced in section 2.2 of these comments, is inserted below. Johnson et al 2009	

Appendix A: Individual Reviewer Comments

COMMENTS SUBMITTED BY

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EPA's Draft Document, *Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen*

CHARGE QUESTIONS

1. **Chapter 1 provides introductory material regarding the approach used in these case studies, definitions of conventional and nanoscale TiO_2, the use of nano-TiO_2 in drinking water treatment and sunscreens, and analytical methods for characterizing nano-TiO_2.**

   - **Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document.**

     In general I find the information provided very clearly presented and very useful for the remainder of the report. Minor comments include:

     a) Section 1.2, page 1-3, Line 20: Not clear what is meant by "…such materials…" Is references being made to all kinds of nanomaterials (first-, second-, third-, fourth-generation of nanotechnology) or is reference being made specifically to nano-metaloxides?

     b) Section 1.5, page 1-7, Line 6-7: Would be good to have a reference for that P25 and Aeroxide T805 have the same particle size and surface areas, but differ in reactivity and it would furthermore be good to know how reactivity was measured?

     c) Section 1.6.2 The work by Tiede et al. and Hasselov et al. should be consulted:

        1. Imaging of engineered nanoparticles and their aggregates under fully liquid conditions in environmental matrices. Tiede K, Tear SP, David H, Boxall AB. Water Res. 2009 Jul;43(13):3335-43. Epub 2009 May 13.
        2. Considerations for environmental fate and ecotoxicity testing to support environmental risk assessments for engineered nanoparticles. Tiede K, Hassellöv M, Breitbarth E, Chaudhry Q, Boxall AB. J Chromatogr A. 2009 Jan 16;1216(3):503-9. Epub 2008 Sep 7.
        3. Detection and characterization of engineered nanoparticles in food and the environment. Tiede K, Boxall AB, Tear SP, Lewis J, David H, Hassellov M. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008 Jul;25(7):795-821.
        4. Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. Hassellöv M, Readman JW, Ranville JF, Tiede K. Ecotoxicology. 2008 Jul;17(5):344-61.
        5. Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health? Boxall AB, Tiede K, Chaudhry Q. Nanomedicine (Lond). 2007 Dec;2(6):919-27
        6. Nanomaterials for environmental studies: classification, reference material issues, and strategies for physico-chemical characterisation. Stone V, Nowack B, Baun A, van den

Brink N, Kammer F, Dusinska M, Handy R, Hankin S, Hassellöv M, Joner E, Fernandes TF. Sci Total Environ. 2010 Mar 1;408(7):1745-54.

- **How might this chapter be improved?**

  It could be made clearer why CEA was chosen as the approach to study and organize the available information in the two case studies. What others approaches are out there and what are their pros and cons compared to CEA and why was CEA selected? It would furthermore be good to know why nano-TiO2 was chosen as a example of a nanomaterial and why the two specific applications (sunscreens and water treatment) were chosen to be subject to more in-depth analysis. Providing such arguments would increase transparency.

  It is not quite clear how primary and secondary information has identified and it might be good to have a methodology section where is clearly stated which literature databases (e.g. pubmed, ISI web of science, ICON's nanoEHS literature database) were searched including which kinds of search terms were used. Sources of secondary information might be TiO2 and sunscreen manufacturers and it might be good to know whether, for instance, US TiO2 manufacturers has been systematically contacted and how this contact came about (questionnaires, personal contact and alike).

2. Chapter 2 presents information on the lifecycle of nano-TiO_2, including potential releases to the environment.

- **To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems?**

  I believe that the chapter is both accurate and sufficient in the manner in which the various stages of the lifecycle of nano-TiO2 is described in regard to its use for arsenic removal.

- **To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens?**

  I believe that the chapter is both accurate and sufficient in the manner in which the various stages of the lifecycle of nano-TiO2 is described in regard to its use in sunscreens.

- **To what extent is the material effectively organized and sufficiently informative to support planning for future research?**

  The material is presented very clearly in regard to what is known, but it is less clear in this chapter what is unknown. This is however mentioned repetitively in the subsequent sections

- **How might this chapter be improved?**

  It might be a good idea to have a section in each chapter of the report clearly stating the research needs identified in the chapter making a clear distinction between 1) information that exists, but not is not publicly available at the point in time and 2) gaps in our scientific and technical knowledge.

3. Information on the fate and transport of nano-TiO_2 in air, water, and soil is discussed in Chapter 3.

- **Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO_2 in the environment.**

  In general, I do believe that chapter 3 accurately characterizes what is known and anticipated behavior of nano-TiO2 in the environment. Minor comments include:

  a) Section 3.1.2, page 3-5, Line 30-32: was any information found on how the use of chlorine in pools might affect dis-/agglomeration of nano-TiO2?
  b) Section 3.2 A clear distinction should be made between soil and sediment
  c) Section 3.2, page 3-7, Line 2: what is meant by "… results being specific to the experimental proctocol…"? Didn't Lecoanet et al. follow the same proctocal in the nanomaterials tested?
  d) Section 3.2.2: It should be noted that TiO2 is expected to precipitate out of the water column and into the sediment

- **To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health?**

  The information is presented in a consistent and thorough manner and all the main exposure routes relevant to biota and human health are presented

- **To what extent is the material effectively organized and sufficiently informative to support planning for future research?**

  Gaps in our knowledge in regard to the subject of chapter 3 are profound and are mentioned consistently throughout the chapter by the authors of the report and in that sense the chapter is organized in a clear manner to support planning for future research

- **How might this chapter be improved?**

  It might be a good idea to have a section in each chapter of the report clearly stating the research needs identified in the chapter making a clear distinction between gaps in our scientific and technical knowledge that can be addressed through more research and which cannot.

4. Chapter 4 provides information on exposure, dose, and translocation of nano-TiO_2 in biota and humans.

- **Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2.**

  Again, in general the chapter is well written and provides a thorough review of the literature on the exposure and what is known about the sub-sequent translocation of nano-TiO2 in biota and humans. Minor comments include:

  a. A distinction is made between dose and exposure and in footnote 11 it is stated that this is consistent with risk assessment usage. References should be provided for this interpretation
  b. Section 4.2.1.2, page 4-5, Line 25-32 and page 4-6, Line 1-13: It should be noted that these dermal exposure estimates are in line with estimates made by Hansen, S.F., Michelson, E., Kamper, A., Borling, P., Stuer-Lauridsen, F. & Baun, A. 2008, Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products. Ecotoxicology 17 (5): 438-447.
  c. Section 4.4, page 4-13, Line 6-15: It should be noted that Mueller and Nowack (2008) assumes that 97% and 90% of the nanoparticles would be cleared in the realistic and the high exposure scenario, respectively. These assumptions have yet to be validated
  d. Section 4.4, page 4-13, Line 6-15: Not clear to me why PNEC estimates made by Mueller and Nowack (2008) is relevant to report here. I recommend that it is deleted or move to the chapter 5
  e. The work by Boxall et al. should be reviewed and cited as well (Boxall, A.B. A., Chaudhry, Q., Sinclair, C., Jones, A., Aitken, R., Jefferson, B., Watts, C. 2008. Current And Future Predicted Environmental Exposure To Engineered Nanoparticles. York: Central Science Laboratory). Based on available information about the applied concentration of nanoparticles in cosmetics, personal care products and paints, Boxall *et al.* (2008) used a long series of algorithms (for among other pesticides, medicinal products, and ultrafine particles) to estimate the predicted environmental concentrations of nanoparticles in soil and water. Although anticipating that 10% market penetration probably provides a conservative estimate (with the exception of sunscreens), Boxall *et al.* calculated the PEC for three scenarios assuming that 10%, 50% and 100% of the products on the market contained nanoparticles. The total predicted concentrations in water were found to be for titanium oxide (24.5-245 μg/L) used in among others paints and sunscreens. It might furthermore be a good idea to look into the work recently reported by Gottschalk, F., Sonderer, T., Scholz, R. W. & Nowack, B. (2010). Possibilities and Limitations of Modeling Environmental Exposure to Engineered Nanomaterials by Probabilistic Material Flow Analysis. Environmental Toxicology and Chemistry, 29, no. 5, pp. 1036-1048.

f. Section 4.6.6, page 4-30, Line 30: It is stated that “Quantitative risk assessment relies on dose-response relationships.” and I wonder to which extend discussion about dose-response relationships is relevant in a chapter on Exposure-dose characterization?

- **To what extent is the material effectively organized and sufficiently informative to support planning for future research?**

  As in the case of the previous chapter, gaps in our knowledge in regard to the subject of chapter 4 are profound and are mentioned consistently throughout the chapter by the authors of the report and in that sense the chapter is organized in a clear manner to support planning for future research

- **How might this chapter be improved?**

  It might be a good idea to have a section in each chapter of the report clearly stating the research needs identified in the chapter making a clear distinction between gaps in our scientific and technical knowledge that can be addressed through more research and which cannot.

5. Chapter 5 characterizes factors that influence ecological and health effects of nano-TiO_2 and discusses the currently available scientific evidence regarding these effects.

- **Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science.**

  In general, the chapter does accurately and sufficiently characterize that the state of the science in regard to what is known about the ecotoxcity of nano-TiO2.

  Minor comments include:

  a) Section 5.2.1.2 The study by Hartmann et al. should be reviewed and cited: N.B. Hartmanna, F. Von der Kammerb, T. Hofmannb, M. Baaloushac, S. Ottofuellingb, A. Baun. Algal testing of titanium dioxide nanoparticles—Testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology 269 (2010) 190–197.

  In regard to the Health effects of nano-TiO2 (Section 5.3) is outside the area of my expertise

- **To what extent is the material effectively organized and sufficiently informative to support planning for future research?**

  I believe that the materiasl is effectively organized and sufficeniently information to support planning for future research, although I do think that it could be made more explicit what the key research gaps and research needs are, for instance in the form of a list of bullets in the end of section 5.2 and 5.3, respectively.

- **How might this chapter be improved?**

  It could be made more explicit what the key research gaps and research needs are, for instance in the form of a list of bullets in the end of section 5.2 and 5.3, respectively.

6. Chapter 6 summarizes the information and research questions presented in the nano-TiO_2 water treatment and sunscreen case studies, as well as discussing the role of case studies in the refinement of research strategies and potential future assessment efforts.

- **We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments.**

  Chapter 6 is divided into two separate parts. One part provides a summary of the various chapters 1-5. This summary is good and I think that the list of important issues/research questions provided in Section 6.1.1, 6.1.2, etc. is good and very helpful in supporting future research strategies and assessments. The second part of chapter 6 is the suppose to be the core result of the whole report i.e. research recommendations and prioritizations and with that in mind section 6.2.1-6.2.3 stands out as rather weak. A prioritization list of research areas is cited to have been made by experts at a NCEA workshop, but it is not clear how the expert were, what the discussions about the various research gaps were and what the arguments for and against ranking one or the other research area higher or lower than another. The issue of prioritization might be the most important part of this report and hence this part should be expanded on.

  Minor comment: In section 6.2.2 and 6.2.3 the authors of the report use the term "…life cycle assessment…" whereas previously in the report the word "assessment" was not used. Instead the word "framework" or "stages" was used which seems more appropriate as life cycle assessment often includes issues not considered in this report e.g. energy uses of different processes

- **How might this chapter be improved?**

  It could be made more clear how the prioritization list of research areas was generated by experts at a NCEA workshop, what the discussions about the various research gaps were and what the arguments for and against ranking one or the other research area higher or lower than another. Furthermore, it would be interesting to discuss how knowing, for instance, whether or not the existing testing protocols are appropriate (as stated in section 6.2.1, page 6-16, Line 7) would change and impact the outcome of the CEA in the two case studies

7. The case studies follow the comprehensive environmental assessment (CEA) approach, which combines a product life-cycle framework with the risk assessment paradigm.

- **Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO2 in particular and nanomaterials in general.**

Before going into detail on how I think that the CEA approach could be improved, I would like to say that I missed some reflections from the authors of the report on what they have learned from trying to complete the CEA in the two cases and how they believe this approach could be improved.

As I read through the report I noted only one or two references to the risk assessment paradigm (section 5.2, page 5-12, Line 21 and section 4.6.6, page 4-30, Line 18). I think that the CEA approach could be strengthen by making it more clear how the risk assessment paradigm is integrated into the lifecycle framework that is the backbone of the CEA approach. It is somewhat unclear why, how, when and which parts of risk assessment (i.e. hazard identification, dose-response assessment, exposure assessment and risk characterization) are used in the various elements of the CEA approach outlined in figure 1.1.

I furthermore think that there is a need for some holistic reflections of the applicability of the CEA approach given the lack of information and scientific data. It would be good to have a section in the final chapter on the pros and cons of using CEA in the two cases and some reflections on what has been learned by trying to complete a CEA. Is it even feasible to use the CEA approach considering that there is a wide range of nanomaterials and applications? To what extend can results be generalized?

Finally, CEA approach needs an element on how to prioritize which knowledge gaps and research needs to address in order to complete the CEA and enable decisions without having to wait endlessly for more scientific research. In theory, completing CEAs and combining a life-cycle framework with a risk assessment paradigm is compelling, but I am struggling with the usefulness of this approach when “all it does” is to raise numerous additional questions without concrete guidelines on how to prioritize these.

8. Please provide any additional comments you would like to make on the draft document.

I would like to acknowledge the pioneering work done here in regard to completing the CEA of two very comprehensive case studies and in regard to preparing an interesting and readable report on this highly relevant subject

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Report Number: EPA-600-R-09-057

Report Title: Nanomaterials Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen

Report Status: Peer Review Draft

General Comments

This is a well written report that provides excellent starting point in outlining and organizing the research needs, priorities and directions related to nano-TiO_2. The organization of this report is clear and easy to understand both from the perspectives of personnel with expertise in this topic and average reader with solid science knowledge but limited knowledge in this topic. The presented information and summaries can easily be used not only to better understand the potential implications of nano-TiO_2 related to arsenic treatment and sunscreen applications, but also to provide context in which the environmental implications of other nanomaterials could be examined. Some comments and suggestions, however, are included in this review document which may help improve the quality of this report.

Charge Question 1

This chapter represents a well conceived introduction with overall accurately and clearly presented information. The goal of this report is clearly emphasized on several occasions in addition to providing supplementary information about potential goal-related misconceptions that may misdirect a reader. This approach represents organizational strength of this chapter allowing the potential reader to remain focused on the goal. The information follows a clear line of thoughts and provides a good introduction, background and support for the next chapters. Several suggestions for improving the quality of this chapter are given below.

On Page 1-15 Ln 5-7, a paper by Hewit is cited with a statement that "Different methods for measuring the same parameter may yield different results for the same material…and therefore stating the testing method is important." This statement is correct and often neglected when presenting and discussing results. On page 1-9, Ln 15-23, the aggregation of nanoparticles is discussed, yet only information about 10 min sonication is provided. Key elements that can determine the size and size distribution of the nanoparticles in a dispersion are related to the power of a sonication (e.g W/L, type…); the initial concentration of a dispersion; the pH of a dispersion; the analytical tool used; and reported distribution (e.g. is it an effective radius reported; number or volume distribution; monomodal or multimodal…). These information are important to obtain a better understanding of the complexity of the issue. Addition of similar information in Table 1-2 would be suitable. For example, what is the method used to determine the particle size? What is the water matrix for the information about the median particle size (is it deionized, buffered…)? What is the concentration of the PBS? Although this table is a mere example, it would prove to be a much stronger illustration if such information is to be included.

Page 1-12, Ln 21-29, discusses the formation of inner-sphere complexation of arsenic species onto nano-TiO_2. Here it is stated that only DMA forms monodentate ligands. In reality, the other arsenic species mentioned here can also form monodentate ligands in addition to the bidentate. The bidentate ligands are more thermodynamically stable and as such they can be the dominating mode of sorption when compared to monodentate ligands. However, where sorbate-to-sorbent ratio is high (e.g. in presence of high concentrations of competing ions), arsenic species may be forced to form monodentate ligands rather than bidentate ligands.

On Page 1-13, Ln 15-19 scattering of nanoparticles is discussed and the reader is redirected to Appendix A. Although statements are made from a reference (Fairhyrst and Mitchnik, 1997, 196248) that the optimal scattering is thought to occur when the particle diameter is approximately half the wavelength of the light to be scattered, the important phenomenon of Rayleigh scattering that relates to nanoparticles is clearly neglected. Discussion related to this scattering and how applies to nanoparticles is essential especially considering the fact that it is valid for particles that are much smaller than the wavelength of electromagnetic radiation in question here (UV-A; UV-B; and UV-C).

On Page 1-16, Ln 7 a statement is made that “Currently technologies are unavailable to measure the total amount of nanomaterials in tissue.” This statement may be a bit misleading considering the fact that studies have shown that metal content in tissues originating from nanomaterials (including nano-TiO_2) can be easily measured. So if the intent of this statement is to minimize or eliminate the above mentioned approach, then this statement may not accurately depict the reality and may require rephrasing.

Consistency when using acronyms is very beneficial in context of document readability. For example, on page 1-18, Ln 12, the acronym for Field Flow Fractionation is FIFFF, yet on the next page and in other places it is FFF. Improving the consistency of the acronyms where applicable may result in better readability.

Charge Question 2

According to the title and the introduction of this chapter, the life cycle stages of nano-TiO_2 are discussed. Although the cradle-to-grave of nano-TiO_2 (with respect to arsenic treatment and sunscreens applications) approach appears to be considered, it appears that emphasis on several stages is either minimized or neglected.

To the knowledge of this reviewer, there are two commercially available nano-TiO_2 based sorbents for arsenic as suggested by the manufacturers. DOW Chemicals produces Adsobsia GTO, while Hydroglobe produces MetsorbG. These sorbents are designed for removal of arsenic in packed bed configurations. The packed bed configurations are the types of systems that are predominantly used in water arsenic treatment for arsenic. It is possible that information on the preparation, distribution and storage of these sorbents can be obtained from the manufacturers or by searching the patent literature. These information can be introduced in Sections 2.2.1 and 2.3.1.

In Section 2.5.1., two scenarios are discussed about disposal of nano-TiO_2 containing sludge. The first discusses physical/chemical water treatment (flocculation) and the second discusses accumulation of nanoparticles in sludge from biological wastewater treatment plants. However, the second scenario does not emphasize this difference. This may be confusing for a reader who does not have a technical proficiency in the field. There are several studies that discuss the interaction and fate and transport of nano-TiO_2 in the environment.

4. Battin TJ, Kammer FVD, Weilhartner A, et al. 2009. Nanostructured TiO_2: Transport Behavior and Effects on Aquatic Microbial Communities under Environmental Conditions; ENVIRONMENTAL SCIENCE & TECHNOLOGY 42, 8098-8104
5. Gottschalk F, Sonderer T, Scholz RW, et al. 2009. Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions. ENVIRONMENTAL SCIENCE & TECHNOLOGY 43, 9216-9222.
6. Kiser MA, Westerhoff P, Benn T, et al. 2009. Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants. ENVIRONMENTAL SCIENCE & TECHNOLOGY 43, 6757-6763.

There is evidence that nano-TiO_2 is accumulated in biomass which is then used in agricultural application. This has to be emphasized.

On Page 2-3 Ln 3-6, Klaessig 2006 is cited. According to this citation, Flame hydrolysis is used for manufacturing of P25 and yields agglomerated particles with mean diameters about 3.6 µm, with the smallest 4% of the particles having average diameter of 160 nm. Here, it is not stated in what environmental conditions these measurements were conducted (see comments in Charge Question 1 Section). There are number of studies showing that discrete P25 nanoparticles can exist in water matrices. These particles have size of 30-50 nm. This contradicts the above cited statement.

On Page 2-4 Ln 7-9, a statement is made that photostability can be increased by doping nano-TiO_2 with metals including iron. Introduction of Fe in nano-TiO_2 may also cause increased photocatalytic activity as a result of creating intermediate energy levels between the valence bands and the conduction band in TiO_2, which can facilitate formation of free radicals by electromagnetic radiation with higher wavelengths. It is prudent to state that doping with some metals can decrease the photostability and facilitate creation of ROS.

On several occasions waste water instead of wastewater term (e.g. Page 2-3 Ln 23) is used; and ground water instead of groundwater term (e.g. Page 2-6 Ln 3) is used.

A paragraph/subsection summarizing the entire chapter would be beneficial and would provide a good ending to this section.

Charge Question 3

This is a short but well organized chapter focusing on the fate, transport and behavior of nano-TiO_2 in the environment. The exposure routes are clearly defined, outlined and easy to follow. Much of the information presented here represents a good base for developing a clear understanding of the future research needs and directions. However, although most of the information presented here are accurate and represent good overview of existing knowledge of fate and transport of TiO_2 nanomaterials, it is evident that many studies published in the last 12 months (approximately) are not accounted. For example, there are 3 studies cited above that focus on environmental implications of nano-TiO_2 in the environment. It would be very beneficial if studies from the last 12 months are to be incorporated into this report.

There is evidence in Kiser et al. (2009) that nano-TiO_2 exists in real world water matrices. These new findings mandate that some of the statements (e.g. page 3-2 Ln 1-3) be changed or restated.

One of the main comments in this chapter is related to the study of Schmidt and Vogelsberger (2006) which focuses on dissolution of TiO_2 nanoparticles. The main concern, here, is related to presentation of the level of dissolution. In their study, Schmidt and Vogelsberger discuss dissolution of TiO_2, however, this dissolution is in the range of µmol/L or nmol/L when saturated suspensions in the range of mg/L (or higher) are considered. The dissolved Ti is negligible when compared to the initial TiO_2 concentrations. The question here is "how relevant this dissolution is to the overall scheme of fate and transport of nano-TiO_2?" The manner in which the information from this paper is presented in this report conveys to the reader that dissolution of nano-TiO_2 is one of the main fate and transport mechanisms. A statement indicating that this dissolution is actually is negligible may contribute to better understanding of almost non-existent dissolution of TiO_2 which is also stated in number of other places in this report.

On Page 3-3 Ln 24-25, the report suggest that most nanoparticles were removed via agglomeration with microorganisms. The proper term here to use is biosorption. There is evidence that the nanoparticle-microorganism interactions are more complex than aggregation, and include sorption and even endocytosis. For example references see:

3. Kiser et al., 2010, Biosorption of nanoparticles to heterotrophic wastewater biomass, Water Research, In press.
4. Asharani et al. 2009, Anti-proliferative activity of silver nanoparticles, BMC Cell Biology, 10, 65

Charge Question 4

This chapter represents a well organized prospectus addressing the potential for nano-TiO_2 exposure and dose in biota and humans. The information in this chapter is clearly presented and, in many cases, well summarized in tables. The routes of exposure are transparently described and easy to understand, especially by readers without significant scientific or technical background. The overall knowledge summarized in this chapter offers a good starting point for future research planning in addition to understanding the future research needs in this area. Addition of schematics and illustrations, like Figure 4-1, could further improve the ease of apprehension of the presented information. Although the importance of exposure to co-contaminants sorbed onto nano-TiO_2 is well presented and emphasized, discussions related to synergistic effects of nano-TiO_2 and co-contaminants appear to be minimized or neglected.

With respect to more specific comments, on Page 4-13 Ln 18, the manner in which the statement is written gives an initial impression to the reader that the section 4.6. summarizes only studies that focused on fish that have been exposed to nano-TiO_2. This, of course, is not true as summarized in the latter part of this paragraph.

On Page 4-14 Ln 4, "stock solution" is used as a term which may confuse a reader of this text because no stock solutions are used in the description; but rather "stock suspension" is discussed. Consistent and correct terminology is important and contributes for improved understanding of the presented information.

On Page 4-14 Ln 15-22, environmental factors were discussed that could explain the differences between the studies conducted by Zhang et al. (2006) and Federici et al. (2007). Here, it is important to note that the available surface area in the experimental tanks could significantly contribute to experimental differences. Many studies have reported that nano-TiO_2 (and other nanoparticles) likes to interact with existing surfaces in the local environment. For example, larger available surface area could allow for higher "loss" of the suspended nanoparticles in the water matrix.

On Page 4-15 Ln 15-16, statement is made that bioaccumulation was not observed in the quantum dots study by Holbrook et al. (2008). Here it is not clear whether bioaccumulation of actual nanoparticles was examined or the quantum dots residual. Many quantum dots, for example CdTe or CdSe, once entering or interacting with biomass or other living organism can be "dissolved" to form ions. In this case, accumulation of the quantum dots into the living organism may be shown via increased concentrations of elements that comprise the quantum dots (e.g. Cd and Te in case of CdTe). This is not the case with nano-TiO_2 because these types nanoparticles do not dissolve.

On Page 4-17 Ln 4, it is stated that adsorption equilibrium of Cd onto nano-TiO_2 occurred within 30 minutes. First, the proper term to use here is pseudo-equilibrium; and second, the time presented here period may be to short. Number of studies have shown that for sorption pseudo-equilibrium to occur minimum 2-6 hours are required. Furthermore, the porosity of the sorbent materials is one of the key factors that control the rate of sorption. In this study, two materials with completely different surface areas are compared (nano-TiO_2 and sediment particles). This comparison is inadequate and the derived conclusions may not be appropriate. Additionally, there are number of sediments that could have performed better than nano-TiO_2. For example, when dealing with sediment, sulfide (present in sediments) can strongly bind available Cd (probably Cd^{2+}), so one can clearly state that sediment with higher sulfide concentration may have higher adsorption capacity.

Defining the term “instillation” in section 4.6.2. may contribute to improved understanding of this section. This term is explained in Chapter 5 and it is commonly used by the scientific community; however, a common reader may not be familiar with terminology.

Table 4-4 provides an excellent overview of TiO_2 absorption/penetration studies; however, dividing this table into 2 separate tables may be beneficial. The first table could focus on sunscreen formulations containing nano-TiO_2; while the second table would could on other nono-TiO_2 formulations.

It would be beneficial if a summary (summarizing the most important issues discussed in this chapter) is presented at the end of this chapter. The manner in which this chapter ends leaves the reader “hanging.”

Charge Question 5

This chapter appears to be the most organized chapter in this report. The approach to describing and outlining both the ecological and the health effects of nano-TiO_2 is well conceived and easy to understand. One of the strengths of this chapter is the clear emphasis on the factors that influence ecological and health effects of nano-TiO_2 at the beginning of this chapter. Additionally, probably the biggest plus of this chapter are the end of section summaries. End-of-chapter summary that summarizes the entire chapter would also be beneficial.

Based on the summarized published literature, the existing research in this area needs to be augmented by employing stronger nano-TiO_2 characterization techniques and consequently correlating the characterization data with the toxicity data. One simple approach to do this and improve the quality of this report is to recalculate and introduce the surface area as a key parameter, in addition to mass, of P25. There are number of studies that show that P25 exhibits a specific surface area of ~ 50 m^2/g. Using this information, one can estimate the studied doses in m^2 rather than in grams. This new data for P25 can be introduced in the tables of this chapter.

Another major recommendation with respect to improving the quality of this chapter is introduction of an appendix with explanation of specific medical terms. On many occasions, specific medical terms were used that are common knowledge among the scientist and medical personnel; however, readers who do not have specific knowledge in this area need a dictionary to understand the medical terminology. For example, medical personnel can easily understand what a pulmonary fibrosis is, but this term could be a puzzle for a reader who has limited medical knowledge.

On Pages 5-6 and 5-7 tables 5-1 and 5-2 are presented summarizing recommendation for nanomaterial characterization. Here, it is not completely clear whether these tables are used as an illustration (i.e. example) or as recommendations of this document. If they are to be used as recommendations by this report, than surface area is one of the properties of nano-TiO_2 that must be characterized, especially for toxicity studies.

In Section 5.1.2.1. dispersions of nano-TiO_2 are discussed with water and oils as the matrix. One important phenomenon that has to be addressed here is that the nanoparticles by themselves can act as a dispersant/surfactant, forming so called Pickering emulsions. In brief, nanoparticles can be the surfactant that helps make liposomes.

On Page 5-8 Ln 20, the sentence starts with lowercase m instead of uppercase M.

When discussing radical formation and scavenging inside the cell (on the same page), it is beneficial to make a statement that one of the reasons why in-cell ROS have not be verified is because of presence of radical scavengers that exist in cells.

One of the statements which appear to exist in number of places in this document, and especially in this chapter is the statement related to organic matter and its interactions with nanoparticles. On number of occasions it is stated that the degree of aggregation generally increases with presence of organic matter. Considering that the most commonly found organic matter in water is the natural organic matter (NOM), it is safe to state that the discussion here is related to NOM rather than non-NOM organic matter in water. It appears that there is a misinterpretation of the NOM interaction with nanoparticles that possibly comes from the paper by Navarro et al. 2008 (cited in this chapter). In the majority of cases, NOM is a complex of organic compounds that contain one or more carboxylic groups and hydroxyl groups. In general, pKa of NOM is ~ 4.5, which makes it negatively charged in natural waters. The NOM has a tendency to sorb onto the nanoparticles (e.g. coat them); change their surface charge and stabilize them (in other words prevent them to aggregate). So unless the organic matter in question in this section is other than NOM (if so it needs to be specified), these statements are not completely true (e.g. Page 5-8 Ln 28-29). Also, addition of salts increases ionic strength, so statements, where “increase of salt concentrations” or “increase of ionic strength” are used together in the same sentence, are mere duplication.

On Page 5-9 Ln 30-33, discussion about sonication and dispersion of nanoparticles is presented. It is essential to state that the particle size distribution during sonication is impacted by both the power input of the sonicator and the initial properties of the nanopowders (which in many cases may be comprised of microscopic particles that have nanocrystaliune structure). In brief, it needs to be emphasized that the dispersion method (e.g. power used; powder used; etc.) is very important.

On Page 5-10 Ln 1-5, in the same paragraph as above mentioned comments, it is not clear what a secondary particle size is. Additionally, the study by Federici et al. (2007) uses electron microscopy to determine dispersion of P25 and from there extrapolates the statement of “good dispersion.” This statement is copied into this report without clearly explaining what this “good dispersion” means.

Splitting Table 5-3 into four different tables each focusing on different exposure or organism, may contribute for better readability and easier access to the desired information presented in this table.

Page 5-24 Ln 4-6: This sentence is not clear.

On Page 5-24 Ln 32-34, toxicity of ZnO to zebrafish eggs is discussed. It may be prudent to state that ZnO has a tendency hydrolyze in water and release Zn^{2+} ions that may be toxic, in contrast to the stable nano-TiO_2 or Al_2O_3.

Page 5-25 Ln 18-28: See the comment on NOM.

In Section 5.2.1.4, it may be beneficial to mention that some of the interactions between nano-TiO_2 and contaminant may result in synergistic effects.

Page 5-28 Ln 1: The sentence starts with lowercase m.

Page 5-28 Ln 8: The sentence starts with lowercase m.

In the summary of section 5.3.2.4., it may be beneficial to emphasize that persistent inflammation is the primary reason for the observed carcinogenic effects of nano-TiO_2.

Charge Question 6

This chapter provides a well rounded summary of the information and research questions/needs presented in the previous four chapters. The integration of the evidence in order to provide a starting point for refinement of future research strategies is clear and well conducted. Overall, there are no major suggestions on how to improve the quality of this chapter.

There are repeating comments from the previous chapters that are applicable to this section of the report which exist as a result of the process of putting the summary together. Addressing the comments from the previous chapters, and addressing the same comments in this section could improve the overall quality of this report. Bulleting may improve the readability of some sections in this chapter. For example, on Page 6-10 Ln 21-28, bulleting the important questions that deserving a further investigation may yield a text that is easier to read. Another example is Page 6-12 Ln13-22.

Charge Question 7

The comprehensive environmental assessment used in this report offers all-inclusive approach to understanding the life-cycle and the risks associated with using nano-TiO_2 in arsenic treatment and sunscreen products. Although the life-cycle stages of nano-TiO_2 are discussed in the second chapter, it appears that emphasis on several stages is either minimized or neglected (see comments for Charge Question 2); and these are included in the next chapter which addresses the fate and transport of nano-TiO_2 in the environment. Maybe a more compact life-cycle approach would be if nano-TiO_2 was evaluated from the cradle-to-grave perspective i.e. by examining the nano-TiO_2 interactions at each stage of its life. This approach coupled together with the risk assessment paradigm could minimize the risks associated with omission of important environmental implications of nano-TiO_2. This could allow for better assessment of the future research directions related not only to these two case studies, but nano-TiO_2 or other nanoparticles in general.

Charge Question 8

The three appendices represent an excellent supplement to the two case studies. However, readers do not always read the appendices. In this case this may be important because there are interesting research questions (e.g. the penetration of hydroxyl radicals) that can help refine certain future research aspects. So inclusion of important information from the appendices into the main text could prove to be prudent and improve the overall quality of this document.

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1. Chapter 1 provides introductory material regarding the approach used in these case studies, definitions of conventional and nanoscale TiO_2, the use of nano-TiO_2 in drinking water treatment and sunscreens, and analytical methods for characterizing nano-TiO_2. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. How might this chapter be improved?

The introduction chapter is reasonable but could benefit from a few modifications.

Page 1-1. Readers unfamiliar with Comprehensive Environmental Assessment (CEA) may have difficulty understanding where it differs from traditional risk assessment (RA). It might be useful to begin with the EPA risk paradigm figure and show where CEA elaborates and expands on this method. Currently, if I did not know a bit about RA and CEA I would find Figure 1 confusing.

Page 1-3. I recommend adding a couple more sentences that expands on the concept stated in lines 8 – 11.

Page 1-4. Lines 16-18 help with CEA. Unfortunately, the reader has to wait for a couple pages before that happens. I suggest your include concepts like this in the CEA section even at the risk of reiterating them here.

Section 1.5 is well done.

Page 1-11. The point made in lines 9-11 that early studies neglected to address adequate particle characterization could also be made for many current studies. I suggest inclusion of a statement that encourages the reader to evaluate particle characterization and reinforces the concept of lines 19-22 on Page 1-15.

Page 1-15. Line 3 states a conclusion about what was addressed in Section 1.3. I have read and reread Section 1.3 and do not agree with this statement. If you want this statement to be true then I recommend you insert a paragraph in Section 1.3 that discusses characterization needs for the use of nanomaterials in research.

Page 1-17. Did you purposely leave out any discussion of the measurement of surface charge or pH_{pzc} in Table 1-3 or Table 1-4?

2. Chapter 2 presents information on the lifecycle of nano-TiO_2, including potential releases to the environment. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems? To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

In general, this chapter points out that there is a lot more that we do not know about the various life stages of nano-TiO2 used in water treatment or sunscreen. The material is well organized and readable. I only have one comment.

Page 2-3. Line 7 refers to the "chloride method" and I assume you mean the method discussed in the prior paragraph. I recommend using that term in the discussion so the reader readily knows which method is being discussed.

3. Information on the fate and transport of nano-TiO_2 in air, water, and soil is discussed in Chapter 3. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO_2 in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This chapter could best be described as a collection of "if…then" statements resulting from the paucity of definitive research on the fate and transport of TiO2. The chapter is an excellent discussion of potential fate and transport pathways and appears to be exhaustive. Further, this chapter accurately discusses the large number of research inadequacies and the discussion facilitates the prioritization of research needs.

The chapter can be frustrating to read as in many cases the lack of conclusive data requires the discussion to be speculative at best. However, in the absence of more research it is difficult to recommend improvements to the chapter.

4. Chapter 4 provides information on exposure, dose, and translocation of nano-TiO_2 in biota and humans. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This chapter benefits by the significantly more published research in this area as compared to that in chapter 3. In general, the chapter is well written and accurately reflects the state of the science (see specific comments below). While few real conclusions are made, there is less conjecture than in the previous chapter. Hence, the chapter does provide an excellent foundation for prioritizing research needs. Suggested edits and improvements are below.

Page 4-3. Line 18-22 makes an excellent point but the statement should include floating aquatic plants.

Page 4-4. I find it strange, although it is discussed later in this chapter, that section 4.2 does not mention sunscreen as an exposure route for humans.

Page 4-15. Line 15 and 16. This sentence is incorrect. Holbrook et al (2008) did show bioaccumulation (as discussed in the previous sentence). I suspect the sentence was supposed to read "**Biomagnification**, however, was not observed in the quantum dot study."

Page 4-30. Section 4.6.6 is extremely well done and illustrates the complexity of this issue. The section might be best concluded by a caution to researchers to pay attention to multiple dose metrics and to regulators to interpret these studies with caution.

5. **Chapter 5 characterizes factors that influence ecological and health effects of nano-TiO_2 and discusses the currently available scientific evidence regarding these effects. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?**

This is by far the most data-rich chapter of this document. It is well organized and I particularly appreciate the comments on page 5-1 lines 15 – 18 that discuss the need for material characterization and warn that many studies do not include this. Hence, a significant amount of data discussed in this chapter must be interpreted with caution. The discussion appears exhaustive and critical.
The discussion of the influence of physicochemical characteristics on TiO2 effects is much appreciated and certainly allows the reader to readily deduce the research needs in this area. The section ends with recommendations for nanomaterial characterization. While several publications are cited I would suggest inclusion of the material at www.characterizationmatters.org since is a compilation of an interdisciplinary workshop that not only included nanomaterial researchers but also representatives from regulatory agencies, funding agencies, and peer-reviewed journals.

Section 5.1. The discussion of the influence of experimental conditions is very useful. The discussion in section 5.1.2.1 could be improved by adding a paragraph on the influence of suspension instability on actual exposure metrics and the resulting inaccuracies in dose-response relationships. The sentence at the bottom of page 5-9 and the top of page 5-10 is unclear and could be improved by deleting the last three words.

Section 5.2. This section is a great compilation of the ecological effects research. It rightfully points out the lack of studies on TiO2 intended for sunscreen use. It could benefit from a discussion of the influence of suspension instability on the dose metric aquatic toxicity studies. This issue results in organism exposure to lower than expected nanoparticle concentrations. Hence, effects are actually attributed to higher concentrations than those actually causing effects.

Section 5.3. This is a very well done section. My only suggestion is to add a discussion of i*n-vitro* studies since there are a large number of them. However, results from these must also be taken with caution because they have not been shown to be predictive of *in vivo* studies. This issue is still worthy of a discussion.

6. Chapter 6 summarizes the information and research questions presented in the nano-TiO_2 water treatment and sunscreen case studies, as well as discussing the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

Section 6.1 is an accurate and very readable summary of the document. I don't see much integration (e.g. estimated exposures being compared with effects concentrations) although the state of this science may be premature for this to happen.

Section 6.2.1 is an interesting report on the workshop and the method and outcome for setting research priorities. However, sections 6.2.2 and 6.2.3 fall short of discussing the next steps for this effort. I expected a more concrete and definitive discussion on how the efforts in the previous 5 chapters would be used to set research priorities within NNI or at least EPA. Instead of such a discussion the document close with a justification of the use of the CEA framework.

The chapter could be improved by discussing the CEA framework, working through the existing data, and discussing uncertainty. The uncertainty discussion could be used to generate a prioritized list of research needs. Without this, the reader is left with an insurmountable mountain of data gaps with no real idea which data are most important to facilitate quantitative risk assessment

7. The case studies follow the comprehensive environmental assessment (CEA) approach, which combines a product life-cycle framework with the risk assessment paradigm. Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO_2 in particular and nanomaterials in general.

I think the overall structure and approach of this document is very good. I like framing the document around CEA. The case studies provide a foundation for the development and refinement of research directions. Unfortunately, no attempt is made to do this.

COMMENTS SUBMITTED BY

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Review of EPA's draft document „Nanomaterial case studies: nanoscale titanium dioxide in water treatment and topical sunscreen"

Charge question 1

1) Remark: A lot of the points listed under this question actually apply to the whole document but are listed here because this chapter contains the overview of the whole document.
2) The document is pretending to make "case studies", however, it is rather using two applications of nano-TiO_2 to organize to some extent the information extracted from the scientific literature and to guide the questions. However, these are not real case studies. From a case study I would expect that only this information is summarized that is pertinent to the case studies. My expectation was that the document contains some general chapters that are then followed by two "case studies" where the information that is relevant for these cases is listed based on the life cycle aspects of the two applications. At the moment just all information about nano-TiO_2 is listed and the case study information gets completely lost. In principle this document is a normal review of nano-TiO_2 with some additional focus on sunscreens. But it's not really a case study.
3) This chapter should also contain a justification why the two case studies were chosen. Why these and not two of the many other uses of nano-TiO_2?
4) Footnote 5 on page 1-5 should be covered in the text. It makes the important distinction between aggregation and agglomeration and this should not just be a footnote but deserves at least a whole paragraph if not more.
5) Chapter 1.4 on conventional TiO_2 needs to be expanded considerably and much more information and details should be given. The distinction between conventional TiO_2 and nano- TiO_2 is often not clear and thus the traditional uses of TiO_2 need to be described in much more details. At this place also a new chapter on natural TiO_2 should be added. This information is needed to establish the baseline of natural TiO_2 exposure. And it is very important because also from conventional TiO_2 use nanoparticulate TiO_2 can be released to the environment. For example the work by Kaegi et al (2008) have shown that from façade paints nano-TiO_2 is released in natural waters. This is a very important information because it means that even when banning all pure nano-TiO_2 uses there is still nano-TiO_2 reaching the environment from traditional TiO_2-uses.
6) Chapter 1.5: This chapter describes the coating of nano-TiO_2 with silica, alumna or other compounds. One aspect that is never mentioned in the whole document is whether a alumina-coated nano-TiO_2 should be considered an alumina or TiO_2 particle? What the environment and organisms see is alumina and if this coating is not degraded then it will remain an alumina particle. Do we need to assess its risk by using data from alumina? And if coated with silica use data from pure silica

rather than from pure TiO_2? It is correct to evaluate this particle together with pure TiO_2? This is an issue that will come up on almost every page of the document and needs to be discussed somewhere. It is absolutely central for he risk assessment of nano-TiO_2. The document contains a lot of information on the actual nano-TiO_2 used in products and his information needs to be used to steer the discussion of the data. That's not really done in the current document and is also due to the fact that not really case studies are discussed (see second remark above). If the whole document is really organized in the two case studies then information on the actual use of certain nano-TiO_2 forms should be used to extract this information form the literature that is relevant for this case study. If no uncoated nano- TiO_2 are used in sunscreens then it means that for this case study all fate and behavior and ecotox data with uncoated TiO_2 should not be used.

7) Table 1-3 and 1-4: nanoparticle tracking analysis (NTA) is missing in both tables.

Charge question 2

1) On a qualitative level this chapter list what is already known about lifecycle of nano-TiO_2 and release to the environment. However, what is missing is a quantitative evaluation. I expected from this chapter to get numbers of release to the environment during the different life cycle stages. The whole chapter remains very vague and again, it is not really a case study but listing some qualitative data. From a case study I expect to see quantitative data, for example a material flow diagram showing the flows from TiO_2 production, formulation, use and disposal to the environment. Even if a lot of data is missing, we can still make some estimations and best guesses.
2) Chapter 2.4.2.: Most nano-TiO_2 in sunscreen will be released during the use phase. Where is it released to? How much ends up directly in the environment (released during swimming in lakes or the sea), how is it washed off during showering, how much is adhering to clothes and washed off during washing? These aspects that are absolutely crucial are not mentioned at all. Again, this is of primary importance if the document should really be a "case study".

Charge question 3

1) The following reference definitely needs to be considered because it is the first to investigate the behavior of nano-TiO_2 under natural conditions in rivers: Nanostructured TiO2: Transport Behavior and Effects on Aquatic Microbial Communities under Environmental Conditions. TOM J. BATTIN, FRANK V.D. KAMMER, ANDREAS WEILHARTNER, STEPHANIE OTTOFUELLING, AND THILO HOFMANN, Environ. Sci. Technol. 2009, 43, 8098–8104. This reference should also be used in chapter 5 because it also contain data on the effects of nano-TiO_2.

2) Chapter 3.1. This chapter discusses fate in water but a lot of the references are for biological growth media. These studies cannot give any clue about the behavior under natural conditions and need to be covered separately, preferably in a sub-chapter in the tox-part of the document. Biological growth media have a composition that is very different from natural waters with very high salt concentrations or organic compounds that are know to have strong effects on the agglomeration behavior.
3) Chapter 3.1.2. In this sunscreen-specific chapter definitely the papers coming from the French group working with a nano-TiO_2 that is actually used in sunscreens need to be presented in detail: "Structural Degradation at the Surface of a TiO_2-Based Nanomaterial Used in Cosmetics", MELANIE AUFFAN, MAXIME PEDEUTOUR, JEROME ROSE, ARMAND MASION, FABIO ZIARELLI, DANIEL BORSCHNECK, CORINNE CHANEAC, CELINE BOTTA, PERRINE CHAURAND, JEROME LABILLE, AND JEAN-YVES BOTTERO, Environ. Sci. Technol. 2010, 44, 2689–2694,
4) Chapter 3.3. This chapter does not present data on fate and behavior in air but only concentration measurements. This does not belong to this section but to a chapter on analysis in the environment (preferably within chapter 4). Also the study mentioned in the first paragraph of section 3.1. (Wigginton et al.) belongs to this new chapter on analysis in the environment. This belongs to the chapter on exposure.

Charge question 4

1) In a chapter on exposure I was expecting to read something about concentrations of nano-TiO_2 in different environmental media. However, section 4.1 is named "biota" with subsection on "aquatic species" and "terrestrial species". This is a very strange organization. In my point of view 4.1. should be about nano-TiO_2 concentrations in water (4.1.1.), sediments (4.1.2.) and soils (4.1.3.). In this part results from measurements and modeling about concentrations should be compiled. Mueller and Nowack (2008) and Gottschalk et al. (2009, 2010) have provided such data on concentrations (model results). Also Boxall et al (2008) have presented modeled data. Later in section 4.5. some modeled data are presented. On p. 4-13 the study by Mueller and Nowack (2008) is presented. However, the risk characterization part of that study does not belong to chapter 4 but to chapter 5 or 6. Only the modeled environmental concentrations belong to chapter 4 and the rest needs to be covered at another place in the document.
2) Chapter 4.3. is a very important one but again it remains qualitative. Based on data by Mueller and Nowack (2008) and Gottschalk et al. (2009, 2010) it is possible to relate the use of nano-TiO_2 in sunscreen to the total nano-TiO_2 use and thus evaluate the importance of sunscreen for total

environmental exposure to TiO_2:

Gottschalk, F. Sonderer, T.; Scholz, R. W.; Nowack, B. (2009) Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ. Sci. Technol. 43: 9216-9222

Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B. (2010) Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. Environ. Toxicol. Chem. 29: 1036–1048.

Charge question 5

1) In this chapter the point 6 mentioned in my comments to chapter 1 is crucial but is not really discussed: what does it mean that some nano-TiO_2 is coated with alumina or silica and what is the actual surface that an organism or cell sees? This issue is not explored but should receive a lot of attention. What if the ecotox and tox-data are grouped according to surface coating? Can we already see some trends? Are some coatings more or less toxic? How different are different TiO_2-formulations?
2) The first paragraph in section 5.1.2.2. has nothing to do with TiO_2 and should be deleted.
3) Chapter 5.2. is called “ecological effects”. Under this title I would expect to read something about effects on ecosystems, however this section is about ecotoxicology and should thus also be named “ecotoxicology”.
4) Chapter 5.2.1.3. There is a study available about nano-TiO_2 effects on willows that should be included: Seeger et al. (2009) J. Soils sediments 9(1),46-53.

Charge question 6

1) Chapter 4 was on exposure, chapter 5 on the effect but what is missing is a chapter on the risk (risk = exposure x effect). This could be part of the integrative summary or could also be a stand-alone chapter. I would suggest to make a new chapter 6 on risk so that the old chapter 6 then becomes chapter 7. At least three studies are available that have already tried to perform such an environmental risk assessment: Mueller and Nowack (2008) and Gottschalk et al. (2009, 2010). The risk assessment result of these studies should be included and critically evaluated. This could also be performed for human risk assessment. Even if the data quality and quantity about exposure and effect is still sparse, it is possible to make some first conclusions about the risk, using established assessment factors to cope with the uncertainty. It is in my opinion a serious omission that nothing is said about risk assessment in the whole document, although it is described in chapter 1 that the CEA-approach is used.

Charge question 7

1) The environment is integrating over all releases of nano-TiO_2 from all its applications. In order to perform an environmental risk assessment of nano-TiO_2, it is thus imperative to have knowledge on the release during all life cycle stages of all nano-TiO_2 applications. It is thus not enough to look only at two case studies. However, these case studies can be used to identify important research questions but again only if a relevant case has been picked. This is definitely the case for sunscreens so the data provided in this document are useful. It is less clear how the drinking water treatment case (which is just a hypothetical one) should be used to guide further research. It would have been much more important to identify which other uses of nano-TiO_2 are important. A second case study with a completely different release scenario during the whole life cycle (e.g. use as UV-absorber in textiles or in paints) would have been much more informative. Both chosen cases are water-centered and thus have similar behavior of the released TiO_2. A use in polymers or in another matrix-bound form would have resulted in a much broader discussion of release. The choice of the second case study, the use in water treatment, is not really understandable and was clearly a bad choice because it resulted in a narrowing of the focus. The whole discussion about release of nanoparticles from materials was therefore not covered at all and this is a serious omission because a large percentage of nanoparticles is used in matrix-bound form. These aspects need to be critically discussed in this chapter 6.
2) As already mentioned in my comments to chapter 1 and in some other places, the most critical missing part of the document is the absence of quantitative information in many places. Even if there is still a large uncertainty regarding many aspects of fate and effects, we can still make some first evaluations of the data and perform a first risk assessment and this is completely missing

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CHARGE QUESTIONS

Please prepare your comments addressing the issues and questions below, and organize your comments in the same order of the charge questions. If a question is outside your area of expertise, please indicate so.

1. Chapter 1 provides introductory material regarding the approach used in these case studies, definitions of conventional and nanoscale TiO_2, the use of nano-TiO_2 in drinking water treatment and sunscreens, and analytical methods for characterizing nano-TiO_2.

a. Is this information accurately and clearly presented?

Yes, I the introductory Chapter 1 is accurately and clearly presented.

b. Please comment on the utility of the chapter in providing background and support for the remainder of the document.

This chapter provides a succinct overview of the purpose and organization of the case studies. However, it may be useful to include more detail explaining why the two case studies were chosen as examples. In particular, why the choice of nano TiO_2 as a water treatment agent, since it is not currently used in this manner? This does not seem to be the most appropriate case study to select since it is primarily hypothetical. Perhaps a more appropriate case study would have been the use of nano iron oxide for cleaning hazardous waste sites.

The chapter should end right after the section with the heading "Sunscreen" and not include the subsequent section entitled, "Analytical Methods" This last section seems out of place in the introduction and would be better as a separate chapter or incorporated into a different chapter. In addition, the section entitled, "Methods of Instrumentation to Assess Workplace Exposure" does not seem to be necessary or relevant for this document (or perhaps move it to Chapter 4, Exposure-Dose Characterization).

c. How might this chapter be improved?

A few specific comments and edits that may improve the chapter are listed below.

- p. 1-1, paragraph 1: There should be more discussion of the types of products that nanomaterials are/will be used in to provide the reader with more background about why nanomaterials are being developed and used. The paragraph should also include what is meant by nanomaterials offering the "potential for benefits and risks" (brief description of what are the benefits, risks?)
- p. 1-5, line 24: can more information be provided about AEROXIDE P25 such as form of TiO_2 (rutile, anatase) and size distribution/mean of the nanoparticles?
- p. 1-6, line 25: change "bulletin" to "Current Intelligence Bulletin"

- p. 1-6, footnote: change "increase" to "increases"
- p. 1-7, line 27: explain what is meant by "the levels of effects" – what are the effects of external surface area? Without further explanation, the purpose of this paragraph describing surface area and its significance is unclear.
- p. 1-9, line 1: should porosity be defined in a footnote?
- p. 1-9, line 16: find a more appropriate reference for the particle diameters than the Klaessig 2006 phone call
- p. 1-9, line 36: add a space between "to" and "150"
- p. 1-15, line 30: explain what is meant by "presence and location" – doesn't TEM also provide information on shape, size, and whether particles are agglomerated/aggregated?
- p. 1-17, Table 1-3: move information in column to directly beneath the "Liquid" header; explain what the dashes (-) mean with a footnote.
- P. 1-20, section 1.6.4: this paragraph uses the term "nanomaterial" rather than "nanomaterials" that had been used throughout the chapter.

2. Chapter 2 presents information on the lifecycle of nano-TiO_2, including potential releases to the environment.

a. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems?

This chapter seems to accurately and sufficiently characterize what is known and unknown regarding the various stages of the lifecycle of nano-TiO2 for arsenic removal in water treatment systems. Because this is a hypothetical/experimental use and is not being widely implemented, there are still a lot of unknowns and data gaps regarding the use of nano-TiO2 for this purpose, and that is conveyed in this chapter. The section entitled "2.4.1 Water Treatment" is lacking in quantitative detail about the process involved with using nanoTiO2 for removing arsenic; in addition, the section does not really explain the mechanics of how nanoTiO2 will be used in water treatment systems, as it only provides hypothetical or experimental processes. This seems to be a significant omission, as it is difficult to develop an adequate case study without this fundamental process information.

b. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens?

This chapter seems to accurately and sufficiently characterize what is known and unknown regarding the various stages of the lifecycle of nano-TiO_2 in sunscreens. One additional piece of information that should be added to section "2.3.2. Sunscreen" is the estimated distribution of sunscreen from internet sales. Also highly recommend obtaining data on the distribution of sunscreen that is more recent than 20 years ago (1990) in this section -- this section should be updated to more accurately reflect current distribution levels of sunscreen.

c. To what extent is the material effectively organized and sufficiently informative to support planning for future research?

The information is logically organized, and includes the appropriate Life Cycle stages and what is known and unknown about each for both case studies. With respect to being informative to support planning for future research, it would be beneficial to include a summary paragraph that highlights what the most critical data gaps are regarding the life cycle stages for both case studies to highlight and direct the need for research to address those data gaps.

d. How might this chapter be improved?

Below is a suggested comment to improve this chapter.

- p. 2-9, line 1-8: this paragraph doesn't seem to fit in here – it is about stability of the coating on a particular manufacturer's nano-TiO_2, and should be moved to a section that describes the chemistry of nano-TiO_2. The data appear to be experimental and it is not clear whether this type of nano-TiO_2 is actually used in sunscreen formulations and how relevant the results of this experiment are to the actual use of sunscreen.

3. Information on the fate and transport of nano-TiO_2 in air, water, and soil is discussed in Chapter 3. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO_2 in the environment.

a. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health?

The information on fate and transport presented in Chapter 3 provides a useful overview of the possible exposure pathways to be considered when evaluating the use of nano-TiO_2 in waste water treatment and in sunscreens. The section on "Air" does not appear to be very informative to the possible fate and transport of nano-TiO_2 in air resulting from its use in water treatment plants or sunscreen. The information in this section is very limited and much more relevant to a facility that would be manufacturing nano-TiO_2 rather than the two case studies in this report. Further, although there are several data gaps where extrapolation of some experimental results may be appropriate, in several instances in this chapter, there are speculations about the possible behavior of nano-TiO_2 in the environment that are not scientifically supported by the information included in the chapter.

b. To what extent is the material effectively organized and sufficiently informative to support planning for future research?

Suggest having a summary paragraph highlighting the most important data gaps that research could be conducted in the future to fulfill the gaps and reduce uncertainties and speculation about fate and transport of nano-TiO_2.

c. How might this chapter be improved?

Below are a few suggested comments for improving this chapter.

- p. 3-3, line11-17: these two sentences refer to a conference proceedings abstract that reported the occurrence of nano-TiO_2 at a waste water treatment plant; however, there is not enough information about the plant, what the levels of TiO_2 were, how the nano-TiO_2 measured, etc. to provide context and understanding about the significance of this reported finding. Also, because this is a non-peer reviewed conference abstract, I suggest removing this from the document. Further, lines 15-17 state what the authors "predicted" regarding nanomaterial concentrations – this appears to be speculation and should be removed from this report.
- p. 3-3, lines 26-31: these sentences are pure speculation from Limbach et al. 2008 about the behavior of nano-TiO_2 based on their experimental results for cerium oxide in waste water and how the high nanoparticle concentration used in the study favors aggregation, and that at more realistic concentration, "a greater percentage of nanoparticles are likely to break through." Because this is pure speculation, it does not belong in this case study document.
- p. 3-3, lines 32-35: There should be more detail about the Harbour et al. 1985 study so that the statement about the behavior of conventional TiO_2 photogenerating long-lived reactive oxygen species in aqueous environments can be put into context; that is, what were the conditions under which TiO_2 generated ROS and how relevant are those conditions to nano-TiO_2 that may be present in water as a result of its use in waste water treatment plants or in sunscreen? There are many variables that may affect the ability to generate ROS (e.g., coatings) that should be considered when extrapolating results from an experimental study to real world situations. Further, that statement, "Similar behavior would be anticipated for nano-TiO_2 is speculation, and should be removed since there are no data to support such a statement.
- p. 3-4, lines 27-31: this sentence includes speculation from Zhang et a. 2008 about the "removal efficiencies would be lower for small aggregates than large aggregates at the same alum concentration"; suggest removing speculation.
- p. 3-8, lines 19-23: a study by Berges et al. 2007 was mentioned in this section as reporting airborne TiO_2 levels "outside the plant"; however, it is impossible to put this information into context and understand its relevance to these case studies, as there is no information about what

type of plant was studied in the Berger et al. 2007 study, duration of the measurement, or how it was obtained. Suggest adding more detail about this study or removing it from this section.

- p. 3-9, lines 4-7: The study by Murr et a. 2004 is cited as evidence that nano-TiO_2 may associate with other airborne nanoparticles; however, there is no detail about the Murr et al. 2004 study, making it impossible to determine how relevant the experimental results are to actual ambient conditions.

4. Chapter 4 provides information on exposure, dose, and translocation of nano-TiO_2 in biota and humans.

a. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO_2.

The information included in this chapter does accurately and sufficiently characterize the information on exposure characterization. One suggestion is to change the title of this chapter to Exposure Characterization, as that is really the focus of this chapter, not dose characterization. Exposure characterization is also a more appropriate title for the specific case studies that are being developed. The organization of the information and the topics are appropriate with one suggested change. On page 4-7, section entitled, “Occupational”: This section seems to be too long and out of place for these case studies, as most of the information focuses on facilities where TiO_2 is manufactured and much of the information is not relevant to the case studies. Suggest making this section shorter and more specific to the occupational settings involved in these case studies.

b. To what extent is the material effectively organized and sufficiently informative to support planning for future research?

It is not clear from reading this chapter what future research is necessary for characterizing exposure to TiO_2. Suggest including a summary paragraph highlighting the most important data gaps and what types of research needs there are regarding being able to characterize exposure for these case studies.

c. How might this chapter be improved?

Below are a few specific suggested comments for improving this chapter:

- p. 4-9, lines 1-11: The study by Berges 2007 appears to be a study of a facility that produces nano-TiO_2 for use in sunscreens, not a facility where the sunscreens are formulated. This distinction should be clearly stated. It is not clear how relevant the reported inhalable and respirable concentrations measured in this TiO_2 production factory would be to a sunscreen formulating facility.

- p. 4-10, lines 7-13: this paragraph about the *in vitro* study by Liao et a. 2009 does not belong in this section – it should be in Chapter 5. Furthermore, the use of *in vitro* data to predict *in vivo* lung inflammatory responses is not appropriate, given that fact that there are several differences between single cell-type responses *in vitro* and *in vivo* whole tissue (lung) conditions. The statement that workers would have significant risk of cytotoxicity response is speculation and results from an *in vitro* study cannot support such a conclusion.
- p. 4-11, Section entitled, "4.4 Cumulative Exposure to Nano-TiO_2 and Other Contaminants" appears to be primarily speculation with little scientific support. The "Trojan horse" effect is not something that has been observed with sunscreen, so this concept of exposure to other contaminants doesn't seem to apply to the sunscreen case study. Further, it is not clear whether there is any evidence to support this happening in waste water treatment plants either.
- p. 4-18, lines 4-12: the document should include a discussion about the differences between the nasal pathway and olfactory bulb in rodents versus humans (e.g., differences in the relative size of the rat olfactory mucosa and olfactory bulb likely predispose rodents, more so than humans, to nasal deposition and olfactory transport.
- p. 4-20, Table 4.3: specify the duration of the study and treatment period for all of the studies
- p. 4-24, Table4-4: add the following study: Bennat C. and Muller-Goymann CC. 2000. Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. Int. J. Cos. Sci. 22:271-283. Also, is it possible to add the names of the authors for the citations that are listed as "Refs in SCCNFP"?
- p. 4-27, lines 13-18: sentence should only include information from the final study (Sadrieh et al. 2010) and not the 2008 meeting abstract.
- p. 4-28, section 4.6.4: it should be mentioned that the gavage dose of 5g/kg is a high dose when discussing the results of this study.
- P. 4-28, Section 4.6.5.: This section should include mention of the point that most of the studies were intravenous or intraperitoneal, routes of exposure that are not relevant for these case studies.

5. Chapter 5 characterizes factors that influence ecological and health effects of nano-TiO_2 and discusses the currently available scientific evidence regarding these effects.

a. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science.

The information included in this chapter seems to accurately and sufficiently characterize the state of the science very well. The discussion of studies in the "Health Effects" section was thorough and the shortcomings and strengths of some of the types of studies (e.g., inhalation studies) was clearly written. The level of detail for the summaries of the inhalation studies seems excessive, given that the inhalation route of exposure is limited to the occupational setting where the nano-TiO2 is added to the sunscreen. The fact that there have not been reports of workers at such facilities experiencing adverse respiratory health effects brings into question the relevance of the inhalation studies.

b. To what extent is the material effectively organized and sufficiently informative to support planning for future research?

Suggest a summary paragraph highlighting the most important research needs.

c. How might this chapter be improved?

p. 5-28, lines 3-7: Is there any scientific basis for the statement that coated photostable nano-TiO_2 in sunscreen could lose its coating? This appears to be speculation, and should be modified to accurately reflect what is known about TiO_2 coating.

- p. 5-62, lines 4-13: suggest removing discussion of abstracts and posters in these case studies; they are not peer reviewed published studies.
- p. 5-64, line 25-26: change lung cancer to lung tumors; should also include discussion of particle overload being a major contributing factor to the development of tumors in these studies.
- For summaries of the various rodent carcinogenicity studies, the wording should be changed from "lung cancer" to "lung tumors"
- p. 5-69, section entitled, "Intramuscular Injection": suggest removing this section; is not a relevant route of exposure.
- p.5-72, lines 1-2: this statement is incorrect, the carcinogenicity of TiO_2 and nano TiO_2 has not been shown "repeatedly in inhalation and instillation studies" – recommend changing to "a few rat studies with TiO_2 have reported the development of lung tumors…."

6. Chapter 6 summarizes the information and research questions presented in the nano-TiO_2 water treatment and sunscreen case studies, as well as discussing the role of case studies in the refinement of research strategies and potential future assessment efforts.

a. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments.

This section clearly summarizes the main components of the two case studies and is well written. More information from section 6.2 about the role of the case studies may be useful to be included in the introduction of the document. The only suggested addition to this chapter is a more clear explanation of what the next course of action is regarding these case studies.

b. How might this chapter be improved?

Try to eliminate speculative statements (e.g., p. 6-6, lines 5-7 "the potential for removal of surface coatings…"

7. The case studies follow the comprehensive environmental assessment (CEA) approach, which combines a product life-cycle framework with the risk assessment paradigm.

a. Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO_2 in particular and nanomaterials in general.

This CEA approach seems to be a plausible and scientifically valid approach for evaluating products incorporating nanomaterials. It provides a framework that nanomaterial producers and users should consider when developing research programs for their materials. The organization , scope, and structure of the case studies seems to be appropriate and logical. The only suggestion for improvement is to more clearly state what the future research needs are and possibly rank the research priorities after each chapter. In addition, caution should be taken to not include too much speculation in the case studies; it is clear that there are many data gaps, so speculating about what may occur is not necessary. It would be more appropriate to identify data needs rather than speculate what may be the outcome.

8. Please provide any additional comments you would like to make on the draft document.

COMMENTS SUBMITTED BY

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Review of EPA's Draft Document,

Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen

Overview: This document will be an excellent resource. The authors write well: the report presents the available technical information clearly and in a very readable manner.

1. **Chapter 1 provides introductory material regarding the approach used in these case studies, definitions of conventional and nanoscale TiO2, the use of nano-TiO2 in drinking water treatment and sunscreens, and analytical methods for characterizing nano-TiO_2.**

1.1 Is this information accurately and clearly presented?

In general, this introduction clearly and accurately introduces the case studies. Some specific comments follow:

- Page 1-1. Consider adding explanation of why these two case studies were chosen, i.e., why are these two specific uses singled out for examination?
- Page 1-2, line 1. Define "secondary contaminant".
- Page 1-1, line 24 and page 1-2, Figure 1-1. Add "sediment" to environmental pathways. See related comment in Section 3.1 below.
- Page 1-5, footnote 5. The footnote indicates that the terms aggregation and agglomeration have not been standardized. Consider citing and using the definitions developed by ASTM International[3], which distinguishes between agglomeration and aggregation of nanoparticles as follows. An agglomerate is a group of particles held together by relatively weak forces (such as Van der Waals force) that can be broken apart. An aggregate is a discrete group of particles composed of individual components that are tightly bonded together and not easily broken apart.
- Page 1-7, lines 25-26. Readers not familiar with the behavior of nanomaterials will need additional explanation to understand the statement "Humic acid-coated nano-TiO_2 had lower zeta potential…" Please see related comment below on Chapter 3 (Section 3.3 of these comments).
- Page 1-11, lines 17-19. Provide a reference for the statement "Further, some particle characterization techniques can affect measurement accuracy…" and/or explanation.
- Page 1-15, lines 11-15. For another excellent overview of analytical methods for nanomaterials, see Chapter 5: Analyses of Nanoparticles in the Environment, by Marilyn Hoyt, in *Nanotechnology and the Environment*[4].

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[3] ASTM International. 2006. Designation: E 2456 – 06. Standard Terminology Relating to Nanotechnology**.**
[4] Sellers, K., et al., 2009. *Nanotechnology and the Environment*. Taylor & Francis Press. Boca Raton, FL.

- Page 1-18, line 17. The meaning of the following statement is unclear "…were also the two techniques that appear to be most prone to artifacts."

1.2 Comment on the utility of the chapter in providing background and support for the remainder of the document. How might this chapter be improved?

This chapter provides substantial background and support for the remainder of the document. No improvements are suggested other than as described above.

2. Chapter 2 presents information on the lifecycle of nano-TiO_2, including potential releases to the environment.

2.1 To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used for arsenic removal in water treatment systems?

An explanation of the process for using nano-TiO_2 in drinking water treatment would provide a reader unfamiliar with water treatment some context for the discussion. First, consider including a process flow diagram for a conventional water treatment plant in Section 2.4.1 with a brief description of the processes so that readers not versed in water treatment will have a basis for understanding subsequent references to the processes of coagulation, flocculation, sedimentation, filtration, and disinfection. Second, additional description of the process(es) by which nano-TiO_2 could be used in treatment, to supplement the brief mention on page 2-6, lines 14-15, would provide further context for potential exposures.

Reference 196092 contains some information on the scale of nano-TiO_2 use in the Photo-Cat system that would provide dimension to the discussion in lines 8-11 of page 2-7: the maximum use level is 75 grams (g) TiO_2 per reactor assembly certified for a minimum flow of 33,600 liters per day (L/day).

Section 2.5.1, regarding the water treatment case study, could characterize the disposal stage of the life cycle more accurately as follows:

- The first paragraph, which describes waste generated from sand filters, is only relevant to this case study if nano-TiO_2 were applied as the first treatment process in the plant. That might not be the case if nano-TiO_2 were applied in a polishing step in a reactor containing nano-TiO_2 fixed in a matrix.
- The second paragraph suggests that contaminants in water treatment sludge placed in a landfill could leach into underlying groundwater. In fact, landfills are designed to collect and treat leachate. While no system is completely leak-proof, this discussion of possible releases from

landfill should acknowledge that lined landfills are designed and maintained to minimize leaching into groundwater.

- The third paragraph indicates that sludge from water treatment could be used for land application. It appears that this paragraph is incorrectly conflating the disposal of water treatment sludge and the disposal of wastewater treatment sludge. The sludge from potable water treatment contains primarily inorganic substances and water treatment chemicals and is not land applied. In contrast, sludge wasted from biological wastewater treatment processes is high in organic matter and is sometimes land applied after suitable treatment as described in this paragraph.
- The fourth paragraph mentions the possibility that nano-TiO_2 residuals in drinking water would eventually reach the ambient environment or sewage treatment facilities. Can the information from bench-and pilot-scale testing be used to bound the possible maximum concentrations which would be released?

2.2 To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-TiO_2 as used in sunscreens?

The discussion of the life cycle of nano-TiO_2 in sunscreen neglects the release of nano-TiO_2 into surface water during recreation while using sunscreen and also neglects the "disposal" of nano-TiO_2 in sewage treatment plants after showering or bathing. The following references, which are not reflected in the draft report, contain information relevant to this discussion and to portions of Chapter 3:

- Johnson, A., et al., 2009. Should we be concerned about the discharge of sunscreen nano TiO_2 to the environment? Poster presented at SETAC Europe: 19th Annual Meeting, 31 May-4 June 2009. Goteborg, Sweden. (These researchers estimated the release of nano-TiO_2 to surface water after sunscreen use. An electronic copy of the poster is appended to these comments.)
- Kiser et al., 2009. Titanium nanomaterial removal and release from wastewater treatment. Environmental Science and Technology. 43(17): 6757 – 6763. (The authors report on the occurrence, characterization, and removal of nano- and larger-sized Ti at wastewater treatment plants. Reference is apparently made to a preliminary report on this work in a conference proceeding abstract [157466], on page 3-3.)
- Limbach et al., 2008. Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency. Environmental Science and Technology. 42(15): 5828-5833. (While this research primarily pertained to the removal of cerium oxide, the authors indicate the relevance of the work to nano-TiO_2. This paper is discussed on page 3-3 [reference 155628].)

- Mueller and Nowack, 2008. Exposure modeling of engineered nanoparticles in the environment. Environmental Science and Technology. 42(12): 4447-4453. (The aim of this study was to use a life-cycle perspective to model the quantities of engineered nanoparticles released into the environment, including nano-TiO_2. This study is mentioned on page 4-12 of the draft document, but is also relevant to Sections 2 and 3.)

2.3 To what extent is the material effectively organized and sufficiently informative to support planning for future research?

With the exception of the comments and recommendations above and in Section 2.4 below, this material is effectively organized and sufficiently informative to support planning for further research.

2.4 How might this chapter be improved?

Consider reorganizing the chapter so that the life cycle is presented for each case study in turn (rather than by reviewing each stage for both case studies).

3. Information on the fate and transport of nano-TiO_2 in air, water, and soil is discussed in Chapter 3.

3.1 Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-TiO2 in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health?

Add a discussion of sediment, as a matrix which could be affected by the release of nanomaterials and could be an exposure route relevant to biota and human health. This would provide context for the discussion of exposure by sediment-dwelling organisms on page 4-3. Relevant references include but are not limited to:

- Boncagni, et al., 2009. Exchange of TiO_2 nanoparticles between streams and streambeds. Environmental Science and Technology. 43(20): 7699-7705. (The authors performed a series of experiments to examine the deposition of P25 and synthesized nano-TiO_2.)
- Gottschalk, et al., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for different regions. Environmental Science and Technology. 43(24): 9216-9222. (In this study, the authors predicted environmental concentrations of nano-TiO_2 based on a probabilistic material flow analysis. The paper discusses assumptions regarding partitioning to sediments.)

Specific comments on the draft chapter follow.

- Page 3-1, lines 20-22. The report indicates that the degree of aggregation generally increases with increases in the amount of organic matter in water. However, some experimental work has shown that increasing the level of natural organic matter in water limits the agglomeration of some nanoparticles. See for example:
  - Hoon Hyung et al., 2006. Natural Organic Matter Stabilizes Carbon Nanotubes in the Aqueous Phase Environ. Sci. Technol. 41:179 -184.
  - Kennedy, A.J., et al., 2008. Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. Environmental Toxicology and Chemistry 27(9): 1932-1948.

Further, on page 3-4 (lines 1-7), the report acknowledges that sorption to humic acid increases the stability of nano-TiO_2 suspensions, which would seem to contradict the assertion on page 3-1, lines 20-22.

- Page 3-2, line 17. Suggest clarifying whether the concentrations cited are the initial concentrations added to the solution, or the "concentrations" of the P25 in suspension.
- Page 3-3, line 13. As noted above, reference is apparently made to a preliminary report on experimental work in a conference proceeding abstract [157466] that was subsequently published as:
  - Kiser et al., 2009. Titanium nanomaterial removal and release from wastewater treatment. Environmental Science and Technology. 43(17): 6757 – 6763.
- Page 3-5, line 3. As noted above, drinking water treatment sludge is generally not land applied.
- Page 3-5, line 15. Suggest inserting the following underlined phrase: "… other inorganic compounds are not readily broken down in that environment <u>and nano-TiO_2 is poorly soluble</u>; however…."
- Page 3-8, lines 3-4 and 11-12. As noted above, drinking water treatment sludge is generally not land applied.
- Page 3-8, lines 19-23. Since air emissions controls can be somewhat dependent upon the local regulations, suggest indicating the location of the facility where Berges et al. made their measurements. Berges et al., 2007 (http://www.dguv.de/ifa/de/fac/nanopartikeln/taipei.pdf; 157594) indicates the facility was in Taipei.

3.2 To what extent is the material effectively organized and sufficiently informative to support planning for future research?

Prioritizing research needs based on the potential impact to various media would require at least a first approximation of the magnitude of potential releases. Further, without some effort to rank-order or prioritize those potential releases the public may be unduly alarmed about some possible exposure routes. Consider adding a summary and conclusions section, which refers to existing life cycle model predictions and, to the extent possible, identifies the major apparent sinks for nano-TiO_2. Even though this summary would be limited by the relative paucity of quantitative information, it would add value.

3.3 How might this chapter be improved?

The chapter could be improved, with respect to the reader new to the science of nanotechnology, by adding a brief discussion of the factors which generally control the fate and transport of nanoparticles. That is, fate and transport discussions primarily refer to suspensions of nanoparticles rather than dissolved concentrations in aqueous solutions (or nonaqueous phase liquids) considered in more conventional fate and transport assessments; the buoyancy of those particles and their tendency to agglomerate via Van der Waals forces dominates their fate and transport. Electrostatic repulsion, represented by zeta potential and affected by pH and high ionic strength, counters the tendency to agglomerate. Sorption to natural organic matter can enhance the stability of nanoparticle suspensions.

Alternatively, such a discussion could be included in Chapter 1.

4. Chapter 4 provides information on exposure, dose, and translocation of nano-TiO_2 in biota and humans.

4.1 Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological effects of nano-TiO2. To what extent is the material effectively organized and sufficiently informative to support planning for future research?

Specific comments on the draft chapter follow.

- Page 4-2, lines 10-11. The draft report says that “Because typical wastewater treatment plants currently do not monitor for or specifically target nanomaterials, nano-TiO_2 might not be completely removed by sewage treatment.” It would be more accurate to simply say that “Discharges of nano-TiO_2 from wastewater treatment plants are not currently regulated. Therefore they are not designed or operated to remove nano-TiO_2, although early research suggests that some removal can occur (Kiser et al., 2009).”
- Page 4-4, lines 18-22. Consider adding the following information to this discussion of nano-TiO_2 in drinking water treatment. Currently, the levels of titanium are not regulated in public water supplies (http://www.epa.gov/safewater/contaminants/index.html). Therefore, water treatment facilities typically do not monitor the levels of Ti in potable water.
- Pages 4-5 and 4-6, discussion of dermal exposure. The estimated ranges of exposures are based on the assumption that sunscreen contains 5% nano-TiO_2, yet the report cites a maximum

concentration of 15% nano-TiO_2 in sunscreen. Why was the maximum (15%) not used to develop upper-bound estimates?

- Page 4-7, lines 14-15. Kiser et al., 2009 cite a second study by Lomer et al., 2000, which indicates that the daily human intake of TiO_2 (average size <200 nm) has been estimated to exceed 5.4 mg/day. They provide the following reference:
  - Lomer, et al., Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. *Analyst* **2000**, *125* (12), 2339–2343.
- Page 4-8, lines 10-17. Suggest concluding the paragraph with a statement that occupational exposure can vary between facilities.
- Page 4-10, lines 14-23. To put the observations of Li et al. into context, indicate that the facility was located in Shanghai.
- Page 4-16, table 4-2. Suggest indicating in this table the means, if any, used to maintain nano-TiO_2 in suspension.
- Page 4-27, lines 1-5. Mortensen et al. qualified their results by saying that under no circumstances is there evidence for massive quantum dot penetration and that quantum dots collected preferentially in the folds and defects in the stratum corneum, as well as in hair follicles.
- Section 4.6.6. Please see the recent (June 2010) publication by OECD, i.e., Publication of the Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials.

4.2 How might this chapter be improved?

This chapter could be improved by moving the important discussion of dose metrics in Section 4.6.6 to the beginning of Section 4.6.

5. Chapter 5 characterizes factors that influence ecological and health effects of nano-TiO_2 and discusses the currently available scientific evidence regarding these effects.

5.1 Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research?

This question is outside my primary area of expertise. With that caveat I offer the following comments.

- Page 5-2, lines 28-30. Consider acknowledging the efforts underway through the OECD Sponsorship Programme. See http://www.oecd.org/document/47/0,3343,en_2649_37015404_41197295_1_1_1_1,00.html. And consider acknowledging the following databases:

- o OECD Database on Research into Safety of Manufactured Nanomaterials, http://webnet.oecd.org/NanoMaterials/Pagelet/Front/Default.aspx?
- o The Project on Emerging Nanotechnologies database on Environment, Health, and Safety Research http://www.nanotechproject.org/inventories/ehs/
- o International Council on Nanotechnology, NanoEHS Database http://icon.rice.edu/virtualjournal.cfm

- Section 5.1.1.4, recommended characterization of nanomaterial [sic] for ecological and toxicological studies. Suggest incorporating OECD guidelines, e.g., Publication of the Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme; First Revision.
- Page 5-7, lines 4-6. Suggest editing to read as follows: "Experimental conditions, particularly the choice of medium/vehicle in which to disperse nano-TiO_2, preparation of testing solutions or suspensions, the formation of agglomerates, and/or measures taken to control the formation of agglomerates can influence the behavior and effects of nano-TiO_2 and other nanomaterials."

5.2 How might this chapter be improved?

This question is outside my primary area of expertise.

6. Chapter 6 summarizes the information and research questions presented in the nano-TiO_2 water treatment and sunscreen case studies, as well as discussing the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

This chapter provides an excellent and very well-written summary of the details presented in previous chapters. One specific comment follows.

- Page 6-6, line 26. The report indicates here that the presence of organic matter in water increases the degree of agglomeration. As noted above in comments on Chapter 3, this is generally not the case.

7. The case studies follow the comprehensive environmental assessment (CEA) approach, which combines a product life-cycle framework with the risk assessment paradigm. Please comment on aspects of this approach that can be improved in future case studies. We would appreciate comment on the overall structure and scope of the case studies and the extent to which the case studies support the development and refinement of research directions for future CEAs of nano-TiO_2 in particular and nanomaterials in general.

The CEA approach provided an excellent framework for organizing and analyzing the literature to create case studies. The document will be a valuable reference.

The two highest-priority topics for further research, i.e., the need to evaluate whether existing human and ecological toxicity test protocols are appropriate for use with nano-TiO_2 and the need to characterize the physicochemical properties of nano-TiO_2 at various stages, follow logically from the information presented. Ongoing work by OECD and other parties will be providing additional information on those topics.

As difficult as it might be considering the state of the science and the number of variables, the CEA approach could potentially be improved by including a rank-ordering of potential exposures. The outcome of the NCEA workshop, as described in the document, hints at this need ("priority topics included… evaluating exposure pathways and populations of greatest concern", page 6-16 line 12). Clearly, the criteria and nomenclature for rank ordering would need to be carefully considered. To illustrate the point qualitatively, in the case study regarding the use of nano-TiO_2 in water treatment the potential for worker exposures would be much greater than the potential for exposure to nano-TiO_2 from water treatment sludge that had leached from the sludge in a landfill and migrated beyond the leachate control system. This kind of prioritization might help to focus research efforts and would also provide valuable context for lay readers who might otherwise misunderstand the potential for exposure and consequent risk.

8. Please provide any additional comments you would like to make on the draft document.

While the report is remarkably well written, some editorial suggestions (e.g., apparent typographical errors) follow:

- Page 1-4, line 21. Spell out acronym "UV" at first use.
- Page 1-11, line 31. Apparent missing word: "… removal in drinking water…" (to distinguish from wastewater treatment or treatment of contaminated groundwater, for example).
- Page 1-12, line 31. Apparently missing radical symbol ("superoxide radical anions [$\bullet O_2^-$}")
- Page 1-12, line 35. Word choice unclear. Should "One generally accepted mechanism of nano-TiO2 antimicrobial property is the…" be "One generally accepted mechanism of nano-TiO2 antimicrobial action is the…" ?

- Page 1-13, line 4. Apparently missing word: "… presence of UV light…"
- Page 1-19, lines 1-2. Word choice unclear. Should "Workplace exposure thus far has focused on measuring nanoparticles in the air. Instruments that can be used for aerosol sampling are available, but…" be: "Workplace monitoring thus far has focused on measuring nanoparticles in the air. Instruments that can be used for aerosol monitoring are available, but…"
- Page 2-1, lines 7-10. Consider moving the sentence "For the rutile-based manufacturing … " from the first paragraph of Section 2.1 to the discussion of manufacturing now in Section 2.2.
- Page 2-2, lines 17-19. Missing words from the sentence "Nonetheless, nano-TiO_2 production based on *a predicted trend of graduate and* a theoretical upper bound…"? [emphasis added]
- Page 2-2, lines 24-26. Consider re-organizing this sentence to more closely parallel (and introduce) the paragraphs which follow, e.g., "Manufacturers and researchers report nano-TiO_2 synthesis by various techniques, including chemical vapor deposition (CVD), flame hydrolysis, the sulfate process, and other processes such as sol-gel, calcination, aerosol pyrolysis, and colloidal synthesis (Wahi et al., 2006, 090580).
- Page 2-3, lines 13-17. Consider moving this paragraph to the end of this subsection, as this paragraph describes post-manufacture processing and the following paragraph returns to the topic of production methods.
- Page 2-4, lines 28-32. Consider moving the sentences "P25 presumably could be stored… good management practices." to follow the first sentence of this paragraph, which also discusses P25.
- Page 2-5, line 13. Noun-verb agreement: "Industry data from the 1990s, although perhaps out of date, shed~~s~~ light on the distribution…."
- Page 2-7, lines 16-26. Consider moving the discussion "Several studies have bench-tested nano-TiO_2 in slurry systems…" to follow the discussion of bench-scale testing which concludes with lines 1-2 on page 2-7.
- Page 2-8, line 22. Noun-verb agreement: "…survey data do~~es~~ not differentiate…"
- Page 3-2, line 21. Apparent typographical error. "… can affect the surface charge~~ing~~ properties…"
- Page 3-2, lines 25-30. Word choice unclear. Should "…solubility increased rapidly..." be "dissolved concentrations increased rapidly…"?
- Page 3-4, lines 32-33. Suggest clarifying meaning by rephrasing "Several different waste streams are generated from drinking water treatment facilities that could contain nano-TiO_2" as "Several different waste streams that could contain nano-TiO_2 could be generated from drinking water treatment facilities."
- Page 3-6, line 30. Suggest clarifying, if appropriate, by adding underlined words: "… after three pore volumes of water passed through the column…"
- Page 4-9, line 15. Suggest adding underlined word: "… the estimated total airborne TiO_2…"

- Page 4-22, line 5. Instead of "A recent report using pig and hairless mice…" suggest "A recent study using pigs and hairless mice…"
- Page 4-22, line 8. Instead of "…exposure of nano-TiO_2 to porcine skin in vitro…" suggest "…exposure of porcine skin to nano-TiO_2 in vitro…"
- Page 4-22, line 13. Missing letter: "… muscle, hear**t**, liver…"
- Page 4-27, line 13. Missing word: "… into the dermis of minipigs…"
- Page 5-3, line 22. Missing words: "… of nano-TiO_2 on the generation of reactive oxygen…"
- Page 5-8, line 20. Capitalize first word in sentence: "**M**ost rutile…"
- Page 5-10, line 31. Missing word: "…UV light…" Similar comment on page 2-11, lines 6, 11, 12.
- Page 6-7, line 12. Apparent typographical error: "…photocatalytic generation of ~~generate~~ reactive oxygen species …"

Appendix:

A copy of the poster by Johnson et al., 2009, referenced in section 2.2 of these comments, is inserted below.

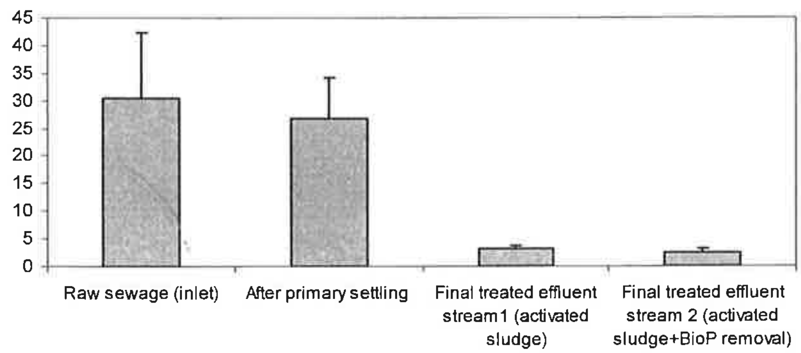
Johnson et al 2009

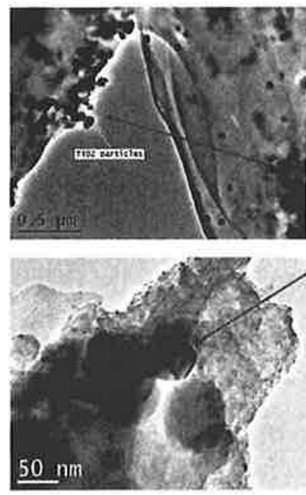
Should we be concerned about the discharge of sunscreen nano TiO_2 to the environment?

Putting sunscreen nano TiO_2 into context

TiO_2 is naturally found in the environment, for example it is present at 5 g/Kg in British river bed sediments. We put lots of colloidal (100-400 nm) TiO_2 into the environment every day from its use as a pigment in paints, paper, inks, cosmetics, and food. Nano TiO_2 is less than 0.1% of our annual consumption.

What happens to nano TiO_2 when it is washed off down the drain and enters a sewage treatment plant?





Settled activated sludge and nano-TiO_2 were closely associated

But nano-TiO2 could not be seen in the aqueous supernatent

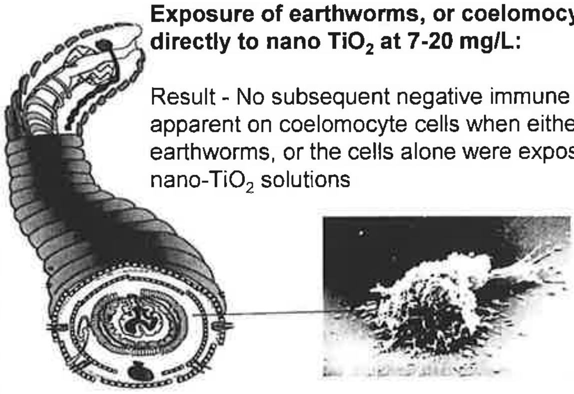
Based on a series of measurements on one day during a dry weather period it would appear that 90% of colloidal (<450 nm) titanium is removed in an activated sludge plant. Electron microscopy shows a close association between 75 nm TiO_2 particles and activated sludge flocs in the lab.

Implication: Greatest environmental exposure to Ti and nano-TiO_2 will be in soil following sludge application to soil as fertiliser

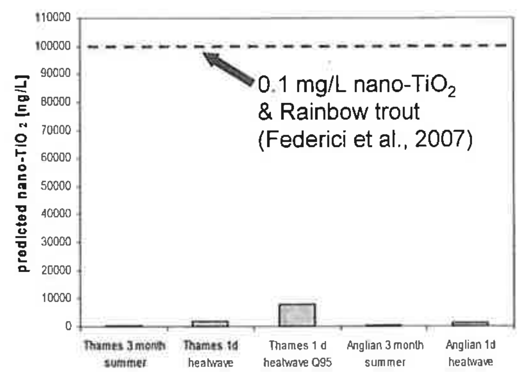
Response of earthworms immune cells (coelomocytes) to nano TiO_2

Exposure of earthworms, or coelomocytes directly to nano TiO_2 at 7-20 mg/L:

Result - No subsequent negative immune effects apparent on coelomocyte cells when either whole earthworms, or the cells alone were exposed to nano-TiO_2 solutions



Proximity of predicted upper limit nano TiO_2 from sunscreen to lowest reported effect concentration to an aquatic organism



Scenarios for predicting highest possible sunscreen nano TiO_2 water concentrations A 1-day heatwave in Thames Valley, UK

- One in four of population apply recommended dose of sunscreen to half of their body (adults)
- Assume that around 1/5 UK sunscreens contain nano-TiO_2 ingredients
- Assume 90% removal in STP
- Would give max concentration of 2,000 ng/L in the Thames region (mean flow, and 8,000 ng/L under Q95 low flow)

Preliminary conclusions

- Plenty of colloidal Ti is going down the sewer into the environment, but vast majority likely to be in 100-400 nm size
- Highest possible modelled water concentration for sunscreen nano-TiO_2 was 8,000 ng/L in the R. Thames, but this is still way below literature effect concentrations
- Most colloidal and nano fraction TiO_2 will enter the environment via the disposal of sewage sludge to land
- Toxicity testing to soil organisms seems most relevant for nano-TiO_2
- Nano-TiO_2 does not harm earthworm immune system

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