**Templates for Mode of Action Analysis using the WHO/IPCS MoA Framework**

The following templates for mode of action analysis are based on the WHO/IPCS Mode of Action Framework and intended for use for both human health and/or environmental mode of action analysis.

Assessment of data sets including reliability should be performed prior to the use of the templates for mode of action analysis, following the available ECHA Guidance in this respect.

The templates can be modified by users where relevant depending on the application.

Before using the templates for mode of action analysis, the WHO/IPCS Guidance on Mode of Action framework and the cases studies included therein

should be consulted. The current document does not provide as such guidance but only short notes taken from the WHO/IPCS Guidance documents and

case studies.

**Guidance for the use of the templates is available at:**

* *New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis (pages 1–18); M. E. Meek, A. Boobis, I. Cote, V. Dellarco, G. Fotakis, S. Munn, J. Seed and C. Vickers; Article first published online: 25 OCT 2013 Available at:* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)
* *WHO/IPCS Guidance on mode of action framework (for cancer and non-cancer risk assessment):*[**http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/**](http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/)

*Part I and Part II Harmonisation Series Guidance available at:*[**http://www.who.int/ipcs/methods/harmonization/areas/cancer\_mode.pdf?ua=1**](http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1)

1. **Problem formulation**

*Short description of the nature of the analysis, the scope and potential application. Initial problem formulation can be modified once the mode of action analysis has been completed if needed.*

*Examples of problem formulation are available at:* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)

*Mode of action is chemical specific and endpoint specific. A chemical may be acting by multiple modes of action; in such cases the mode of action analysis may need to be performed for each mode of action separately if the critical effects are different.*

1. **Hypothesised Mode of action Statement**

*Short description of the mode of action that will be analysed using the templates further below.*

Examples are available within the case studies of *Part I and Part II Harmonisation Series Guidance available at:*[**http://www.who.int/ipcs/methods/harmonization/areas/cancer\_mode.pdf?ua=1**](http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1)

1. **Summary of data for use in Mode of Action Analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Route/Dose** | **Incidence** | **Comments** | **References** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

*Summarise the information available that will be used in the mode of action analysis. Within the comments column you can also insert aspects on data reliability; however this should also be reflected in table 5d, within the explanatory text on confidence/uncertainty for each key event and the corresponding supporting data.*

Example is available within *Part I case study (4-aminobiphenyl and DNA reactivity) of Harmonisation Series Guidance available at:*[**http://www.who.int/ipcs/methods/harmonization/areas/cancer\_mode.pdf?ua=1**](http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1)

1. **Listing of key events identified for a specific Mode of Action**

|  |  |
| --- | --- |
| ***Key Event 1*** |  |
| ***Key Event 2*** |  |
| ***Key Event 3*** |  |
| ***Key Event 4*** |  |

*Following collection of all available information, analyse and map the available data and information per key event identified for a specific hypothesised mode of action.*

*According to WHO/IPCS MoA framework, key events are measurable events that are critical to the induction of the (adverse) effect*

*List key events in order from earliest to latest key event; Last key event should be the (adverse) effect. Provide a short name for each key event that will be used in the tables further below.*

Example is available within *Part I case study (4-aminobiphenyl and DNA reactivity) of Harmonisation Series Guidance available at:*[**http://www.who.int/ipcs/methods/harmonization/areas/cancer\_mode.pdf?ua=1**](http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1)

1. **Bradford Hill Considerations for Weight of Evidence Analysis of available data/information for Mode of Action Analysis in experimental species**

**5a. Dose Response Relationships and Temporal Association**

*In this section, concordance of dose-response relationships together with temporal association will be presented. Try to correlate the dependency of each key events with dose (increasing severity with increasing dose can be shown for each key event vertically) as well as the temporal relationships for each of the key events and the final (adverse) effect (i.e. key events occur in sequence earlier key events appearing before later key events. This can be shown by looking for each dose, horizontally across the table, the time at which each key event occurs). A key event cannot play a role in an (adverse) effect if it is manifested only after toxicity has occurred or if it occurs only at doses higher than those inducing toxicity.*

***Dose response relationship****: Are the key events observed at doses below or similar to those associated with the (adverse) effect?*

***Temporal association****: Are the key events observed in hypothesised order?*

**Species (name)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Dose (units)*** | ***Key Event 1 (time observed and severity)*** | ***Key Event 2(time observed and severity)*** | ***Key Event 3(time observed and severity)*** | ***Key Event 4(time observed and severity)*** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

*If needed, repeat table where information on dose-response relationships and temporal association is available for additional species/systems.*

*See example in figure 5* *within: New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis (pages 1–18); M. E. Meek, A. Boobis, I. Cote, V. Dellarco, G. Fotakis, S. Munn, J. Seed and C. Vickers; Article first published online: 25 OCT 2013 Available at:* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)

**5b. Consistency & Specificity – Biological Plausibility**

***Consistency & Specificity***

*(In this section the weight of evidence linking the key events with the toxicological response should be addressed; e.g. consideration of consistent observations in a number of studies with different experimental designs. Consistency is the repeatability of the key events in a MoA in different studies / species / strains etc.)*

*Is the sequence of events reversible if dosing is stopped or a key event prevented?*

*Is the incidence of the (adverse) effect consistent with the incidence of each key event? (e.g. at similar doses the incidence/severity of later key events would not be expected to be greater than that of earlier key events).*

*Is the pattern of effects across species/strains/systems consistent with the hypothesised mode of action?*

***Biological Plausibility***

*According to the WHO/IPCS MoA Framework the following aspects should be considered in this section:*

*Does the hypothesised mode of action make sense based on broader knowledge (e.g. biology, established mode of action)?*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***Key Event 1*** | ***Key Event 2*** | ***Key Event 3*** | ***Key Event 4*** |
| ***Consistency & Specificity*** |  |  |  |  |
| ***Biological Plausibility*** |  |  |  |  |

*See example for the parts of consistency and biological plausibility in figure 6* *within: New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis (pages 1–18); M. E. Meek, A. Boobis, I. Cote, V. Dellarco, G. Fotakis, S. Munn, J. Seed and C. Vickers; Article first published online: 25 OCT 2013 Available at:* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)

*And within case studies of Part I and Part II Harmonisation Series Guidance available at:*

[**http://www.who.int/ipcs/methods/harmonization/areas/cancer\_mode.pdf?ua=1**](http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1)

**5c. Qualitative and Quantitative human concordance**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | ***Qualitative Concordance*** | |  |  |  |
| ***Key Event (name)*** | ***(Evidence in Experimental Species)*** | ***(Evidence in Humans)*** | ***Quantitative Species Concordance (experimental species and humans)*** | ***Quantitative Dose Response*** | ***Confidence/ Uncertainty*** |
| ***Key Event 1*** |  |  |  |  |  |
| ***Key Event 2*** |  |  |  |  |  |
| ***Key Event 3*** |  |  |  |  |  |
| ***Key Event 4*** |  |  |  |  |  |

*See example in Table 2* *within: New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis (pages 1–18); M. E. Meek, A. Boobis, I. Cote, V. Dellarco, G. Fotakis, S. Munn, J. Seed and C. Vickers; Article first published online: 25 OCT 2013 Available at:* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)

*Assessment of concordance for each key event between and within species taking into account both chemical-specific and more generic information such as anatomical, physiological and biochemical variations. An example is available at* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)**.**

*In this section assessment of potential interspecies and interindividual differences in kinetics and dynamics are considered for each key event.*

*The conclusions for concordance analysis between and within species can be recorded using words like: plausible, likely, unlikely, evidence based etc.*

*In addition, for each key event confidence on the concordance analysis is filled in; this is usually done in a qualitative way (high, medium, low) and should be in line with the plausibility of each key event taking into account inconsistences and data/information availability (including quality of the data available). Uncertainty/confidence, if needed can be further elaborated under section 7 in free text format.*

*In this section the following needs to be considered:*

* *Is the weight of evidence sufficient to establish the hypothesised mode of action in experimental species?*
* *Can human relevance of the specific mode of action be reasonably excluded on the basis of fundamental qualitative differences in key events between experimental species and humans?*
* *Can human relevance of the specific mode of action be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?*

*For the latter, depending on problem formulation, if the scope of the MoA analysis is for use in risk assessment of a chemical, and if the experimental MoA is likely to operate in humans, consideration of use chemical specific adjustment factors instead of default assessment factors should be considered (additional guidance available at:*

*WHO/IPCS Chemical Specific Adjustment Factors:* [*http://www.who.int/ipcs/methods/harmonization/areas/uncertainty/en/*](http://www.who.int/ipcs/methods/harmonization/areas/uncertainty/en/)

*and within ECHA Guidance at: Part B, Chapter R.8* [*http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment*](http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) *)*

*In addition, the scope of the mode of action analysis may also need to include consideration of PBPK modelling approaches for example in case of species/systems extrapolations. Guidance on the use of PBPK modelling in risk assessment is available via WHO/IPCS at* [*http://www.who.int/ipcs/methods/harmonization/areas/pbpk/en/*](http://www.who.int/ipcs/methods/harmonization/areas/pbpk/en/)

*and within ECHA Guidance at: Part B, Chapter R.8* [*http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment*](http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment) *.*

1. **Other potential Modes of Action**

*Discuss if alternative modes of action for the specific (adverse) effect under consideration are likely. If there is supporting evidence a separate MoA analysis needs to be performed. Alternative modes of action should be distinguished from additional components of a single MoA that likely contribute to the observed effects; these should be addressed in the analysis of the principal MoA.*

**Comparative Weight of Evidence Table**

|  |  |  |
| --- | --- | --- |
| ***Bradford Hill Considerations*** | ***Supporting Evidence*** | ***Potentially Inconsistent Evidence*** |
| ***Dose Response & Temporal Concordance*** |  |  |
| ***Consistency & Specificity*** |  |  |
| ***Biological Plausibility*** |  |  |

|  |  |  |
| --- | --- | --- |
| ***Bradford Hill Considerations*** | ***Supporting Evidence*** | ***Potentially Inconsistent Evidence*** |
| ***Dose Response & Temporal Concordance*** |  |  |
| ***Consistency & Specificity*** |  |  |
| ***Biological Plausibility*** |  |  |

*This table can be used when two (or more) hypothesised modes of action need to be compared to enable to conclude on the most likely one.*

*An example is available in figure 6 within the WHO/IPCS publication at:* [**http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc**](http://onlinelibrary.wiley.com/doi/10.1002/jat.v34.1/issuetoc)

1. **Uncertainties/Inconsistencies and Identification of Data Gaps**

*Describe any remaining uncertainties and identify potential data gaps taking into account the conclusions drawn in section 6. In case for certain key events the available evidence is weak in experimental species and/or the human concordance is not strong enough for the purpose of the specific problem formulation, additional information and/or data may be needed to increase the confidence.*

Examples are available in *Part I and Part II Harmonisation Series Guidance available at:*

[**http://www.who.int/ipcs/methods/harmonization/areas/cancer\_mode.pdf?ua=1**](http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf?ua=1)

*Although uncertainties/inconsistencies in most cases are described in a qualitative way (using words like high, medium, low), if the data available allows quantification of uncertainty may be possible especially if this is needed from the problem formulation step. Further guidance on uncertainty analysis is available at:* [*http://www.who.int/ipcs/methods/harmonization/areas/hazard\_assessment/en/*](http://www.who.int/ipcs/methods/harmonization/areas/hazard_assessment/en/)

1. **Conclusions in relation to problem formulation**