**Breakout session discussion guidance**

The aim of the breakout sessions is to discuss and possibly conclude on topics and elements for a pragmatic guidance document on NAM-enhanced read across (RAX), including identifying and filling knowledge gaps. The discussion should map out conditions (circumstances/areas/problem formulations) where NAM-enhanced RAX is acceptable and argue why.

**GENERAL DISCUSSION TOPICS (all case studies)**

Please consider the below described circumstances/areas/problem formulations two general possible scenarios may exist:

**Scenario 1:** category approach- many to one prediction- i.e. many source compounds do set the basis with in vivo endpoint data of acceptable quality.

**Scenario 2:** analogue approach: one to one - one source compounds has in vivo endpoint data of acceptable quality.

**I. What are the generic requirements for a NAM assay/outcome to be acceptable in a RAX justification?**

Given that validation approaches for NAM approaches are currently being developed in OECD are there topics to consider specifically in relation to NAM-supported RAX, which are not already covered? Are there specific needs to make it possible for the regulator to readily check the reliability of the data in terms of for example;

1. Documentation/transparency of the NAM
2. Applicability domain, e.g. how discriminating is the NAM in terms of chemical structure or biological activity
3. Performance

**II. What are the requirements under the following RAX conditions?**

Below scenarios are to be discussed dependent on whether an AOP or Mode of Action is known or not.

Mode of action involves identification of specific effects between exposure and effect from the early effects, late responses and to the pathology. An example is for example decrease in thyroid hormones resulting in an increase in thyroid stimulating hormone levels resulting in thyroid follicular cell hypertrophy and hyperplasia.

AOP are biological pathways, which can be tipped by a stressor often with focus on the MIE. An example is inhibition of the sodium iodide symporter leading to impairment of learning and memory through several KE involving decreased circulating thyroid hormone levels.

1. **When an AOP is known for the shared apical effects & target organs**
2. Which testing scope is needed?
	1. Is testing according to AOPs sufficient? Or is testing in assay systems covering the shared toxicological effect patterns sufficient?
	2. Can recommendations be made on whether all KEs of an AOP need to be tested and identify cases where this would not be necessary?
	3. Does the testing scope change in case a negative trend is the read-across outcome?
	4. Concerning the primary effect: should test assay systems provide evidence that the read-across endpoint is the lead effect? Is this needed for scenario 1 as well as for scenario 2?
	5. Supporting evidence: what other effect data (other than the RAX effect) can strengthen the case? E.g. data from another guideline test.
3. Toxicokinetics: PBPK modelling informs about the plasma and organ concentration in humans. Under which conditions is IVIVE-PBPBK model acceptable? Is full PBPK modelling needed for RAX justification or are in vitro toxicokinetic parameters such as plasma protein binding, and intrinsic hepatic clearance sufficient?
4. Metabolism: under what circumstances would test assay systems be considered reliable if it is not known whether the parent molecule or a metabolite is causing the toxicity?
5. **When a Mode of Action or a specific shared apical effects & target organs is known.**

Same questions – i. to iii. – as above ad 1).

1. **When a Mode of Action is not known.**

This is in particular often the case for adverse toxicological changes like significant decrease in body weight (greater than 10%) for which as mode of action is unknown.

1. Which testing scope is needed?
	1. Is testing in assay systems covering the shared toxicological effect patterns sufficient?
	2. Does the testing scope change in case a negative trend is the read-across outcome?
	3. Concerning the primary effect: should test assay systems provide evidence that the read-across endpoint is the lead effect? Is this needed for scenario 1 as well as for scenario 2?
	4. Supporting evidence: what other effect data (e.g. data from other guideline test) can strengthen the case?
2. Toxicokinetics: Does PBPK modelling informs about potential target organs and by this define testing scope?
3. Metabolism: under what circumstances would test assay systems be considered reliable if it is not known whether the parent molecule or a metabolite is causing the toxicity?
4. Can omics data to guide hypothesis building and testing?
5. **When the source chemical(s) hardly show(s) toxic effects, i.e. only at very high doses for example up to the testing limit of 1000 mg/kg bw/day.**
6. Which testing scope is needed?
	1. Is testing assay systems covering the minimal toxicological effect patterns sufficient?
	2. Should test assay systems provide evidence that other effects than observed for source chemical(s) do not occur? Is this needed for scenario 1 as well as for scenario 2?
	3. Supporting evidence: what other effect data (e.g. data from other guideline test) can strengthen the case?
7. Toxicokinetics: is a PBPK model needed for RAX justification or are in vitro toxicokinetic parameters such as plasma protein binding, and intrinsic hepatic clearance sufficient?
8. Metabolism: would test system metabolic competence be relevant for concluding on low toxicity potential?
9. Can omics data to guide hypothesis building and testing?
10. **When in vivo data is sparse , e.g. when there is uncertainty for source compound(s) that all required effects are covered by in vivo data.**
11. How can NAMs help establish the RAX case?
12. In the context of RAX what does sufficient evidence of no effect equate to in pragmatic testing terms.
13. What other evidence would give reassurance of an absence of other ‘unexpected’ effects? For example, when and when not can screening of potential mechanism of toxicity by in vitro transcriptomics data provide reassurance?
14. **Use of additional safety factors.**
15. Could additional safety factors or other estimates that quantify the predicted uncertainty be applied, such that sufficient confidence could still be given for a specific risk assessment scenario?
16. To which components could or should this be appropriately applied to?

**III. What data integration and analysis are required?**

Clearly, building hazard assessments on a battery of individual test systems also requires new tools to integrate the data that is obtained this way, and to qualify and quantify the associated uncertainty that is intrinsic to every test model per se. This also applies to the incorporation of toxico-dynamic as well as -kinetic concepts and models that represents an integrated part of the hazard characterization approach.

1. How should data from different NAMs, i.e. in vitro test data, and (validated) TK calculations be integrated? Would a decision tool like Dempster Shafer, that quantifies uncertainty and trust, be of help here?

**CASE-STUDY SPECIFIC TOPICS**

**Do you support the way that NAMs have been applied in this RAX case study?**

If yes, why do you support (what are the factors that provide confidence)?

If no, why do you not support (what are the factors that lead to uncertainty)?

Additional case study specific topics may be brought in and discussed.