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Introduction to the Mode of Action Analysis and Conclusion on the ED Criteria

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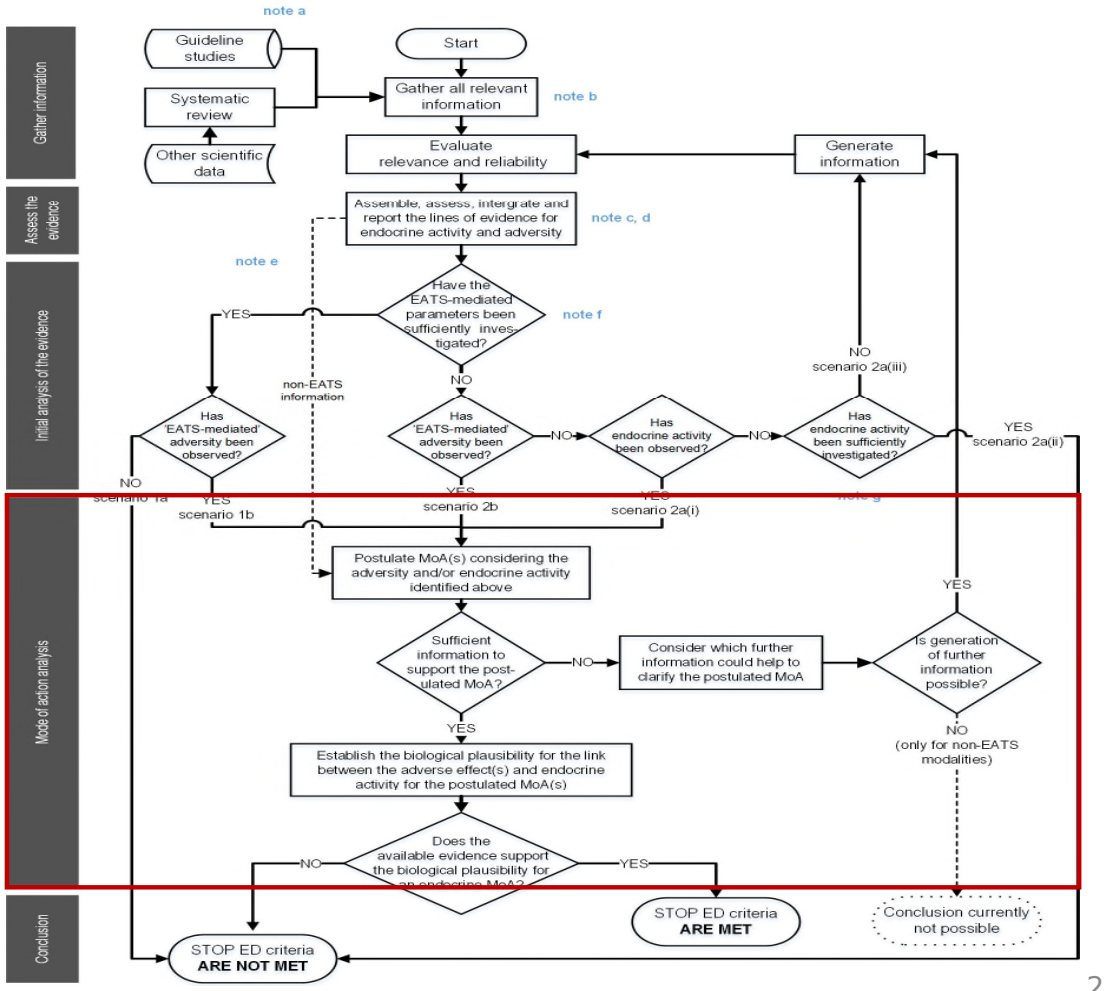
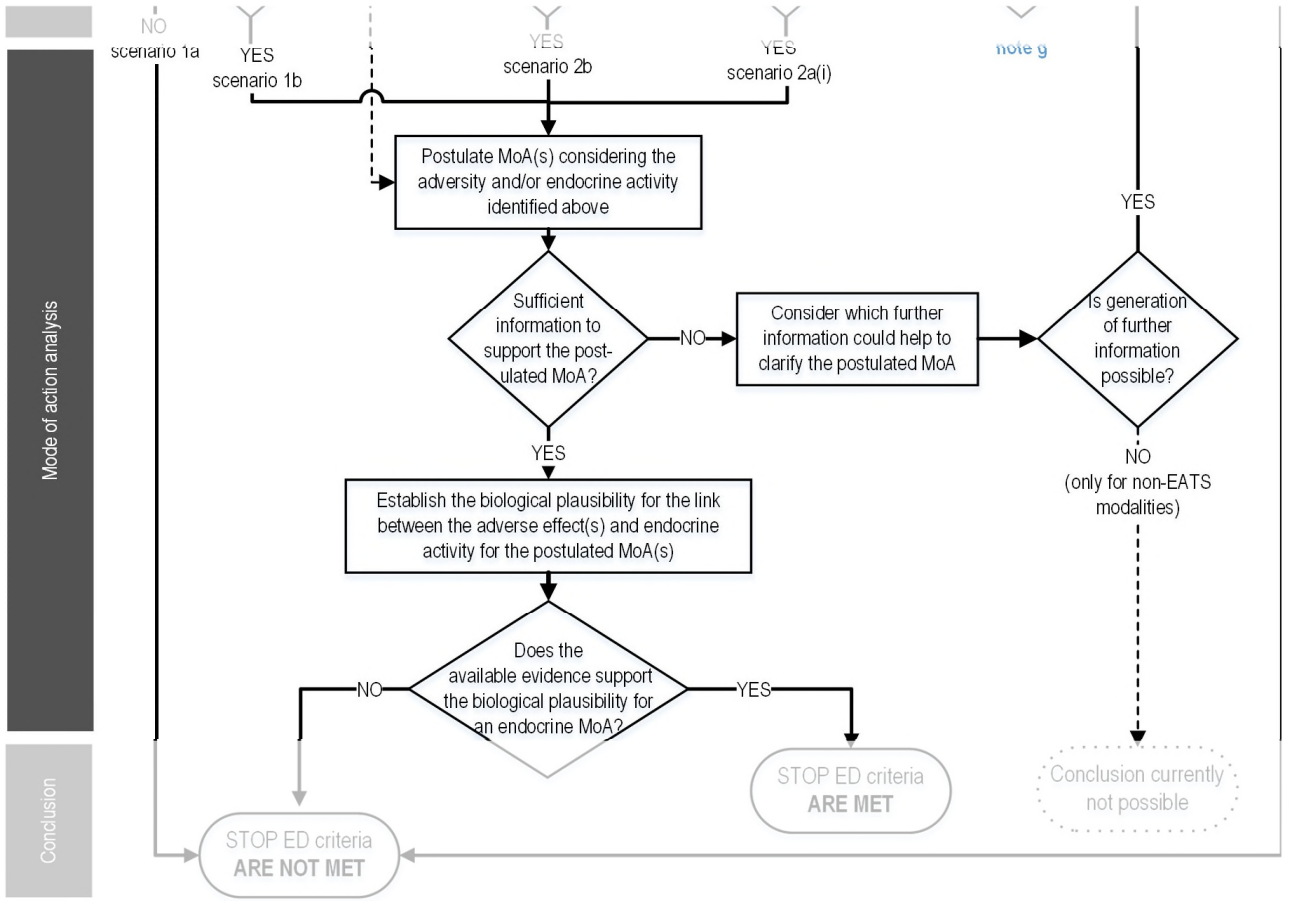
Flowchart illustrating the ED assessment strategy



Mode of Action Analysis

- **Mode of action analysis is required if 'EATS-mediated' adverse effect(s) or endocrine activity (or both) have been observed**
- **Multiple MoAs may need to be considered**
- **Alternative non-endocrine MoA**
- **Adversity based on non-EATS endocrine parameters**

Mode of Action Analysis



Mode of Action Analysis

Aim: Establish the link between the lines of evidence for adversity and endocrine activity. This link should be:

- Biological plausible

  and

- Established using a WoE approach

Additional data generation may be needed:

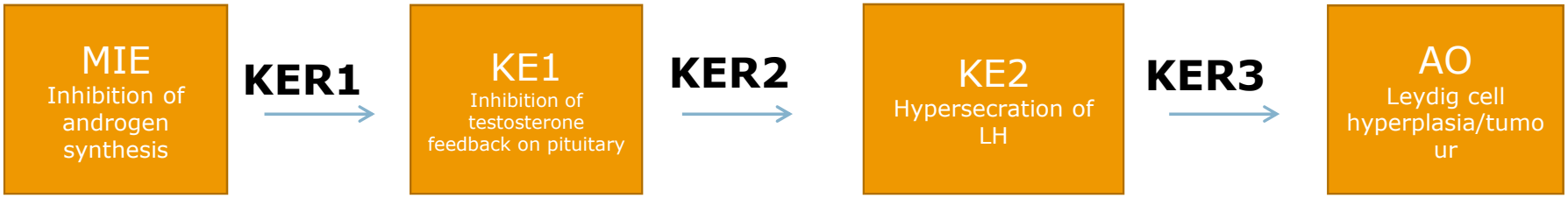
- Proposed frameworks for the MoA (IPCS and AOP)
- Proposed approach for the WoE (modified Bradford Hill considerations).

Mode of Action Analysis

Following identification of **endocrine activity and/or an adverse effect**, there is a need to establish the biological plausibility of the link between the two.

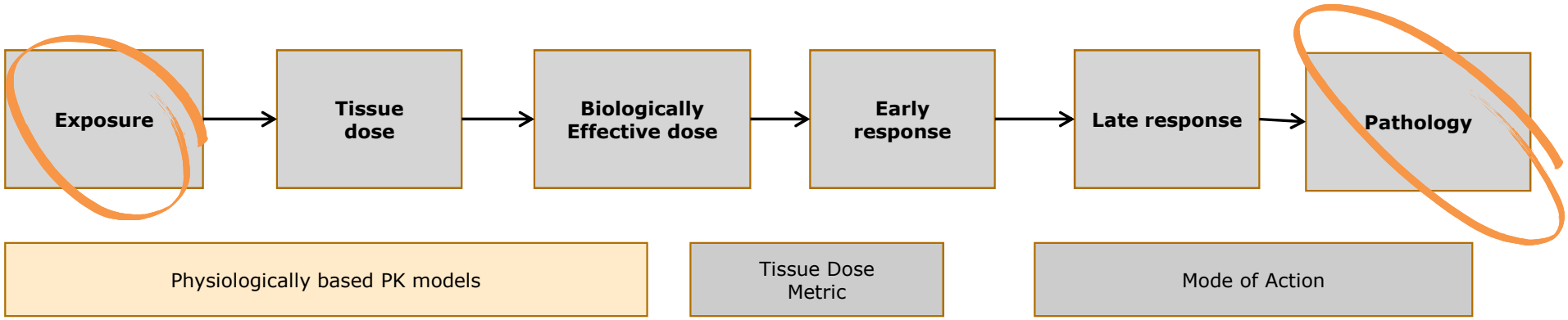
In practice this means establishing a series of KEs (including the MIE and the AO)

- KE: measurable and essential
- KEs are essential but not always sufficient



Exposure-Response Continuum

Using AOPs to support MoA analysis (1)



Toxicokinetics
Chemical specific:
Absorption, Distribution, Metabolism, Excretion
(MoA)

Toxicodynamics
Effect on the tissue
Chemical agnostic biological pathway **(AOP)**

Using AOPs to support MoA analysis (1)

The AOP Wiki have many AOPs under development:

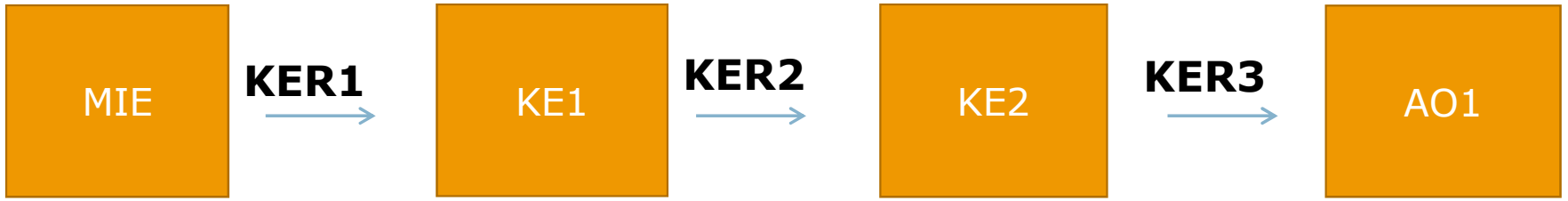
- **About 30 AOPs involving EAS-system**
  - Most advanced for fish
- **About 50 AOPs involving the T-system**
  - Neurodevelopment and brain function
  - Cancer
  - Cardiovascular system

Using AOPs to support MoA analysis (2)

Part of the already described AOP may be used to describe the MoA

- KEs already described; key aspects already documented e.g. essentiality of the KE.
- May provide evidence for biological plausibility for key event relationships

Mode of Action Analysis is about the Key Event Relationships



Line of evidence:
ER1 agonist

Hypothesised:
Negative feedback
on LH secretion

Hypothesised:
Decreased
endogenous estradiol

Line of evidence:
Impaired fertility

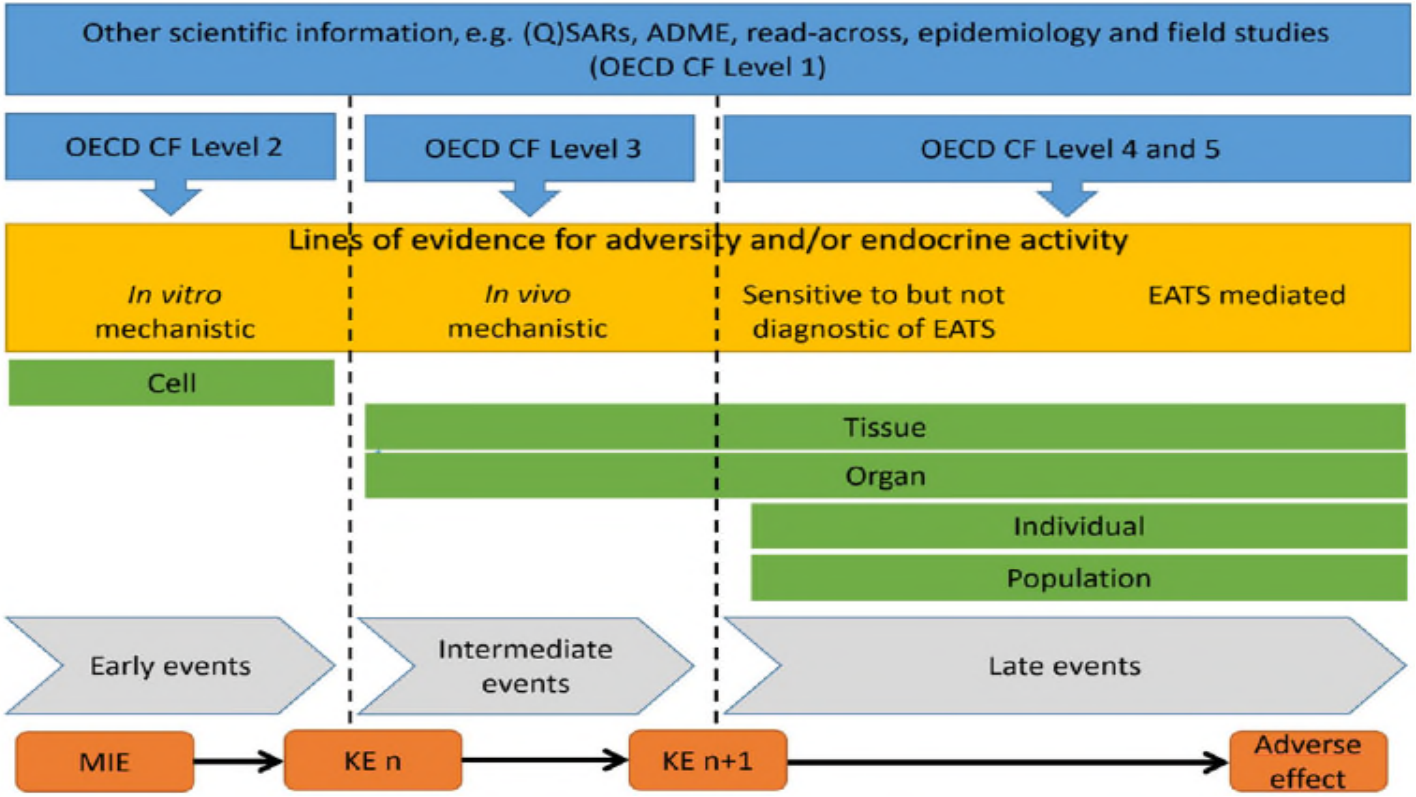
Line of evidence:
Deep venous thrombosis

Line of evidence:
Mammary neoplasms (adenocarcinoma)

10

Postulate a MoA(s) based on the available evidence

- Arrange the lines of evidence into a logical sequence of events from early events to late events.
- There may be multiple AOs - focus on the most relevant AO.
- Postulate a sequence of key events
- There may be a need to create intermediate key events based on existing knowledge



Postulate MoA(s)

It may **not be necessary to establish the whole sequence and relationship of events leading to adverse effect(s) to conclude** on the biological plausibility of the link between endocrine activity and adverse effect.

Existing knowledge on endocrinology / toxicology may be sufficient to assess the biological plausibility (e.g. if MoA is mainly postulated and empirically supported on the basis of EATS-mediated parameters) .

In the case of adversity based on 'EATS-mediated' parameters, the underlying knowledge (i.e. by coherence analysis (Susser, 1991)) of the likely endocrine nature of the effects may be such that judgement can be reached on the biological plausibility of a link without recourse to a detailed MoA analysis.

Document the MoA

[Summary of the hypothesis] **The molecular initiating event is unknown; however, the substance increases serum oestradiol in a dose-dependent manner. This results in continuous estrogen receptor 1 activation in estrogen sensitive tissues (numerous tissues are affected however this mode of action focuses on the uterus). The increased estrogen signalling ultimately results in cancer**

	Brief description of key event (KE)	**Supporting evidence**
Molecular initiating event (MIE)	Inhibition of androgen synthesis (postulated MIE)	None (no data provided, but hypothesised based on current knowledge and former experience with chemicals)
KE 1	Increased serum oestradiol	Increased serum estradiol (OECD TG 407)
KE 2	Uterine hypertrophy	Increased uterine weight (OECD TG 407 and 408)
KE 3	Uterine hyperplasia	Histopathology (OECD TG 408 and 453)
Adverse effect (AE)	Uterine neoplasia	Histopathology (OECD TG 453)

Weight of evidence

The ED criteria state that a weight of evidence approach shall be applied for the assessment of the available scientific data

In the Guidance, weight of evidence methodology as indicated in the criteria is used in two different contexts:

- Firstly, weight of evidence is applied for the evaluation of the line(s) of evidence for adversity and/or endocrine activity to determine whether there is sufficient empirical support for the assembled lines of evidence; and
- Secondly, weight of evidence is used for the mode of action analysis, to establish the link between the adverse effect(s) and the endocrine activity.

Expert judgement could be necessary when considering the available lines of evidence, including the overall evaluation of the consistency of the dataset as a whole.

Modified BH Considerations	Conclusions
Biological Plausibility	KER is consistent with current biological understanding – plausible.
Essentiality of Key events	Effects are reversible if the stressor is removed (e.g., Villeneuve et al. 2009; EHP 117: 624-631)
Concordance of Empirical Observations	**Dose response** – The key events observed at doses below or similar to those associated with the apical effect? **Temporality** – The key events are observed in hypothesized order? **Incidence** – The frequency of occurrence of the apical effect less than that for the key events?
Consistency	Same pattern of effects has been observed in several test species (e.g., fathead minnow, zebrafish, medaka)
Analogy	Similar pattern of effects observed for three well known aromatase inhibitors (FAD, LET, PRO)

Biological plausibility of a KER

- The biological connection between upstream and downstream KEs
- Based on the "broader knowledge" of normal biological function
- The most important element for the overall confidence in a postulated MoA.

-strong – if is there is extensive understanding of the KER based on extensive previous documentation and broad acceptance

-moderate – if the KER is plausible based on analogy with accepted biological relationships, but scientific understanding is not completely established

-weak – the structural or functional relationship between the KEs is not understood.

Essentiality of KE

If a KE is blocked are the downstream KEs and the AO prevented?

Direct experimental evidence can support essentiality; e.g. reversibility studies, antagonism, knock out models.

-Strong - if there is direct evidence from specifically designed experimental studies illustrating essentiality for at least one of the KEs (e.g. stop/reversibility studies, antagonism, knock-out models, etc.).

-Moderate - if there is indirect evidence that sufficient modification of an expected modulating factor attenuates or augments a KE.

-Weak - if there is contradictory experimental evidence of the essentiality of any of the KEs or there is evidence for no reversibility

Empirical support for a KER (1)

Dose response and temporal concordance

[Species X] dose–response and temporal concordance between the key events				
	KE 1	**KE 2**	**KE 3**	**Adverse effect**
	Increased serum oestradiol	**Uterine hypertrophy**	**Uterine hyperplasia**	**Uterine neoplasia**
Dose (mg/kg/day)				
10		– (90 days)	– (90 days)	
30	+ (28 days)	+ (28 days)		– (2 years)
90	++ (28 days)	++ (28 days) +++ (90 days)	+ (90 days)	+ (2 years)
180		+++ (28 days)	++ (90 days and 2 years)	++ (2 years)
360	+++ (28 days)	+++ (90 days)	+++ (90 days)	

Only key events with available data for dose-response and temporal concordance are included.
– indicates no effect; +, ++ and +++ indicates the effect size, i.e. severity.

Empirical support for a KER (2)

Dose-response and temporal concordance

-strong – if there is extensive evidence for temporal, dose–response and incidence concordance and no or few critical data gaps or conflicting data
-moderate – if there is evidence inconsistent with the expected pattern for which, however, an explanation can be found (e.g. based on experimental design, technical considerations, differences among laboratories).
-weak – if there are significant inconsistencies in the empirical support (e.g. no dose-response and temporal concordance, inconsistencies among studies) that cannot be explained.

Consistency, analogy and specificity of KEs

-consistency same pattern of effects across several species
-analogy – same sequence of KEs for other substances with a similar MoA
-specificity – the AO is a consequence of the postulated endocrine MoA, and not the an indirect result of other toxicity

For details on how these elements are weighted see the ED Guidance.

Conclusion on the MoA analysis

- Establish a biologically plausible link between the adverse effect and the endocrine activity

- The WoE should reflect;
  - The **biological plausibility** for each KER
  - The empirical support for each KERs
  - Essentiality of KEs
  - Consistency, analogy, and specificity of the MoA

- List uncertainties

- Use a tabular summary

CONCLUSION on ED criteria

Conclusion on the MoA analysis

- Establish a biologically plausible link between the adverse effect and the endocrine activity

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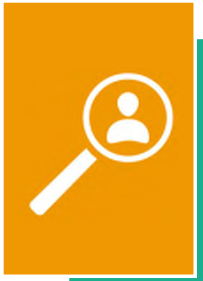

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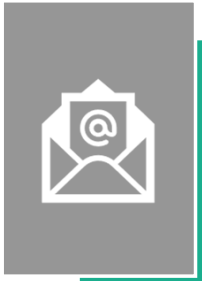

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