Human health endpoints

Webinar: updated REACH Guidance for nanomaterials: what you need to know

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Human-health endpoints

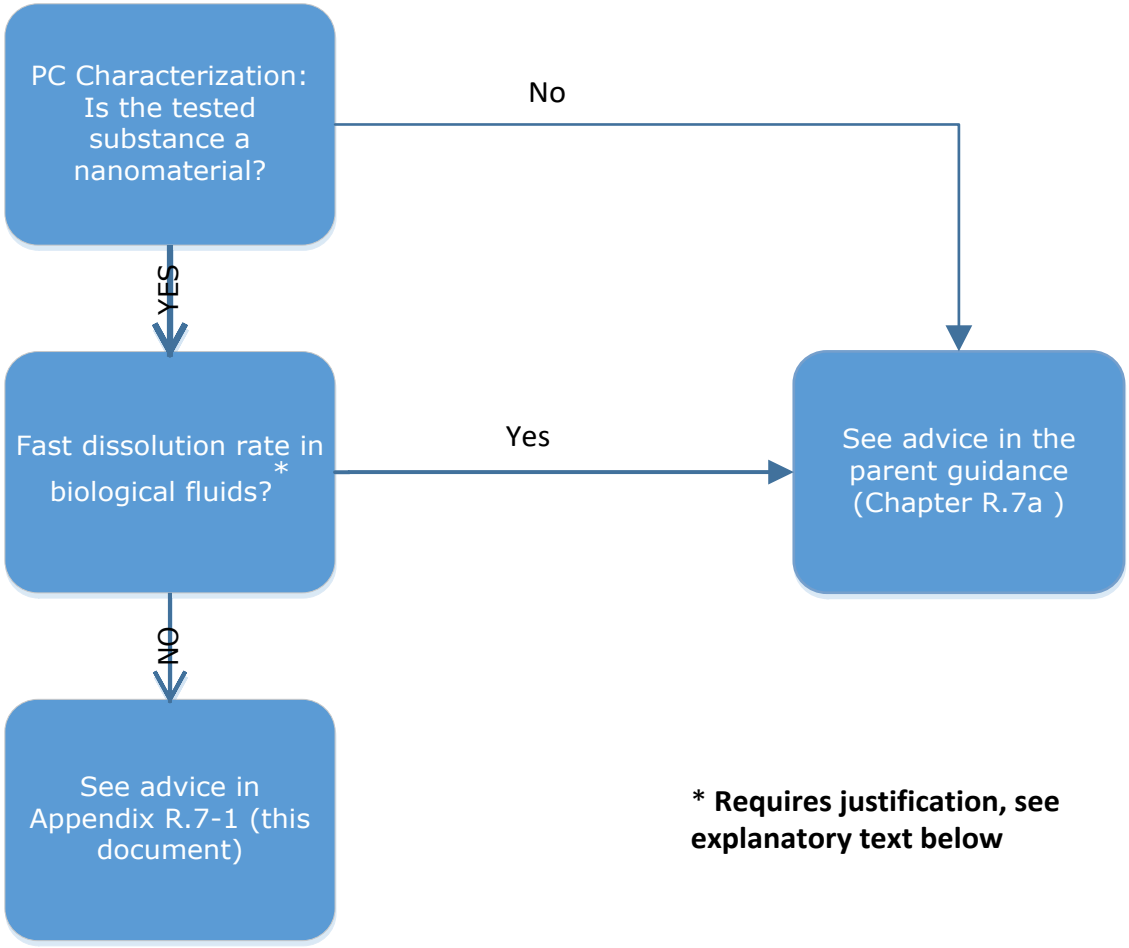
- Doesn’t exclude parent guidance
  - If no specific endpoint advice, parent guidance applies

- Covers sections of appendices to Chapters R7.a (human health endpoints) and R.7c (toxicokinetics) and includes new sections.

Decision tree: testing human health endpoints

- Testing strategy dependent on dissolution potential in relevant biological fluids and testing media
- No exact cut-off value proposed. Dissolution rate needs to be close to instantly dissolved
- Determination of dissolution rate gives insight on how a certain particle may interact with its biological environment

Decision tree for testing the human health endpoints



Advisory note: testing and sampling strategy and sample preparation (I)

Reporting

- Parameters needed in the endpoint study record:
  - Chemical composition as described in Guidance for identification and naming of substances under REACH and CLP
  - Size (as a minimum the D50, but particle size distribution is recommended)
  - Shape and aspect ratio
  - Surface chemistry

Advisory note: testing and sampling strategy and sample preparation (II)

Biological sampling

- Samples from *in vivo* toxicological studies collected according to OECD test guidelines (TG)
  - If indication that NM distributed in other tissues, not listed in test guidelines, collection of additional tissues is recommended
- Keep samples to allow later analysis (e.g. storage by chemical or physical tissue fixation for microscopy, freezing for burden analysis)

Advisory note: testing and sampling strategy and sample preparation (III)

Use of non-animal testing approaches

- Follow ongoing developments and validation efforts by OECD and EU reference laboratory for alternatives to animal testing (EURL-ECVAM).
- Monitor regulatory acceptance of new methods.
- Implementation of non-animal approaches requires prior consideration of all available information, including context-specific characterisation (critical for grouping, read-across and QSARs).
- Results from non-testing and in vitro methods can be useful in weight of evidence and give key information when planning an animal test.

Repeated dose toxicity I

- Testing to be performed via inhalation, unless convincing information (e.g. uses, dissolution rate, etc.) that justifies another route.
- Any modification of protocols described in OECD inhalation TG 412 and TG 413 should be justified.

Repeated dose toxicity II

- Dose range finding studies or repeated dose studies with poorly soluble particles (PSP):
  - Recommended to collect additional toxicokinetic data.
  - Recommendations for NM applicable to Chapter R7c Endpoint specific guidance on toxicokinetics.
- Data on lung burden and clearance may be useful arguments in the context of read-across.

Repeated dose toxicity III

- PSP: recommended to collect samples at several time points and/or in different organs.
  - Use of extra animals for the additional analyses generally not recommended but important to balance between performing additional analyses and following an indication of toxicity
- Bronchoalveolar lavage (BAL) analysis recommended
- Use more than one different dose-describing metric and include mass metric.

Advisory note: consideration of lung burden within inhalation toxicity assessment (I)

- Moved under section “repeated dose toxicity”
- Scientific background redrafted and shortened
- Includes reference to maximum tolerated dose for new studies and dosimetry considerations
- Clarifies when advice is relevant for interpretation of available information

Advisory note: consideration of lung burden within inhalation toxicity assessment (II)

- While mass is always required, advised to use more than one different dose-describing metrics
- Choice for selected method should be justified
- Includes overview of recommendations for lung burden

Mutagenicity I

- Ames test (TG 471) not recommended for NM
- Measures of cytotoxicity based on cell proliferation described in TGs are appropriate for determining top concentration for *in vitro* tests of NM.
- Extent of cellular uptake is critical to interpret test results

Mutagenicity II

- *in vitro* micronucleus (recommended modification): if cytochalasin B is used, it should be added post-treatment (or delayed co-treatment).
  - Ensures a period of exposure of the cell culture system to the NM in the absence of cytochalasin B

Mutagenicity III

- Before *in vivo* genotoxicity study, toxicokinetic investigations should determine if the NM reaches the target tissue if the target tissue is not the site of contact.
- Without toxicokinetic information the genotoxic effects should be investigated in the site of contact tissue.
- Selected route of administration should be justified
  - Exposure of target tissues should be addressed.

Toxicokinetics (R.7c) I

- Detecting and quantifying NM in biological tissue is analytically and technically challenging.
- Methods used and their limitations should be adequately documented.
- Solubility and dissolution rate in relevant biological fluids and testing media is essential to understand a particle's kinetic.
- PSP: translocation across biological barriers is essential information.

Toxicokinetics (R.7c) II

- Toxicokinetics data is valuable to justify use of toxicological data between different forms of a substance.
- Collect as much toxicokinetics data as possible from the experiments required under REACH
- Important to balance between performing additional analyses and following an indication of toxicity.

Main changes
General advisory notes

- Decision-tree based on solubility and dissolution - a starting point for nanomaterial (NM) testing
- Advisory note on testing and sampling strategy and sample preparation
- Reorganisation of general advice for non-testing methods under section 3.1.1. Not by each endpoint to avoid repetition

Main changes

Individual endpoints

- Update of advisory note on consideration of lung overload
- Update of section on repeated dose toxicity
- Update of section on mutagenicity
  - Relocation of information on bacterial assay interference
  - Inclusion of general recommendations for mutagenicity testing agreed at OECD/WPMN workshop (Nov 2013)

R.7c (toxicokinetics)

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