README FILE FOR THE NANOPUZZLES LIST OF EVALUATED REFERENCES

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Overview of this document

This file documents the spreadsheet of evaluated references prepared within the NanoPUZZLES EU project [http://www.nanopuzzles.eu] for the purpose of selecting nanotoxicology articles from which (meta)data were extracted for the purposes of developing nano-QSAR models and/or ISA-TAB-Nano datasets. Each reference documented in this final spreadsheet corresponds to a primary experimental reference.

The notes provided regarding the meaning of the columns in this spreadsheet were adapted from those provided for the purposes of a "quality check" procedure carried out within the NanoPUZZLES project prior to the preparation of the first public release of this spreadsheet (NanoPUZZLES.Spreadsheet.of.Evaluated.References.Public.v1.0.xlsx).

Important caveats

Please note that this spreadsheet and the evaluation process were iteratively updated and contributed to by a diverse team, hence the manner in which certain columns were populated may not always be perfectly consistent.

In addition, along with the general caveats noted here under "Important Caveats", possible problems associated with the population of specific columns– which were identified subsequent to completion of the spreadsheet – are documented via notes starting as follows: "**Column specific caveats:**".

In general, columns in this spreadsheet referring to the availability of certain (meta)data, or compliance with certain criteria, were populated with "Y" (otherwise "N") if these (meta)data were available, or this criterion was applicable, for *any* of the nanomaterials, or studies, corresponding to the experiments reported in the article in question. Hence, these may have been populated with "Y" even if the corresponding (meta)data was only available, or the corresponding criterion applicable, to one of the nanomaterials studied, or one of the studies. Entries of "Y?" and "N?" were created when the curator was unsure of the answer but felt it was probably "yes" and "no" respectively.

In general, the columns in the spreadsheet are concerned with the data which are reported to have been generated by the authors of the current reference *and, ideally, which were available from the publication or the supporting information*. Hence, if the authors refer to data reported in earlier publications or simply discuss a particular kind of data or method, this was should not have been considered relevant. *However*, where certain articles indicated data were generated and these were known to be publicly available elsewhere – even if not directly available from the evaluated article – these available (meta)data were taken into account when populating this spreadsheet. *Conversely*,

in some cases, “no. Nanomaterials” may have been populated based upon the authors’ descriptions of the experiments they carried out – even if corresponding data were not ultimately available for that publication. See the spreadsheet column “Miscellaneous comments” for further details.

It cannot be claimed that synonyms for the (meta)data searched for in order to populate a given column, beyond the iteratively updated guidance notes for which the latest version is provided in this document, were consistently looked for. For example, “endotoxin” may otherwise be referred to as “lipopolysaccharide” (LPS) [Petersen 2014], but these synonyms were not consistently looked for in the examined references.

It should also be noted that a loose and iteratively updated notion of “nanomaterial” was used when preparing this spreadsheet, such that the nanomaterials which were studied in the relevant references may only be nominal nanomaterials or otherwise not consistent with some definitions of a nanomaterial. Please see the guidance notes regarding “*Columns under the “n.o. Nanomaterials” section*” for the different kinds of criteria which were used to identify nanomaterials declared to have been studied in the evaluated references.

A basic “quality check”, of the correctness of the information concerning the availability of data for key biological endpoints and the number of nanomaterials, was carried out for 72 of the 336 references. A more thorough “quality check” was carried out for 39 of these 72 references. More than 50% of the 72 references contained at least some errors in the assigned annotations.

In light of these key caveats, the annotations in this spreadsheet should only be taken to be indicative of the (meta)data availability corresponding to a given reference.

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Specific Guidance for Specific Columns

Columns under the "Comments" section:

'Are there any 'nanomaterials' (based on the authors' classification) counted under "no. Nanomaterials" with no dimensions inside the 1 - 100 nm range?

See the comments under **Columns under the "n.o. Nanomaterials" section:** below.

Miscellaneous comments

All other comments will have been recorded here. These may include caveats specific to that reference or, otherwise, explanations of why certain columns were populated with certain entries.

Columns under the "Reference details" section:

These should be self-explanatory. N.B. As long as the first page number is provided and is correct, the lack of the last page number should not be considered an error.

Columns under the "Type of reference" section:

Primary experimental reference? [Y/N]

Is this a "primary experimental reference"? If yes –select "Y" from the dropdown box. If not – select "N" from the dropdown box.

A primary experimental reference will report the generation of new experimental data. The reference is not a primary experimental reference if the paper says "these data were previously reported in …." or something similar.

Please note that a "review" is NOT a primary experimental reference.

A modelling (e.g. nano-QSAR) reference (see below for additional explanation) *might* be a primary experimental reference – if the experiments used to generate the data are reported in the reference and no indication is given that the dataset was obtained from another paper.

Modelling Study? [Y/N]

Is this a modelling study or not? "? If yes –select "Y" from the dropdown box. If not – select "N" from the dropdown box.

A modelling study means a reference which describes a computational model e.g. a "nano-QSAR", "QNAR", "quantitative structure-activity relationship" etc. (N.B. A "structure-activity relationship" is not necessarily a modelling study i.e. a "structure-activity relationship" is not the same thing as a "quantitative structure-activity relationship".)

Columns under the "Available endpoint data?" section:

Genotoxicity? [Y/N]

Does this reference report any genotoxicity data for nanoparticles or nanomaterials? If yes –select "Y" from the dropdown box. If not – select "N" from the dropdown box.

Please note, that you should still select "Y" in this box even if negative toxicity data were obtained i.e. if the nanomaterials were determined not to be toxic. This box is only concerned with whether or not a *genotoxicity* study on nanomaterials was described in this reference.

If the reference specifically refers to "genotoxicity", "genotoxic" or "genetic toxicology" data having been generated for nanomaterials or nanoparticles, enter "Y" in this box.

Otherwise, please consider the following explanation of what "genotoxicity" means.

Genotoxicity refers to any kind of toxicity which causes changes and/or damage to DNA molecules or chromosomes.

Genotoxicity includes "mutagenicity" e.g. the nanomaterials were assessed for their "mutagenic potential", i.e. their ability to cause "mutations", or were determined to be "mutagens" or not to be "mutagens". "Mutagenicity" may be assessed using the "Ames test" (or "OECD 471" or "bacterial reverse mutation test") or the "HPRT forward mutation assay" (or "OECD 476").DNA damage may be assessed using the "Comet" assay. Chromsome damage may be assessed using the "chromosome aberration" test (or "OECD 473") or the "micronucleus assay" (or "OECD 487").

Please refer to Doak et al. [Doak 2012] for further information.

Cytotoxicity? [Y/N]

Does this reference report any cytotoxicity data for nanoparticles or nanomaterials? If yes –select "Y" from the dropdown box. If not – select "N" from the dropdown box.

Please note, that you should still select "Y" in this box even if negative toxicity data were obtained i.e. if the nanomaterials were determined not to be toxic. This box is only concerned with whether or not a *cytotoxicity* study on nanomaterials was described in this reference.

Please note that, in contrast to Lewinski et al. [Lewinski 2008], we do not consider genotoxicity as a type of cytotoxicity. Hence, if genotoxicity data were reported and no other cytotoxicity data were reported, enter "N" in this box.

If the reference specifically refers to "cell viability" data or "cell death" data having been generated for nanomaterials or nanoparticles, enter "Y" in this box. Likewise, if the reference specifically refers to "cytotoxicity" data having been generated for nanomaterials or nanoparticles (and you have checked this does not actually refer to genotoxicity data), enter "Y" in this box.

Otherwise, please consider the following explanation of what "cytotoxicity" means - principally based on Lewinski et al. [Lewinski 2008] except for the fact that genotoxicity is not considered a type of cytotoxicity. The references to different assays were also informed via consideration of Domey et al. 2014 [Domey 2014] Hu et al. 2009 [Hu 2009], Thill et al. 2006 [Thill 2006] and Kroll et al. 2009 [Kroll 2009].

Cytotoxicity refers to toxicity which kills or damages cells. If the cells are killed, the cytotoxicity may be considered a “lethal” effect. (This includes necrosis and apoptosis [Kroemer 2005].) “Sub-lethal” cytotoxicity includes “inflammation” - which might be determined using an ELISA assay to detect various cytokines such as IL-8, IL-6, TNF-alpha. “Sub-lethal” cytotoxicity also includes “oxidative stress” which might be detected via measuring “reactive oxidative species (ROS)”, “lipid peroxidation” or via using a “glutathione (GSH) assay”.

The following assays might be used to detect “lethal” cytotoxicity: MTT,MTS,WST,XTT, Neutral red (= toluylene red),Trypan blue,LIVE/DEAD,LDH (= Lactate dehydrogenase),Cell Titer 96 ,Alamar blue (= Resazurin),Metabolic activity (e.g. ATP assay, 14C-Glucose mineralization test),Colony forming unit (CFU) counting, Apoptosis/necrosis assays (e.g. Annexin V/propidium iodide staining, Caspase-3). Other assays which might be used to detect cytotoxicity include “Digital holographic microscopy” and "Electrochemical impedance spectroscopy". (However, these are not necessarily always used to detect cytotoxicity – so check if they were used to generate cytotoxicity data if these assays are mentioned!)

“Aquatic toxicity? [Y/N]”

Does this reference report any aquatic toxicity data for nanoparticles or nanomaterials? If yes – select “Y” from the dropdown box. If not – select “N” from the dropdown box.

Aquatic toxicity refers to any toxicity caused to marine organisms e.g. fish, Daphnia magna (D. magna) etc.

Please note, that you should still select “Y” in this box even if negative toxicity data were obtained i.e. if the nanomaterials were determined not to be toxic. This box is only concerned with whether or not a *aquatic toxicity* study on nanomaterials was described in this reference.

Any data for endpoints considered for modelling within NanoPUZZLES? [Y/N]

If **Cytotoxicity? [Y/N]**, **Genotoxicity? [Y/N]**, **Aquatic toxicity? [Y/N]** was set to “Y”, or **Any data for miscellaneous biological endpoints (other than genotoxicity, cytotoxicity, aquatic toxicity) which were considered for modelling within NanoPUZZLES?** not blank, this should be set to “Y”.

Any data for miscellaneous biological endpoints (other than genotoxicity, cytotoxicity, aquatic toxicity) which were considered for modelling within NanoPUZZLES?

Initially, cytotoxicity and genotoxicity were the endpoint prioritised for modelling within NanoPUZZLES, with aquatic toxicity being investigated later in the project. However, publications providing a significant amount of nanomaterial data for other – potentially toxicologically relevant – biological endpoints were also identified as possible sources of data for developing models.

Columns under the “n.o. Nanomaterials” section:

For each of the different categories (“Metal oxides”, “Metals”, “Silica”, “Carbon nanotubes (single-walled and multiwalled)”, “Fullerenes and fullerene derivatives”,” Other”, “Unclear”), you should enter the number of nanomaterials (a nanoparticle is a type of nanomaterial) for which experimental assessment was described in the current reference.

Please note that a tested chemical may be considered a nanomaterial if any one of the following conditions applies:

1. The authors of the publication describe it as a “nanomaterial”, or “nanoparticle”, or “nanotube”, or “nanocube”, or “nanowire” etc.
2. At least one of its reported size values (e.g. “size”, “length”, “diameter”) lies in the range 1 – 100 nm.
3. It is a “fullerene” or a “fullerene derivative” e.g. a “fullerenol”.

Please further note that if there is a contradiction between criterion [1] and [2] (e.g. a “nanoparticle” for which the only size value reported is 200 nm = 0.2 µm), this should be reported in the **“'Nanomaterial(s)' with No Dimensions Inside of 1 - 100 nm Range?”** column.

N.B. This issue is complicated by the fact that different size measurements may give different values. If, say, multiple “size” values are provided and only some of them are in the 1- 100 nm range, please ignore the larger or smaller size values when populating this column: **“'Nanomaterial(s)' with No Dimensions Inside of 1 - 100 nm Range?”**

Please also note the following points:

1. If nanoparticles with the same chemical composition but different (nominal) sizes, or other physicochemical/structural characteristics such as crystal phase are presented, these count as distinct materials. For example, if the reference reports studies on “TiO2 (10nm, rutile phase), TiO2 (40nm, rutile phase), TiO2 (10nm, anatase phase), TiO2 (40nm, anatase phase)”, you would enter “4” in the column “Metal oxides”.
2. “Silica” = “silicon dioxide” and “quartz” is a form of “silica”.
3. Some nanomaterials are a combination of different types of chemical components. For example, metal oxides might have ligands attached to their surface or be coated in some other material. If a given nanomaterial could be reported in more than one of these categories (“Metal oxides”, “Metals”, “Silica”, “Carbon nanotubes (single-walled and multiwalled)”, “Fullerenes and fullerene derivatives”,” Other”, “Unclear”), it should preferably be assigned on the basis of the chemical composition of the core – but this is not essential. *Never* count a nanomaterial more than once.

Columns under the “Availability of electron microscopy (e.g. TEM, SEM, STEM) or atomic force microscopy (AFM) images of the studied nanomaterials” section:

“Any electron microscopy or atomic force microscopy image?”

Are electron microscopy or atomic force microscopy images *of the nanomaterials studied in this reference* provided? If yes –select “Y” from the dropdown box. If not – select “N” from the dropdown box.

TEM = transmission electron microscopy

SEM = scanning electron microscopy

AFM = atomic force microscopy

STEM = scanning transmission electron microscopy

"Ex-Situ electron microscopy or atomic force microscopy images as required for descriptor calculations?"

Are "ex situ" electron microscopy or atomic force microscopy images *of the nanomaterials studied in this reference* provided? If yes –select "Y" from the dropdown box. If not – select "N" from the dropdown box.

N.B. "Ex situ" means outside of cells or biological tissue.

Columns under the "Detailed consideration of Important Physicochemical/Structural Measurements [adapted from Stefaniak et al. 2013 Table III: DOI: 10.3109/17435390.2012.739664]" section:

N.B. The guidance provided for populating these columns was largely derived from Stefaniak et al. 2013 [Stefaniak 2013]

"Chemical composition "

If any information is provided about the core chemical/elemental composition (or molecular structure, where relevant) and/or the *original* surface composition (i.e. not including adsorbed species) of the studied nanomaterials, please select "Y". (This includes details about impurities or purity, but this information is not required in order to select "Y" in this column.)

For example, in Zhou et al. 2008 [Zhou 2008], the nanomaterials are described as "functionalized multiwalled **carbon** nanotubes" (i.e. the chemical identity of the core of the nanomaterial is a multiwalled carbon nanotube) and the surface molecular composition is given in figure 2. Hence, this column should be set to "Y" for Zhou et al. 2008.

"Surface area"

If any surface area information is reported for any of the studied nanomaterials, enter "Y".

"Particle size / size distribution"

If any size information (e.g. "size", "diameter", "length", "radius") is reported for any of the studied nanomaterials, enter "Y".

If any of the following kinds of information are reported for any of the studied nanomaterials, please enter "Y".

"size range" for a single nanomaterial (e.g. nanomaterial X, 30 – 40 nm)

"polydispersity index" = "PDI" from dynamic light scattering (DLS)

"particle size = 13.1 ± 2 nm"

A graph indicating the distribution of particle sizes for a single nanomaterials (check the figures in the publication or the supporting information/supplementary information).

"Morphology/shape/form"

If any shape information is provided for any of the studied nanomaterials, enter "Y".

This would include, for example, any reference to "spherical nanoparticles", "nanowires", "nanocubes", "nanotubes", "cylindrical particles" etc.

This would also include any reference to "aspect ratio" or "physical form".

"Adsorption data"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"CORONA" (e.g. "CORONA composition", "protein CORONA")

"percentage adsorbed" = "adsorption percentage"

"adsorption constant"

"binding" data e.g. protein binding or small molecule binding

"Agglomeration/aggregation state"

If any of the following kinds of information are reported for any of the studied nanomaterials, please enter "Y".

"average agglomeration number"

"agglomeration strength"

"aggregation strength"

"Crystal structure"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"crystal structure"

"crystal phase"

"crystallite size"

"physico-chemical structure"

"rutile" or "anatase" nanomaterials

"Surface charge/zeta potential"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"charge"

"surface charge"

"zeta potential"

"Dispersability (dry/wet)"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"logP"

"logK[o/w]"

"hydrophobicity"

"hydrophilicity"

"lipophilicity"

"amphiphilic character"

"partition coefficient"

"dustiness"

"Surface reactivity/catalytic activity"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"surface tension"

"surface reactivity"

"reactive oxygen species generation" = "ROS"

"photocatalytic activity"

"redox potential"

"catalytic activity"

"Solubility/dissolution"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"solubility"
"dissolution"
"biodurability"
"dissolve"
"dissolving"

"Density"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"density"

"Porosity"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"porosity"
"pore size"

"Stability"

If the reference specifically talks about the "stability" of the nanomaterials – unless they are broken down because of "dissolution" (see above) – enter "Y" in this column.

Otherwise, if the reference talks about the nanomaterial being broken down due to thermal (i.e. breakdown due to heat), photochemical (i.e. breakdown due to chemical reactions – including light induced breakdown) or biodegradation (i.e. breakdown due to biological activity) mechanisms, enter "Y" in this column.

Column specific caveats: There was a typo in the original guidance notes used to populate the spreadsheet: "or biodegradation" was actually "and biodegradation".

"Surface_morphology/structure"

If any of the following kinds of information is reported for any of the studied nanomaterials, please enter "Y".

"surface morphology"
"surface structure"
"surface roughness"

Columns under the "Type of cytotoxicity data" section:

These columns only need to be populated if the corresponding "Cytotoxicity? [Y/N]" column entry is "Y".

[N.B. The following is based on Lewinski et al. [Lewinski 2008], Domey et al. 2014 [Domey 2014] and Kroll et al. 2009 [Kroll 2009], unless otherwise indicated.]

"Lethal cytotoxicity data?"

If the answer to ANY of the following questions is "Yes", enter "Y" in this column.

(1) When describing the assay, does the reference talk about measuring "cell viability", or the "viability of cells", or "cell death", or "apoptosis", or "necrosis" or "membrane damage" or (specifically for red blood cells) "haemolysis" (= "hemolysis")[1]?

(2) When describing the assay, does the reference talk about measuring "metabolic activity" or "mitochondrial activity"?

For example, in Zhou et al. 2008 [Zhou 2008], the following statement is provided:

> "To avoid the artifactual interference from MTT assay, **we used the WST-1 assay**(50) to evaluate the acute cytotoxicity of the f-MWNT library in macrophages. The assay measures the **viability of cells** by determining the activity of the mitochondrial dehydrogenases"

[1] http://bioportal.bioontology.org/ontologies/OAE?p=classes&conceptid=http%3A%2F%2Fpurl.obolibrary.org%2Fobo%2FOAE_0002506

In Table 1 of this article, "cell viability" data is also given for the "MWNTs" (i.e the carbon nanotubes as defined in the introduction).

Hence, the correct entry in this column for Zhou et al. 2008 [Zhou 2008] is "Y".

Columns under the "Assays used: cytotoxicity assays (c.f. Kroll et al. 2009 [DOI: 10.1016/j.ejpb.2008.08.009], Lewinski et al. 2008 [DOI: 10.1002/smll.200700595], Domey et al. 2014 [DOI: 10.1007/11663_2013_8] , c.f. ENV/JM/MONO(2009)20/REV)" section:

[N.B. The following is based on Lewinski et al. [Lewinski 2008], Domey et al. 2014 [Domey 2014] and Kroll et al. 2009 [Kroll 2009], unless otherwise indicated.]

These columns only need to be populated if the corresponding "Cytotoxicity? [Y/N]" column entry is "Y".

For these columns, simply consider the cytotoxicity assays used to generate cytotoxicity data for the nanomaterials in the paper. You do not need to populate all of these columns – only the columns corresponding to the names of the cytotoxicity assays used in the publication.

If the name seems to correspond to one of the predefined columns, select "Y" for that column.

If not, enter the name (as reported in the paper) in the "Other cytotoxicity assays" column. N.B. If multiple assay names need to be reported in this column, please separate the names using a semi-colon (";") e.g. "assay name [1]; assay name [2]".

Columns under the "Assays used: Genotoxicity assays (c.f. Doak et al. 2012 [DOI: 10.1016/j.mrgentox.2011.09.013], Golbamaki et al. 2015 [DOI: 10.1039/C4NR06670G])" section:

N.B. See Golbamaki et al. [Golbamaki 2015] and Doak et al. [Doak 2012] for further information.

These columns only need to be populated if the corresponding "Genotoxicity? [Y/N]" column entry is "Y".

For these columns, simply consider the genotoxicity assays used to generate cytotoxicity data for the nanomaterials in the paper. You do not need to populate all of these columns – only the columns corresponding to the names of the genotoxicity assays used in the publication.

If the name seems to correspond to one of the predefined columns, select "Y" for that column.

If not, enter the name (as reported in the paper) in the "Other genotoxicity assays" column. N.B. If multiple assay names need to be reported in this column, please separate the names using a semi-colon (";") e.g. "assay name [1]; assay name [2]".

Columns under the "Type of aquatic toxicity data" section:

These columns only need to be populated if the corresponding "Aquatic toxicity? [Y/N]" column entry is "Y".

"Embryonic zebrafish mortality"

Are embryonic zebrafish mortality (= death of zebrafish embryos) data presented? If so, select "Y" for that column.

N.B. Further information about zebrafish toxicity data can be found in the following references [Harper 2008 ,Truong 2011 ,Liu 2013].

"Other aquatic toxicity data"

Please record any other kinds of aquatic toxicity data using a semicolon (";") delimited list as above e.g. "type of aquatic toxicity data [1]; type of aquatic toxicity data [2]".

Columns under the "Consideration of potential artefacts and causes of misinterpretation (c.f. Petersen et al. 2014 [DOI: 10.1021/es4052999])" section:

These columns were informed by Petersen et al. [Petersen 2014]

"Is any information provided regarding impurities or purity (not including endotoxin contamination)?"

Enter "Y" in this column if the paper suggests experiments were carried out to determine the level of impurities in the nanomaterials which were tested in the article or any information is otherwise provided regarding the impurities contained within the nanomaterials which were tested in the article. *Please note, "Y" should be entered in this column even if the reference suggests "pure" nanomaterials or that experiments to measure impurities were carried out and none were detected.*

"Is any information provided regarding endotoxin contamination?"

Enter "Y" in this column if the paper suggests experiments were carried out to determine the level of "endotoxin" in the nanomaterials which were tested in the article or any information is otherwise provided regarding the level of "endotoxin" contained within the nanomaterials which were tested in the article. *Please note, "Y" should be entered in this column even if the reference suggests that experiments to measure levels of "endotoxin" were carried out and no "endotoxin" contamination was detected.*

Column specific caveats: In hindsight, the instructions provided for populating this column should also have referred to "lipopolysaccharide" or " LPS", which are effectively synonyms for "endotoxin" [Petersen 2014].

"Was potential assay interference accounted for?"

Enter "Y" in this column if the paper suggests tests were carried out to determine whether or not the tested nanomaterials "interfered" with the toxicity assays used to generate toxicity data for the nanomaterials which were tested in the article. Possible kinds of "interference" which they may have looked for include the following:

- interactions between the tested nanomaterials and the assay reagents (e.g. if the nanomaterial oxidised the test reagent)
- the nanomaterial might produce a signal which is similar to the assay measurand (i.e. the entity which is measured in an assay experiment) e.g. it might absorb/emit light at the measured wavelength, fluoresce, generate reactive oxygen species *in the absence of cells*[Kroll 2009], or otherwise produce a signal which cannot be distinguished from the assay measurand
- the nanomaterial might cause cells to agglomerate (this should not be confused with agglomeration of the nanomaterials themselves)

Please note, "Y" should be entered in this column even if the reference suggests that experiments to measure "interference" were carried out and no "interference" was detected.

Also, enter "Y" in this column if they suggest that assay "interference" was avoided via their experimental design e.g. via using a "control" incorporating the tested nanomaterial in the absence of biological cells.

Column specific caveats: Other than those references linked to external NanoPUZZLES work (see columns under "Relation to other NanoPUZZLES work section"), for which this was checked, revisit any references for which "Was potential assay interference accounted for?" = "Y" and if the only basis for saying this was that cell agglomeration was observed, change this to "N" if the corresponding assays were not based on cell counting [Petersen 2014].

"Was potential nutrient depletion accounted for?"

Enter "Y" in this column if the paper suggests tests were carried out into whether or not the tested nanomaterials caused "nutrient depletion". *Please note, "Y" should be entered in this column even if the reference suggests that experiments to measure "nutrient depletion" were carried out and no "nutrient depletion" was detected.*

"Was potential post-exposure period toxicity accounted for?"

Enter "Y" in this column if the paper suggests tests were carried out into whether or not the tested nanomaterials caused toxicity "after the exposure period" i.e. during the assay test protocol period. *Please note, "Y" should be entered in this column even if the reference suggests that experiments to measure "Post-Exposure Period Toxicity" were carried out and no "Post-Exposure Period Toxicity" was detected.*

Column specific caveats: In contrast to the guidance provided when populating the spreadsheet, Petersen et al. [Petersen 2014] actually considers "post-exposure period" toxicity a form of assay interference.

Columns under the "Other data quality considerations" section:

Were the data generated according to Good Laboratory Practice (GLP)?

Did the publication explicitly state that they generated the data according to GLP [OECD 1998]?

Columns under the "Relation to other NanoPUZZLES work" section:

These document the "priority references" which were used to develop the final nano-QSAR models and/or ISA-TAB-Nano datasets within the context of the NanoPUZZLES project.

1. If a model had been published at the time of writing, the corresponding literature reference is provided.
2. The NanoPUZZLES ISA-TAB-Nano [Thomas 2013,https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano,Marchese Robinson 2015] datasets should be available from https://figshare.com/ : search for "NanoPUZZLES project".

Columns under the "NanoPUZZLES evaluation details"

These refer to the "quality check" exercise referred to under the "Important caveats" section near the start of this README file.

References

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