Strategies to increase the technical quality of new approach methodologies (NAMs) and nanoecotoxicology tests

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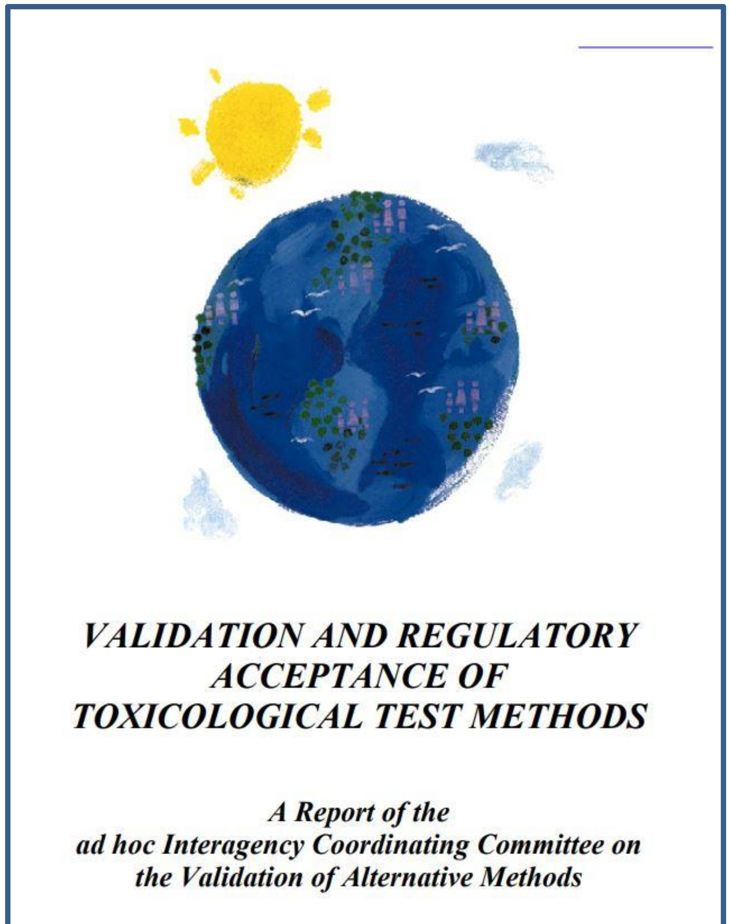
ICCVAM: Validation Workgroup

Updating the Interagency coordinating committee on the validation of alternative methods (ICCVAM) Report

ICCVAM Sponsor Agencies:
CPSC, FDA/CFSAN

Participating Agencies:
EPA/OPP, EPA/ORD, ATSDR, VA ORD, DOD, NIST, OSHA, NIEHS, NIH, FDA/CDER/CTP/OCS/ CDRH



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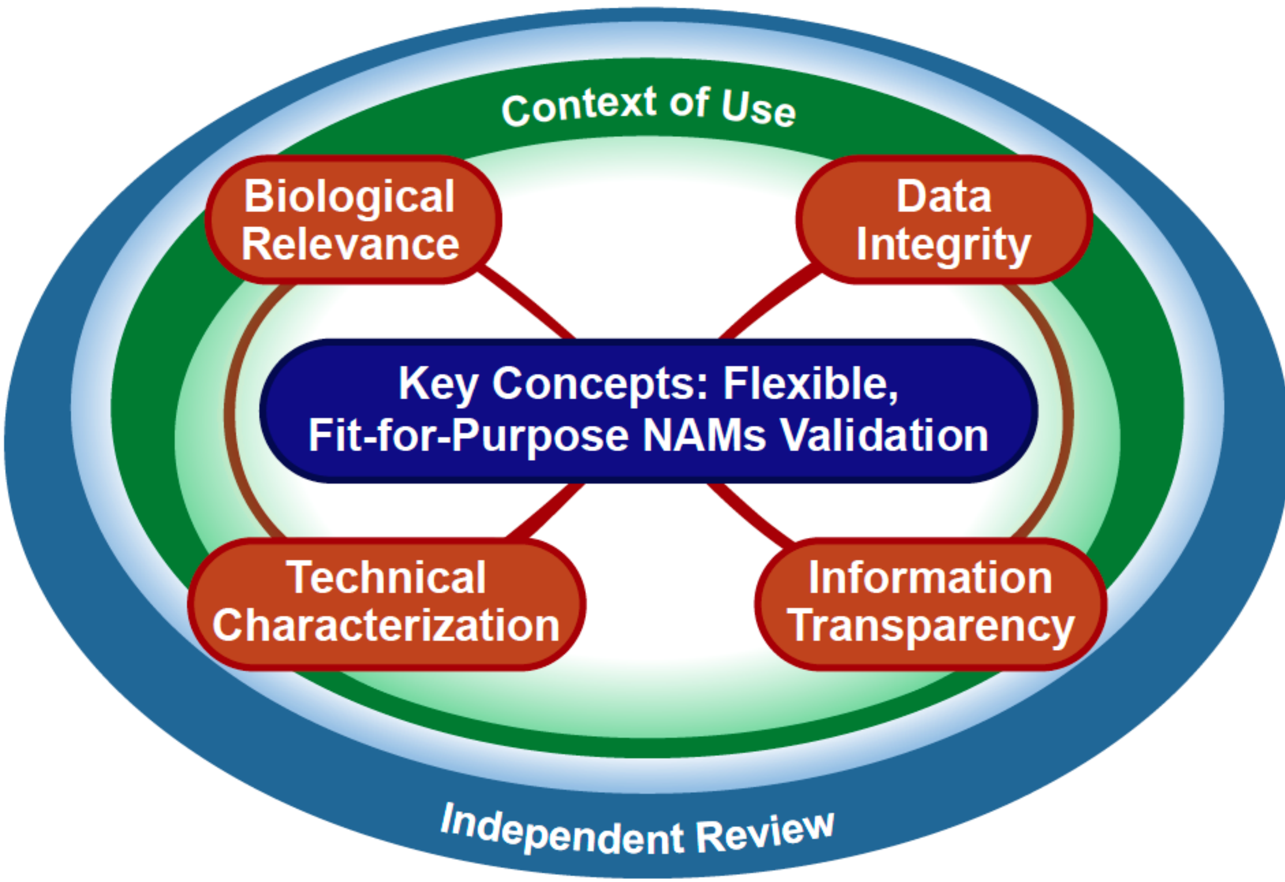
Key Concepts to Consider During Development and Implementation of Flexible, Fit-for-Purpose NAMs Validation Strategies



Final Draft ICCVAM Validation Report, Figure 1
(adapted from van der Zalm et al. 2022 Arch Tox)

https://ntp.niehs.nih.gov/go/ICCVAM-submit

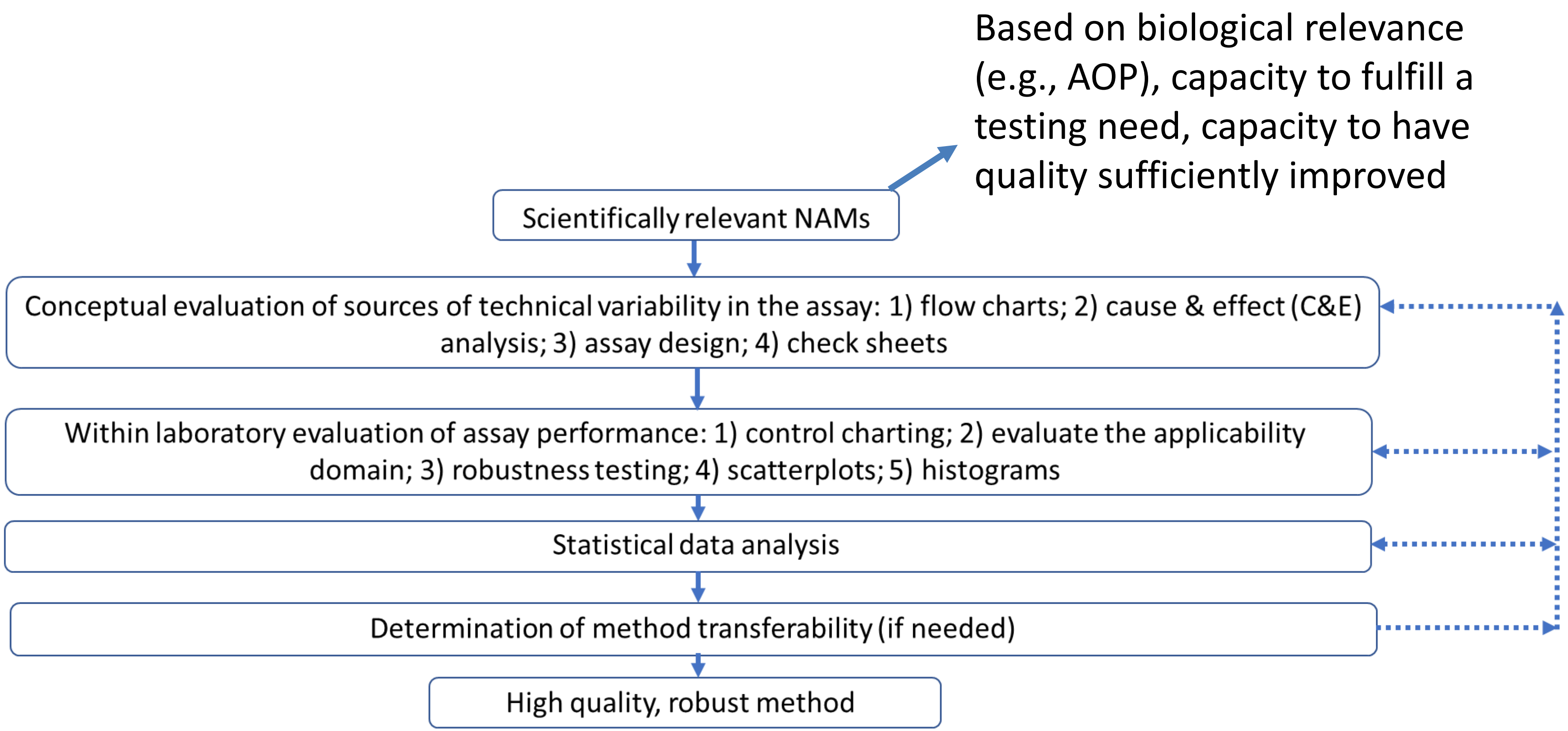
Technical Framework for High Quality NAMs

Collaborative project with CPSC, NICEATM, DOD, EMPA, NIST

- To yield reproducible NAM results across time and among laboratories, the framework includes a series of inter-related steps that describe
  - How to apply basic quality tools (cause-and-effect analysis, flow charts, control charts, etc) to improve confidence in NAMs
  - Approaches for adding statistical confidence to decisions based on NAM results
  - There may be tradeoffs though with more controls potentially leading to higher costs

Petersen, E. J., Elliott, J. T., Gordon, J., Kleinstreuer, N., Reinke, E, Roesslein, M., Toman, B. 2023, Altex, 40 (1), 174-186. https://doi.org/10.14573/altex.2205081

Technical Framework For High Quality NAMs



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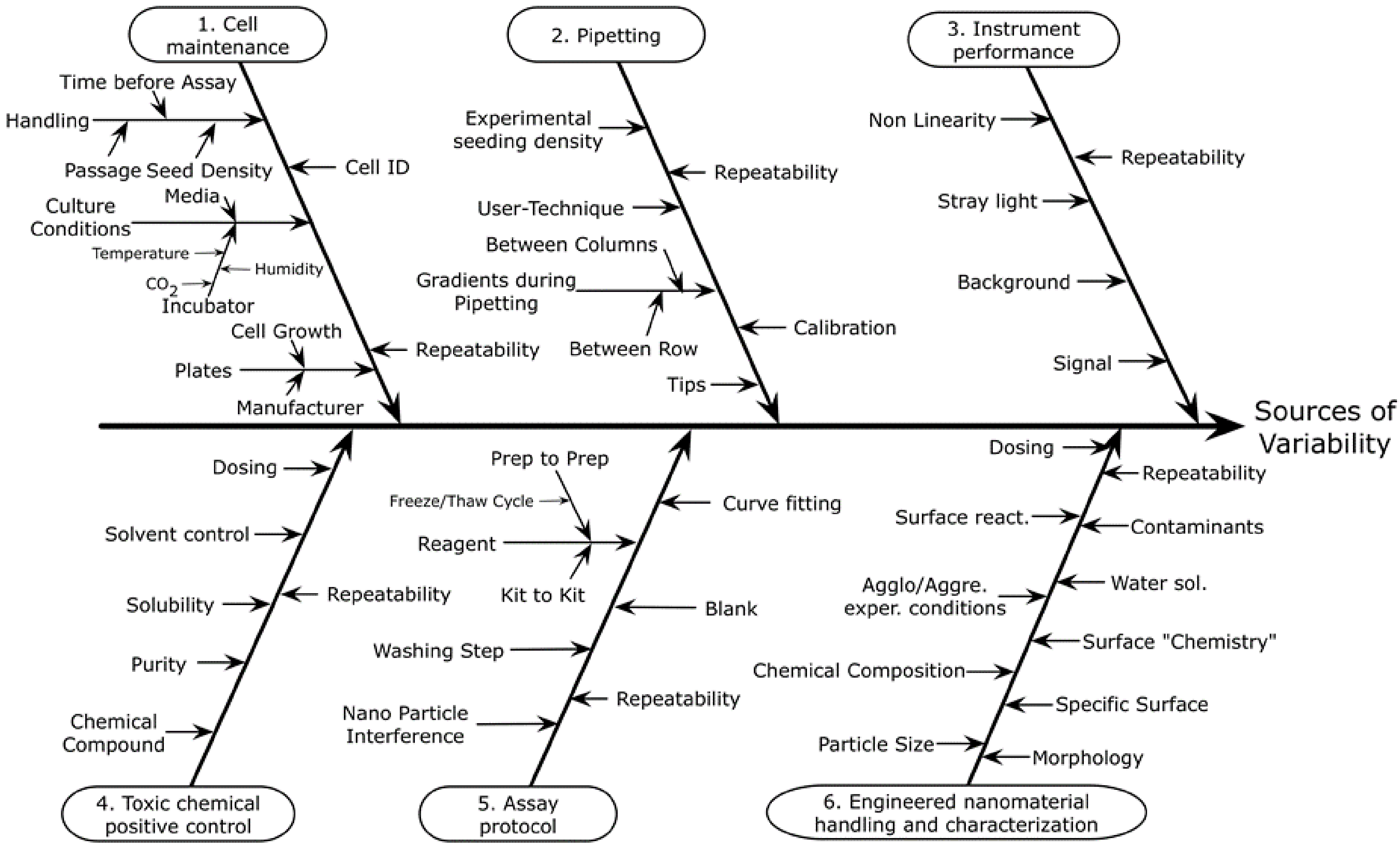
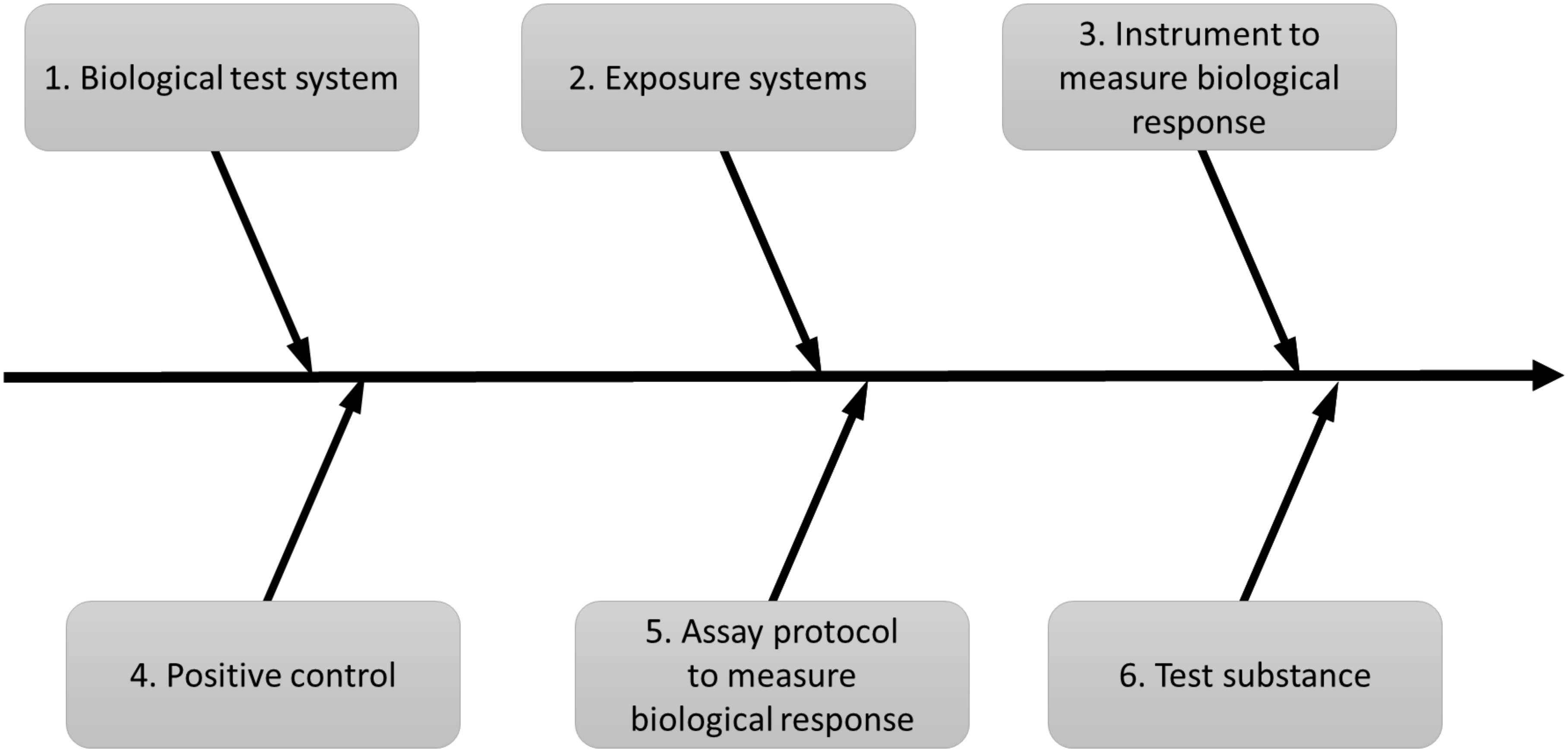
Example: cause-and-effect analysis



Robustness testing can evaluate each of the branches

Rosslein, M., Elliott, J. T., Salit, M., Petersen, E. J., Hirsch, C., Krug, H. F., Wick, P. The use of cause-and-effect analysis to design a high-quality nano-cytotoxicity assay, Chemical Research in Toxicology, **2015,** 28(1), 21-30.

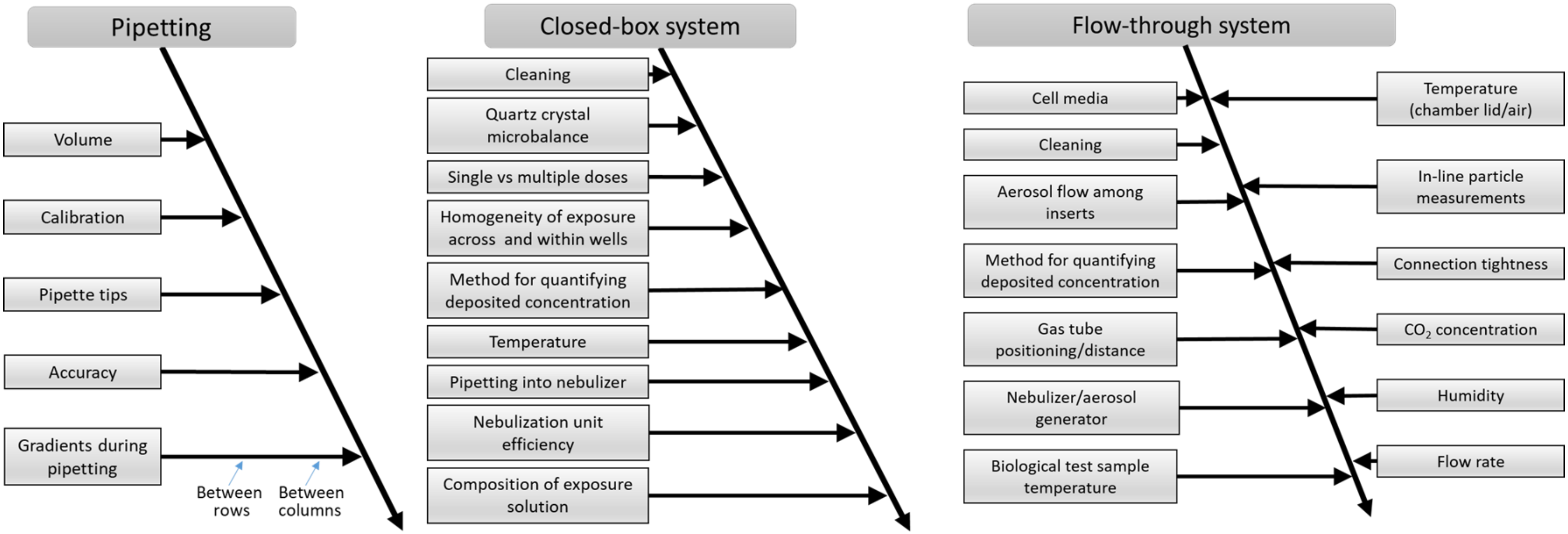
Use case: inhalation NAMs



- *Collaboration with NIST, BfR, CPSC, and PISC*
- *Different branches may be shared among NAMs*
- *Sources of variability and control measurements may also be shared among NAMs with similar branches*

Petersen, E. J., Sharma, M., Clippinger, A. J., Gordon, J., Katz, A., Laux, P., Leibrock, L. B., Luch, A., Matheson, J., Stucki, A. O., Tentschert, J., Bierkandt, F. S., Use of cause-and-effect analysis to optimize the reliability of *in vitro* inhalation toxicity measurements using an air-liquid interface. **2021**. Chemical Research in Toxicology, 34, 1370-1385.

Use case: inhalation NAMs



- *Different potential branches are described for the main factors that vary among methods*
- *Common control measurements and troubleshooting are also described*

Petersen, E. J., Sharma, M., Clippinger, A. J., Gordon, J., Katz, A., Laux, P., Leibrock, L. B., Luch, A., Matheson, J., Stucki, A. O., Tentschert, J., Bierkandt, F. S., Use of cause-and-effect analysis to optimize the reliability of *in vitro* inhalation toxicity measurements using an air-liquid interface. **2021**. Chemical Research in Toxicology, 34, 1370-1385.

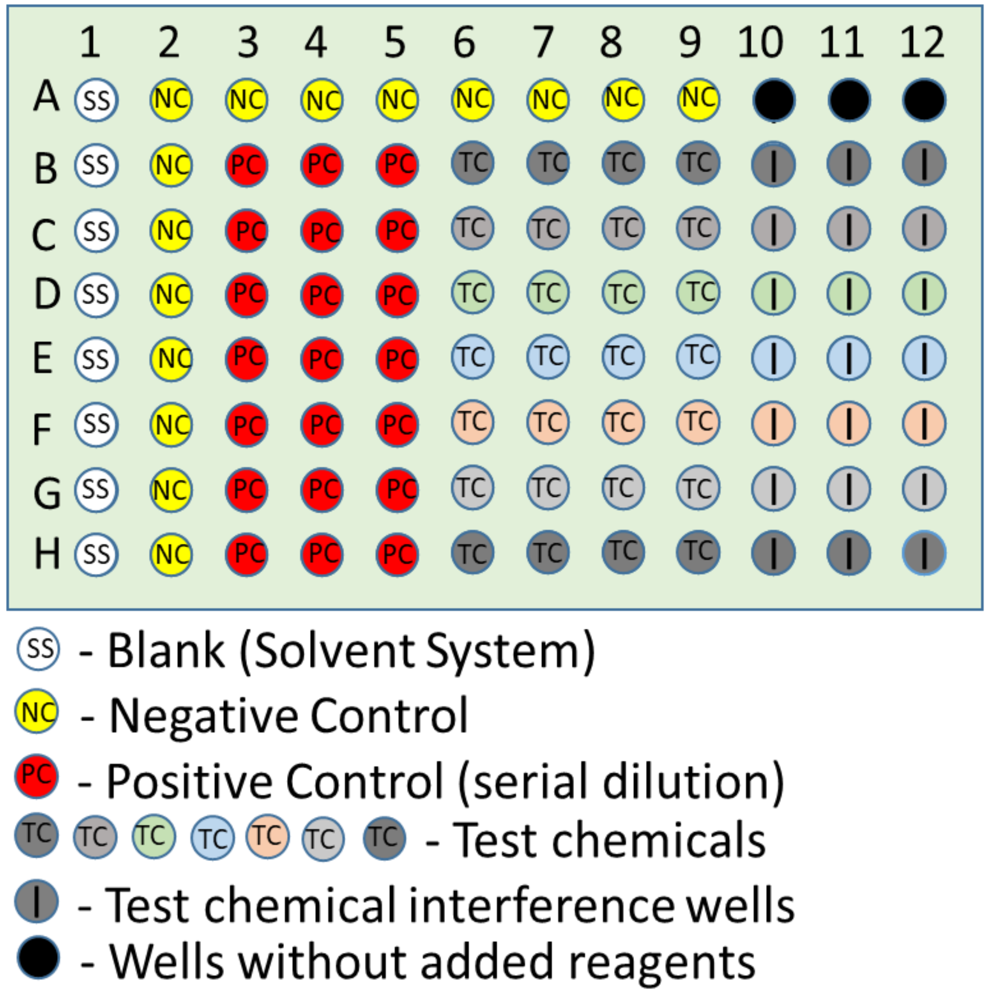
Example: flow chart

1. Add acetonitrile to solvent system and negative control wells
2. Add solvent system (50 % Phosphate buffer: 50 % acetonitrile) to wells
3. Add positive chemical control or test chemicals to relevant wells
4. Add the probe molecule (NBT or PDA) to relevant wells, and cover plate with plate seal
5. Place the plate in the plate reader, and take kinetic measurements for 50 min.

Control measurements should cover each step in the flow chart

Petersen, E.J., Uhl, R., Toman, B., Elliott, J.T., Strickand, J., Truax, J., Gordon J. Development of a 96-Well Electrophilic Allergen Screening Assay for Skin Sensitization Using a Measurement Science Approach. Toxics, **2022**, 10(5), article number 257.

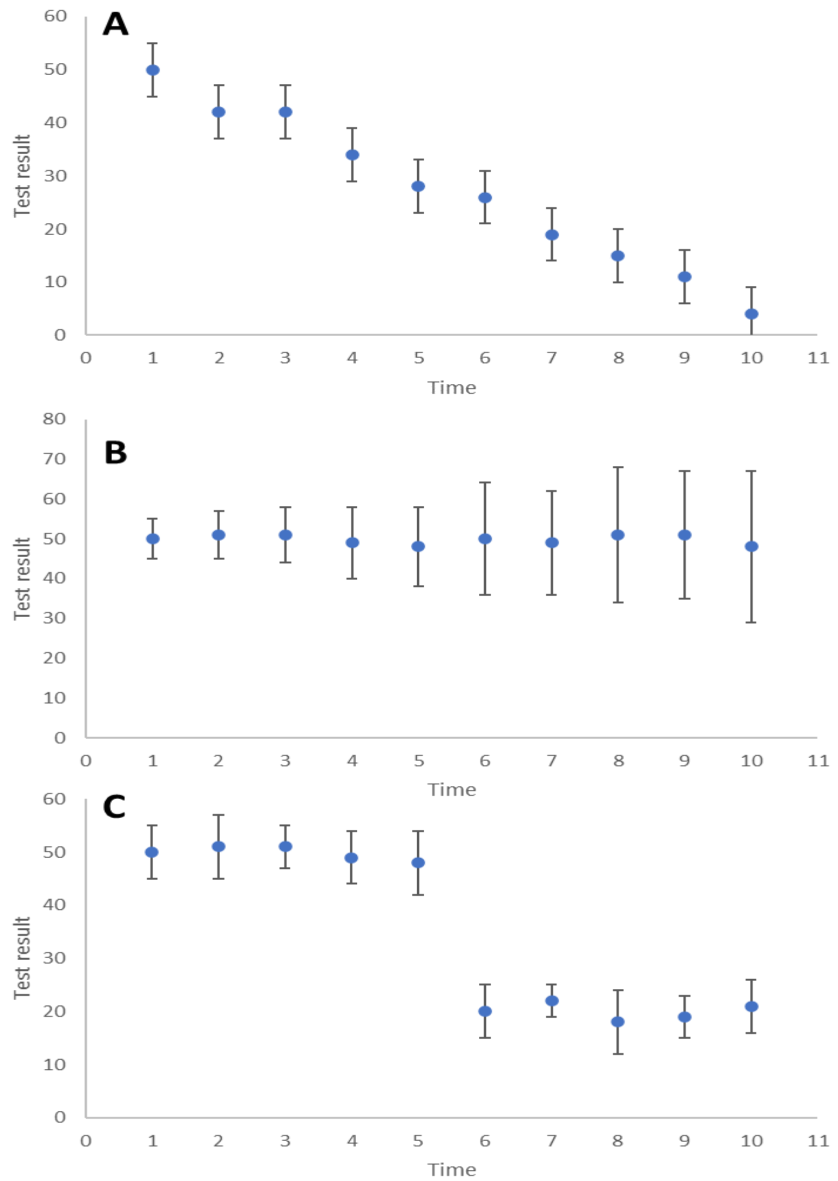
Example: plate design



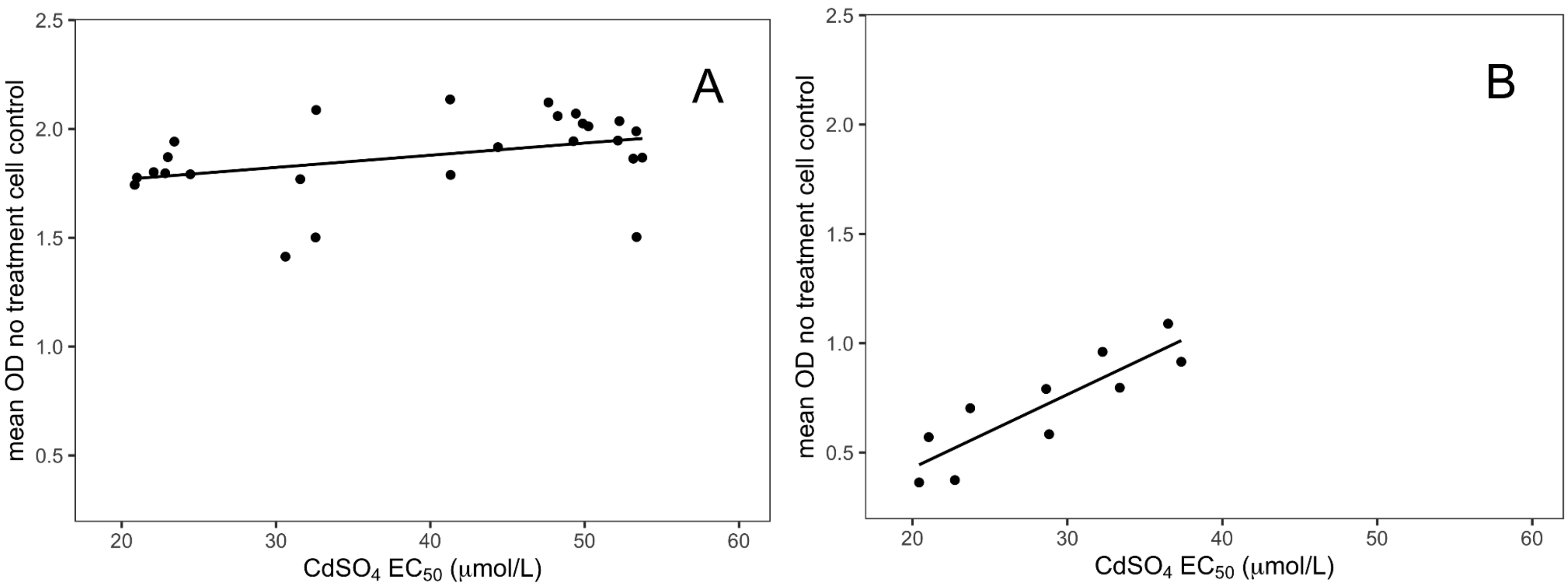
Control measurements evaluate key sources of variability each time the assay is performed

Petersen, E.J., Uhl, R., Toman, B., Elliott, J.T., Strickand, J., Truax, J., Gordon J. Development of a 96-Well Electrophilic Allergen Screening Assay for Skin Sensitization Using a Measurement Science Approach. Toxics, **2022**, 10(5), article number 257.

Example: control charting



Example: scatter plot



There is either a lack of an interaction between the EC_{50} values (part A) or an interaction (part B) depending upon the range of mean OD values which reflect the number of cells.

Ranges in specifications can be set to avoid interactions among variables

Elliott, J. T., Rosslein, M., Song, N. W., Toman, B., Kinsner-Ovaskainen, A., Maniratanachote, R., Salit, M. L., Petersen, E. J., Sequeira, F., Lee, J., Kim, S. J., Rossi, F., Hirsch, C., Krug, H. F., Suchaoin, W., Wick, P. Toward achieving harmonization in a nano-cytotoxicity assay measurement through an interlaboratory comparison study, **2017**, Altex, 34(2), 201-218.

Developing a guidance document for aquatic toxicity testing

Contributions from over twenty colleagues from eight countries

Guidance document 317

- OECD GD 317 published on-line July 2020: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)8&doclanguage=en

OECD
Organisation for Economic Co-operation and Development

ENV/JM/MONO(2020)8

Unclassified — English - Or. English

20 July 2020

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

GUIDANCE DOCUMENT ON AQUATIC AND SEDIMENT TOXICOLOGICAL TESTING OF NANOMATERIALS

Series on Testing and Assessment
No. 317

JT03464103

Petersen, E. J., Goss, G. G., von der Kammer, F., Kennedy, A. J. New guidance bring clarity to environmental hazard and behavior testing of nanomaterials. **2021**. Nature Nanotechnology, 16(5), 482-483.

Overview of Sections

1. Introduction
2. Scope
3. Background
4. Analytical and measurement techniques
5. Test dispersion preparation
6. Conduct of the test
7. Data analysis and reporting (Nanomaterial-specific)

Key topics covered in the guidance document

- Characterization of the as-produced test material and the test material in stock and test dispersions
- Robust monitoring of exposure concentration and consistency (e.g., if the concentration remains within 20 %) during the experiment to determine need for water exchanges, time-weighted averages, etc.
- Test dispersion preparation approaches for materials with different levels of stability in suspension
- A hierarchy of modifications to the test media (e.g., pH, ionic strength, addition of natural organic matter) for particles that are not sufficiently stable in suspension

Key topics covered in the guidance document

- Discussion of potentially relevant control experiments to avoid artifacts and, if needed, understand mechanism of toxicity
- Detailed suggestions for additional assays-specific modifications for a range of OECD test guidelines
- Methods for spiking sediments for sediment exposures
- Key issues related to data analysis and reporting including different dose metrics

Summary

- Quality tools enable more confidence in measurement systems
- Technical framework focused on quality in NAMs
- Plate design allows direct encoding of control measurements for each test sample
- New OECD guidance document can help increase confidence in nanoecotoxicology measurements