Noncancer MOA Decision Points: Examples from the Draft Formaldehyde Inhalation Assessment of Respiratory Tract Effects

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Acknowledgements

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Disclaimers:

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

The analyses in this presentation are at a draft, deliberative stage, and do not necessarily represent approaches that will be used in subsequent stages of review.

Introduction

Problem: in MOA analyses, there is a need to counterbalance rigor with efficiency (i.e. fit-for-purpose analyses), and to clearly present and justify decisions

- *This presentation illustrates subjective decision points and documentation*

Formaldehyde inhalation is a unique example:

- Robust database including numerous human, animal, and in vitro studies

  MOA Impact: greater number and flexibility of decision options than typical chemicals

- Scrutiny of decisions is arguably more robust than exists for other reviews

  MOA Impact: possible need for additional or more detailed analyses and documentation

- Formaldehyde is endogenously produced

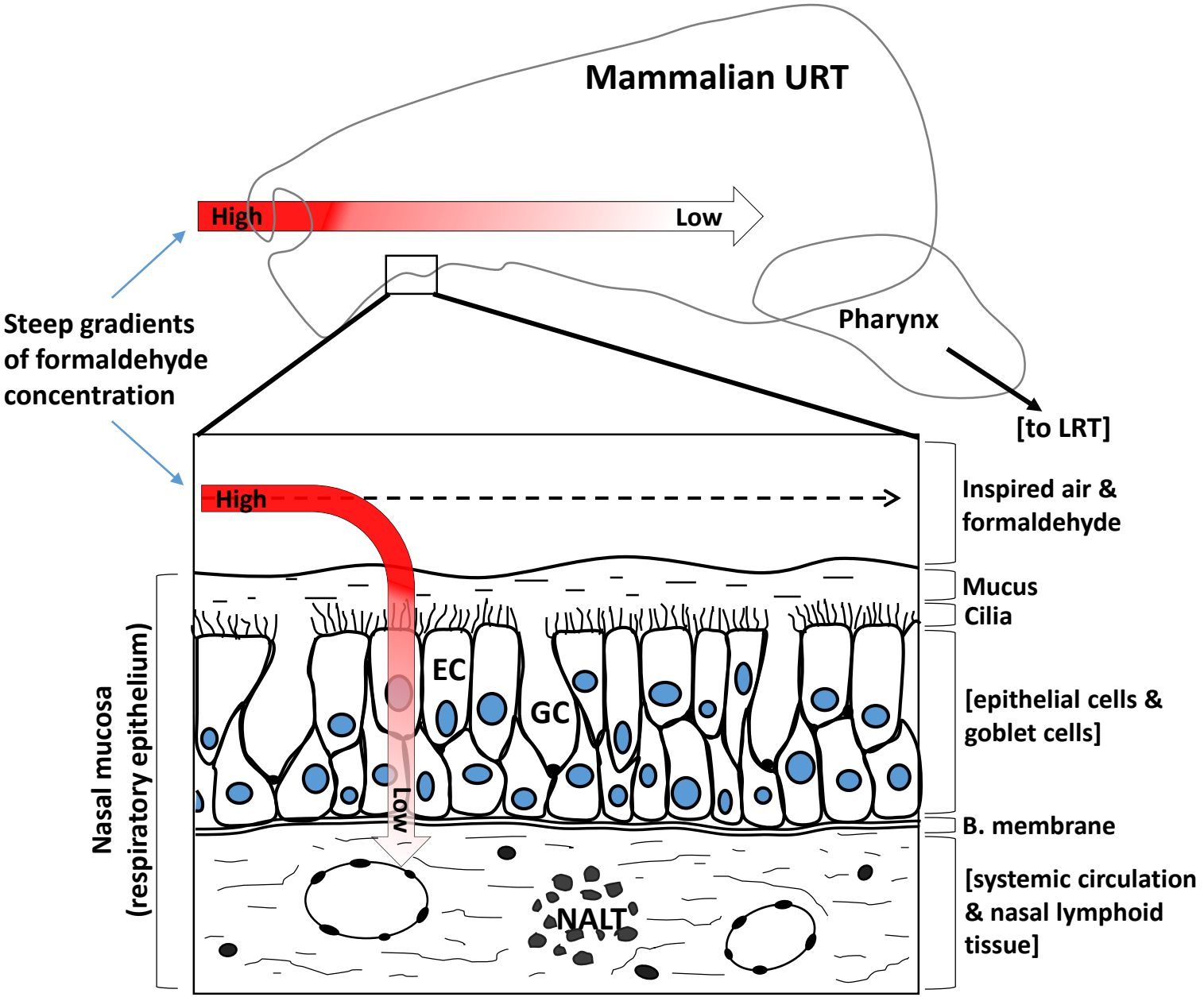
  MOA Impact: May have implications for dose-response decisions (not addressed herein)

- Formaldehyde is a highly reactive chemical

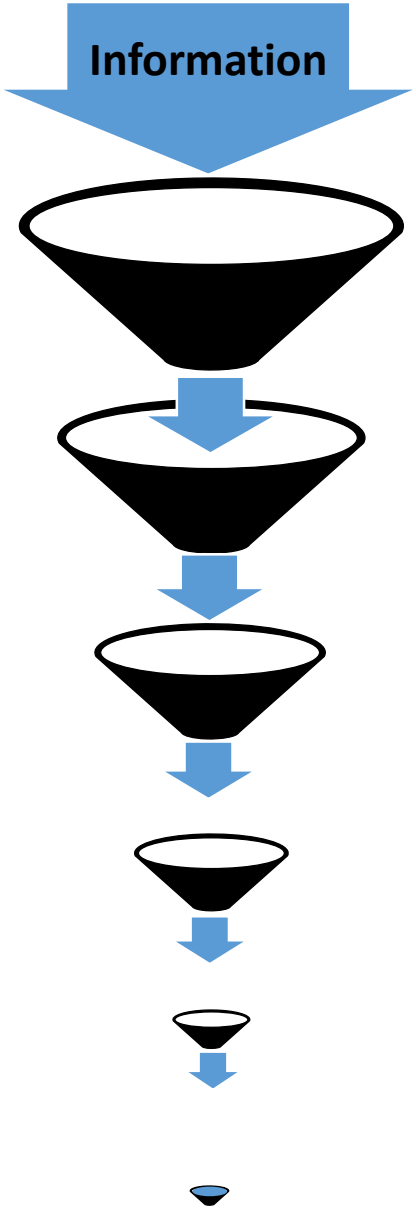
  MOA Impact: TK considerations are an essential component of the MOA analyses

The Focus of this Presentation is on the Systematic Approaches, not Formaldehyde

Toxicokinetics interpretation: The majority of inhaled formaldehyde reacts in the upper respiratory tract (URT) and very little (if any) reaches the LRT or systemic circulation



Example Decision Process for MOA Evaluation



Decisions #1-3: Focus the review and conduct the search

Decisions #4-5: Organize and consider individual studies

Decisions #6-7: Group and summarize the available data

Decisions #8-9: Assemble (e.g., connect) the evidence

Decision #10: Evaluate (e.g., for strength) the evidence

Decisions #11-12: Describe & apply the results of the evaluation

Where to Start?

Decision Point "1": At what level is a MOA evaluation performed?

- Several potential health effects relate to airway changes; expected overlap of mechanisms

Decision: A single evaluation encompasses all of the respiratory tract-related health effects

[Alternatives: MOA analyses for each individual health effect or even more specific endpoints]

Decision Point "2": **What (if any) constraints should be placed on data considered?**

- Non-inhalation studies poorly replicate inhaled formaldehyde distribution. The mammalian respiratory tract, circulatory and immune systems, are most relevant to human toxicity.

Decision: Given the database size, exclude non-inhalation exposure, non-gaseous in vitro and non-mammalian studies. Restrict to respiratory tract, circulatory, or immune models

[Alternative: Consider and analyze all available relevant data, possibly in a tiered fashion]

__Decision point "3"__ (not addressed): How is the search(es) conducted and documented?

Individual Studies

Decision Point "4": How are individual mechanistic studies considered? Should this differ from evaluations in other applications (e.g., endpoints used to describe health hazards)?

- Mechanistic studies are more abundant and generally assume a lesser role in assessments

Decision: Decision criteria identify "more", "less" or "least" informative studies (latter are excluded). Criteria differ for epidemiology and toxicology, and emphasize exposure issues

[Alternatives: Evaluations could be more/ less rigorous; trade-offs are efficiency and clarity]

__Decision point "5"__ (not addressed): How to determine which study data are "mechanistic"?

Example of simplified decision criteria for individual studies by study design

intent: identify mechanisms most likely to be associated with constant, chronic inhalation human exposure

Observational Studies
Generally (not strictly scored),"less informative" if multiple (2) unmet preferences; "least informative" if majority unmet:
Exposure duration • duration ≥ 5 days (acute exposures noted) • daily exposures of several hours
Exposure levels • inhaled concentration accurately quantified in exposed group • use of an appropriate referent group • exposure contrast expected to allow for detection of differences across groups
Comparability • endpoint result comparisons can discern effects of formaldehyde exposure alone (e.g., controlling for co-exposures, blinding)
Sample size • > 10 persons/ group to (theoretically) reduce variability
Reporting • clear description of methods • detailed, quantitative reporting of results

Experimental Studies (human or animal controlled exposure)
Generally (not strictly scored),"less informative" if multiple (2-3) unmet preferences; "least informative" if majority unmet:
System • in vivo with nose-only or whole-body inhalation exposure
Test article • paraformaldehyde (PFA; note: experiments of non-URT tissues/ models were automatically "less informative" if PFA was not indicated)
Exposure paradigm • duration of ≥ 5 days (acute exposures noted) • periodicity of ≥ 5hours/ day and ≥ 5 days/ week (if ≥ 1 day)
Exposure levels • inhaled concentration was quantified (as ppm, mg/L or mg/m^3) • at least one tested exposure level of ≤ 3 mg/m^3 (note: studies only testing above 10 mg/m^3 were considered "excessive")
Comparability • endpoint result comparisons can discern effects of formaldehyde exposure alone (e.g., controlling for other experimental manipulations, including chamber air exposure).
Sample size • > 10 humans or > 5 animals/ group to (theoretically) reduce variability
Reporting • clear description of methods • detailed, quantitative reporting of results

Illustration of individual study documentation (supplement to MOA analysis) by study design

Note: general type of outcome presented is *URT-specific inflammation or structural changes*

Study	System	Exposure	Endpoint	Results	Utility and Rationale
Observational Epidemiology Studies					
(xxxxx et al., 2004)	Symptomatic and non-symptomatic Human workers with carbamide-FA glue (n=29)	Exposed workers: 0.87± 0.39 mg/m^3 (n=21 non-exposed); duration mean: 12.7± 9.6 years	Assessment of chronic URT inflammation	Increase in subjective symptoms and objective clinical findings of chronic, URT inflammation (hypertrophy/ atrophy of mucus membranes; rhinitis) and decreased neutrophil function but N/C in leukocyte cell counts; symptomatic workers exhibited decreased resistance to infections (increased frequency and duration)	**More informative [mixture exposure]**
(xxxxx et al., 2000)	Primary school personnel in Sweden (n=234)	0.003-0.016 (mean= 0.0095) mg/m^3; duration unclear (working at least 20h/wk; assumed length months or more)	Assessment of acoustic rhinometry and factors in nasal lavage	Formaldehyde was significantly associated with multiple measures of nasal obstruction. Formaldehyde was positively associated with biomarkers for eosinophils (eosinophil cationic protein; lysozyme); N/C in a neutrophil marker (myeloperoxidase) or albumin	**Less Informative [mixture exposure (formaldehyde was independently associated with these changes, but so were NO_2 and Aspergillis)- did not evaluate confounding; some school measures below detection limit]**
Controlled-Exposure Studies in Humans or Primary Human Cells					
xxxxx and xxxxx (1983)	Human Healthy students (n=16: 5 males, 11 females)	Paraformaldehyde 0.3, 0.5, 1, or 2mg/m^3 for 1-3, then 4-5hr (internally controlled)	Mucus flow rate (also expiratory flow- not described here)	Nasal mucus flow rate in anterior nose was decreased at 1-3 hr at ≥0.5mg/m^3; rate did not decrease further at higher FA levels or with longer exposure (~5hr)	**More informative [short duration]** NOTE: ACUTE; latin square design
(xxxxx et al., 1999)	Human cells ex vivo from healthy volunteers (n=12)	Formalin gas: 0, 0.1, 0.5, or 5 mg/m^3 for 1 or 3 hr	Ex vivo ciliary beat frequency (CBF)	Decreased CBF in cultured respiratory epithelial cells at 5 mg/m^3 at 2hr only	**Less informative [ex vivo assay; formalin; short duration]** NOTE: ACUTE
xxxxx et al., 2014	In vitro human nasal epithelial cells (n=3 healthy donor) over 3T3 cell feeder layer	Formalin 0, 0.5, 1, or 3 mg/m^3 for 1hr	Cilia beating frequency (CBF)	CBF increased immediately after exposure to 1 FA and increased for 1hr, which persisted 20 min after washout; Slight decrease in CBF at 3 FA (authors attributed this to toxicity); N/C with 0.5 FA	**Least Informative [in vitro; formalin; short duration; small sample size]** Note: ACUTE
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
xxxxx et al., 1989	Young adult male rhesus monkeys (n=3/group)	PFA 0 or 6ppm for 1 or 6 weeks (6hr/d, 5d/wk)	Nasal histopathology and proliferation	Proliferation, cilia and goblet cell loss, hyperplasia and neutrophil inflammatory response at 1wk; squamous metaplasia more developed at 6wk and lesions more extensive and more posterior Lesions/ proliferation in larynx, trachea, carina mild at 1wk, more severe at 6wk	**More informative [high exposure level]** Note: n=3 monkeys/ group is considered a reasonable sample
xxxxx et al., 2003	Male BN and F344 rats; n=4/group	Formalin aerosol 1% for 3hr/d for 5d vs. water	Nasal mucosa cytokines and structure	Degeneration and neutrophil inflammation (F344> BN); Decreased IFN-γ and IL-2 in BN; N/C in F344; N/C in IL-4 or IL-5 in BN or F344	**Least Informative [formalin; short periodicity; small sample size; high, unknown exposure levels]**

Mechanistic Events

Decision Point "6": How are data grouped, and how are groupings judged/ documented?

- For this database, TK is critical, and multiple studies of varied design are often available for related endpoints, which may allow for more granular grouping than is usually possible.

Decision: Data grouped into "mechanistic events" of related endpoints by tissue region are analyzed on criteria emphasizing consistency across study types to arrive at conclusions

[Alternatives: Group data by endpoint similarity across tissue regions; use a different grouping (e.g., human vs. animal) or weighting (i.e. more or less stringent criteria) for decisions]

__Decision point "7"__ (not addressed): How to identify "mechanistic events" or "key events"?

Example of decision criteria for evaluating mechanistic events across lines of evidence

Summary Conclusion	Description of Evidentiary Support
"Reliable"	direct evidence supporting an effect in multiple, consistent *more informative* studies
"Probable"	direct or indirect (e.g., genetic changes) evidence supporting an effect in at least one *more informative* study, with consistent changes supporting an effect from *less informative* studies
"Suggestive"	• direct or indirect evidence supporting an effect in 1 *more informative* study • evidence suggesting an effect in multiple, consistent *less informative* studies • evidence of an effect from a *more informative* study and/or multiple *less informative* studies for which a comparable set of studies appear to be directly conflicting
"Minimal"	• evidence suggesting an effect in a single, *less informative* study • a set of *less informative* studies providing inconsistent results
"Inadequate"	• evidence cannot be interpreted (e.g., no data; a pattern in the results across studies could not be deciphered) • data that is suggestive of no change in the parameter (note: "reliable" evidence of no change is not considered "inadequate")

Key Note: **"Inadequate" evidence is not included in subsequent analyses**

Illustration of mechanistic event summary documentation (integral to MOA analysis)

Mechanistic Event		Study-Specific Findings from "More" or "Less" Informative Experiments	Summary of Evidence	Conclusion
URT Sensory Nerve-Related Changes				
Trigeminal Nerve Stimulation	More	*Human*: No direct data *Animal*: Increased afferent nerve activity: (xxxxx, 1991) acute ~20% at 0.62 mg/m^3 and ~50% at 2.21 mg/m^3 ; (xxxxx, 1975) acute (threshold response) at 0.31 mg/m^3	Increased activity of trigeminal nerve afferents at levels below 0.5 mg/m^3 after acute exposure in animals; effects of prolonged exposure are unexamined	Reliable
	Less	*Human*: N/A (see summary to the right) *Animal*: N/A (see summary to the right)	Sufficient information for 'Reliable' from *more informative* studies	
TRPA1 Activation	More	*Human*: None *Animal*: None	No evidence to evaluate	Suggestive
	Less	*Human*: None *Animal*: Formaldehyde activates the transient receptor potential cation channel, TRPA1, in in vitro models relevant to inhalation exposure of the URT: (xxxxx, 2007), and in vivo using formalin as a pain stimulus (not shown); Inhibition of TRPA1 and TRPV1 reduce FA exposure-induced immune-related responses in mice (xxxxx, 2013; xxxxx, 2005): 1 or 3mg/m^3 for 2 or 4wk	Indirect data identify TRPA1, and possibly TRPV1, as a molecular target(s) of formaldehyde exposure	
Neuropeptide Release from Sensory Nerve Endings	More	*Human*: None *Animal*: **in serum:** Increased substance P in mice with subchronic exposure (xxxxx, 2004): subchronic at 2.46 mg/m^3	Substance P was increased in serum with subchronic exposure in one mouse study at 2.46 mg/m^3	Probable
	Less	*Human*: **in URT:** Substance P in nasal lavage is increased in human volunteers with ocular exposure (xxxxx, 2005): 4d (5min/d) at 3mg/m^3, not at 1 mg/m^3 *Animal*: **in URT:** Formaldehyde stimulates release of calcitonin gene related-protein in in vitro models relevant to inhalation exposure of the URT: (xxxxx, 2011); Experiments using the related chemical, acrolein, suggest this is TRPA1-mediated **in LRT:** Increased substance P and CGRP, both amplified with OVA (xxxxx, 2013; xxxxx, 2011), and both were dependent on TRP activation (xxxxx, 2013): short term at 3mg/m^3	Data suggest formaldehyde activates TRPA1 channels on sensory neurons, leading to release of CGRP and substance P, with acute or short-term exposure at > 1 mg/m^3	

Combining Mechanistic Events

Decision Point "8": How are relationships between events identified and judged?

- Associations between mechanistic events are poorly studied; subjective inferences w/o data

Decision: Potential associations are identified based on tissue region and related biological roles. Basic decision criteria for evaluating relationships are applied using expert judgment.

[Alternatives: Perform more or less (or none) rigorous analyses of potential associations]

Decision Point "9": What considerations are weighed when constructing "MOAs" from individual events and, more generally, what presentation is the most informative?

- Analysis should include regional specificity, multiple potential hazards, and differing levels of confidence in the various mechanistic events and associations

Decision: Underlying structural organization by tissue region including more apical toxicity endpoints. Confidence is included, as is a separate illustration of the "strongest" data

[Alternatives: Skip this (interim) step and jump right into constructing MOA(s)/ AOP(s); use a different underlying structural organization (e.g., by exposure level) in a parallel approach]

Example of decision criteria for mechanistic relationships

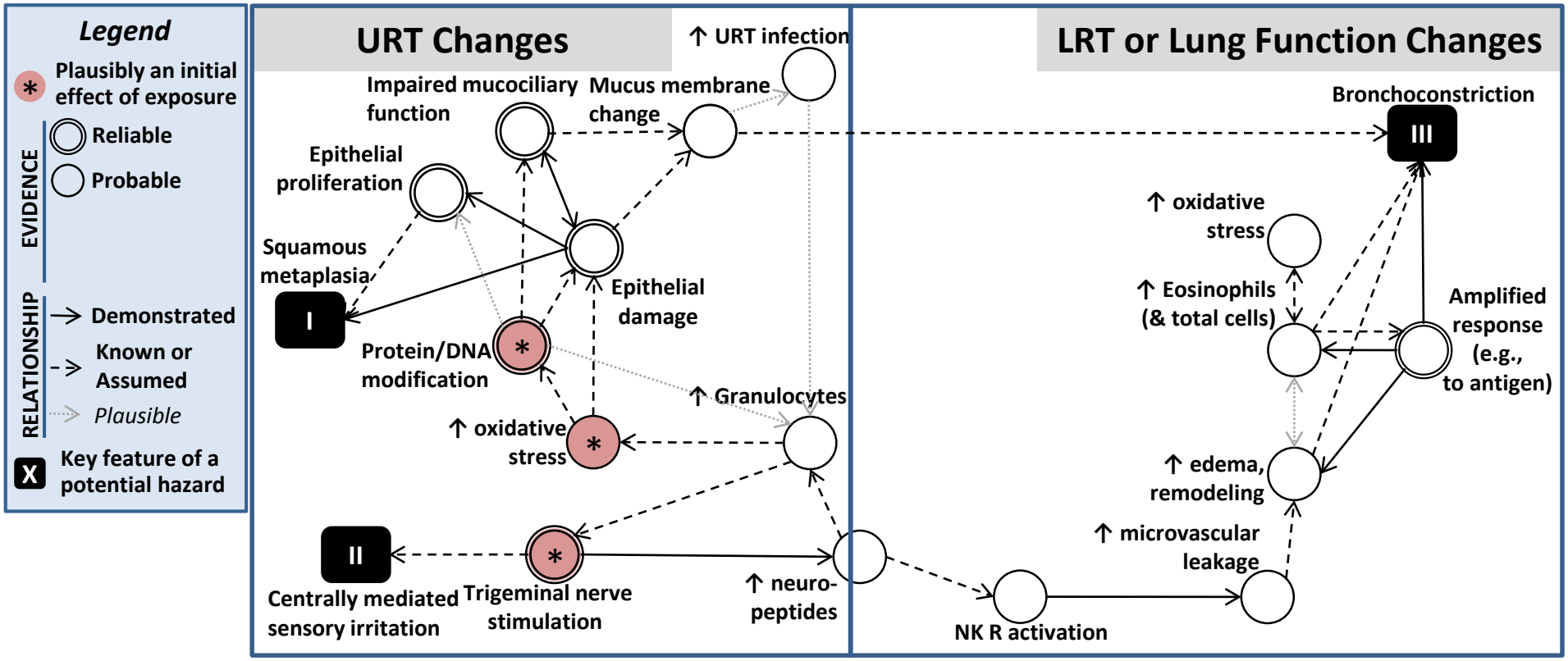
Note: these criteria are more subjective than those used to evaluate “mechanistic events”

Summary Conclusion	Description of Evidentiary Support
“Demonstrated”	Formaldehyde-specific data demonstrate a linkage (e.g., inhibition of event “A” prevents event “B” from occurring; “A” and “B” are linked by concentration, location, and temporality)
“Known or Assumed"	• An association between events “A” and “B” is known based on established (basic) biology • An association has been demonstrated for similar chemicals and/ or effects
“Plausible"	An association is justifiable based on underlying biology, but it is not well-established

Key Note: **Events for which an association does not seem likely (based on underlying biology) are not linked**

Illustration of integrated mechanistic displays across potential health hazard endpoints

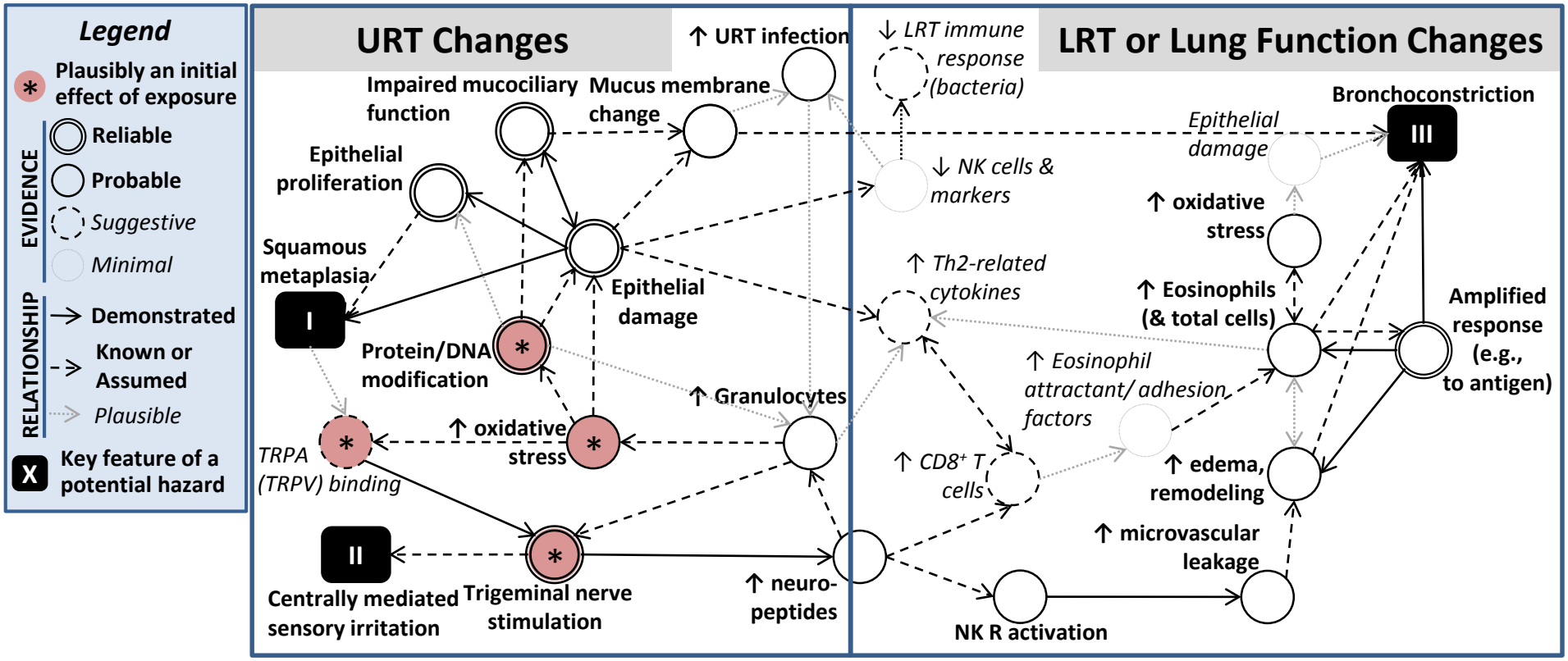
Presentation of the "reliable and probable" ("strongest") mechanistic evidence in the URT and LRT



Key Note: **Illustrations include "plausibly an initial effect…"; these mechanistic events that are interpreted as the most likely to be due (or most closely related) to direct interaction with inhaled formaldehyde molecules**

Illustration of integrated mechanistic displays across potential health hazard endpoints

Presentation of "all available" mechanistic evidence in the URT and LRT



Evaluating Mechanisms or MOAs

Decision Point "10": How is the most relevant sequence (or network) of mechanistic events distilled from the larger set of mechanistic information?

- Involves tradeoffs (e.g., between speculation and potentially erroneous assumptions of no association), predominantly due to missing experiments or unclear data.

Decision: For the more apical toxicity endpoints, the shortest sequence of the most reliable events from the "plausible initial effects…" events is assembled, regardless of tissue region

[Alternatives: apply one of many different approaches for constructing MOAs/ AOPs, some of which are more qualitative and less constrained than the approach used here]

Note: consolidating based on the most reliable chemical-specific data here might exclude data or associations that are much more relevant to the true biological progression of toxicity

Illustration of extracting the "most likely" mechanism(s) for effects

[Squamous metaplasia]

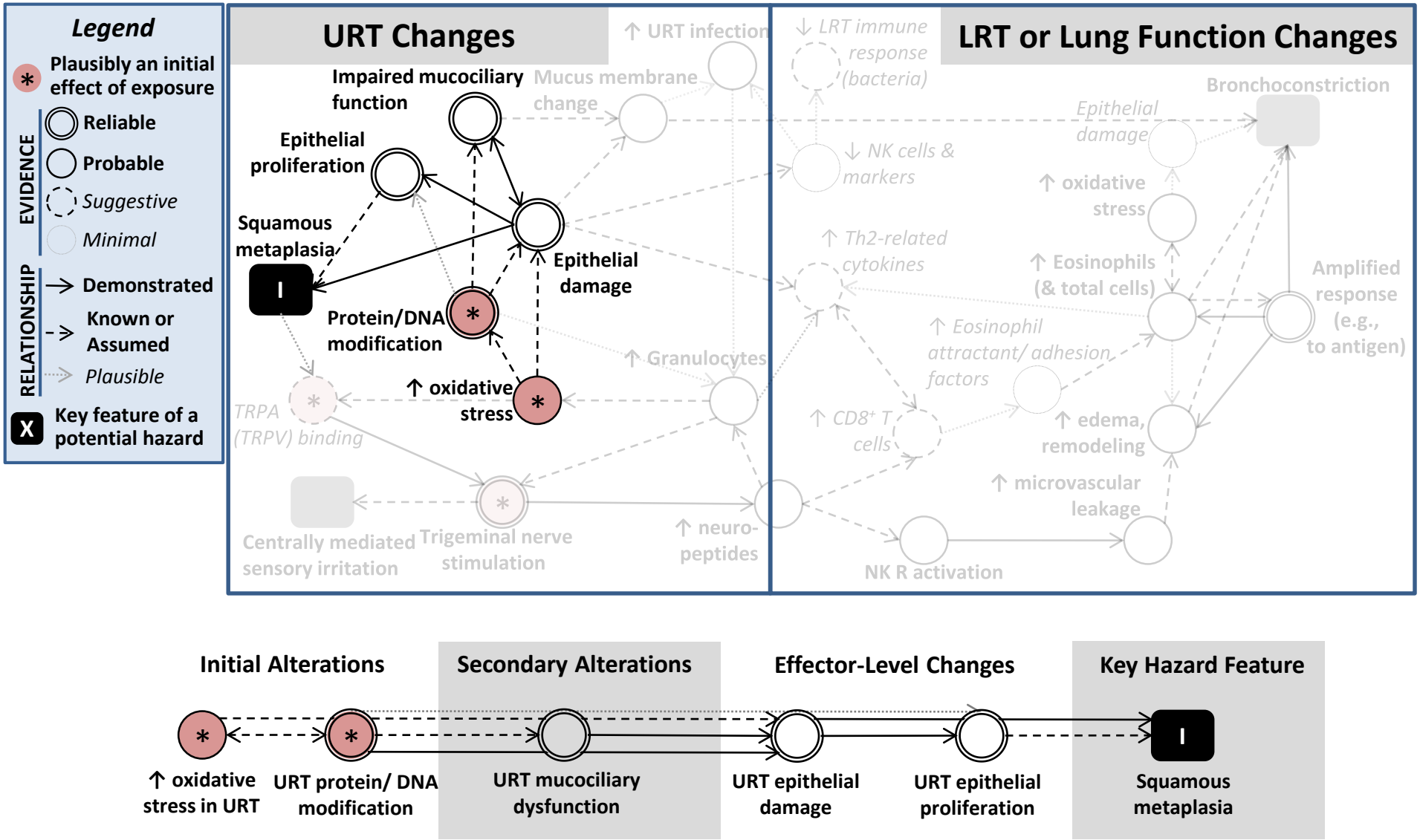


Illustration of extracting the "most likely" mechanism(s) for effects

[Sensory irritation]

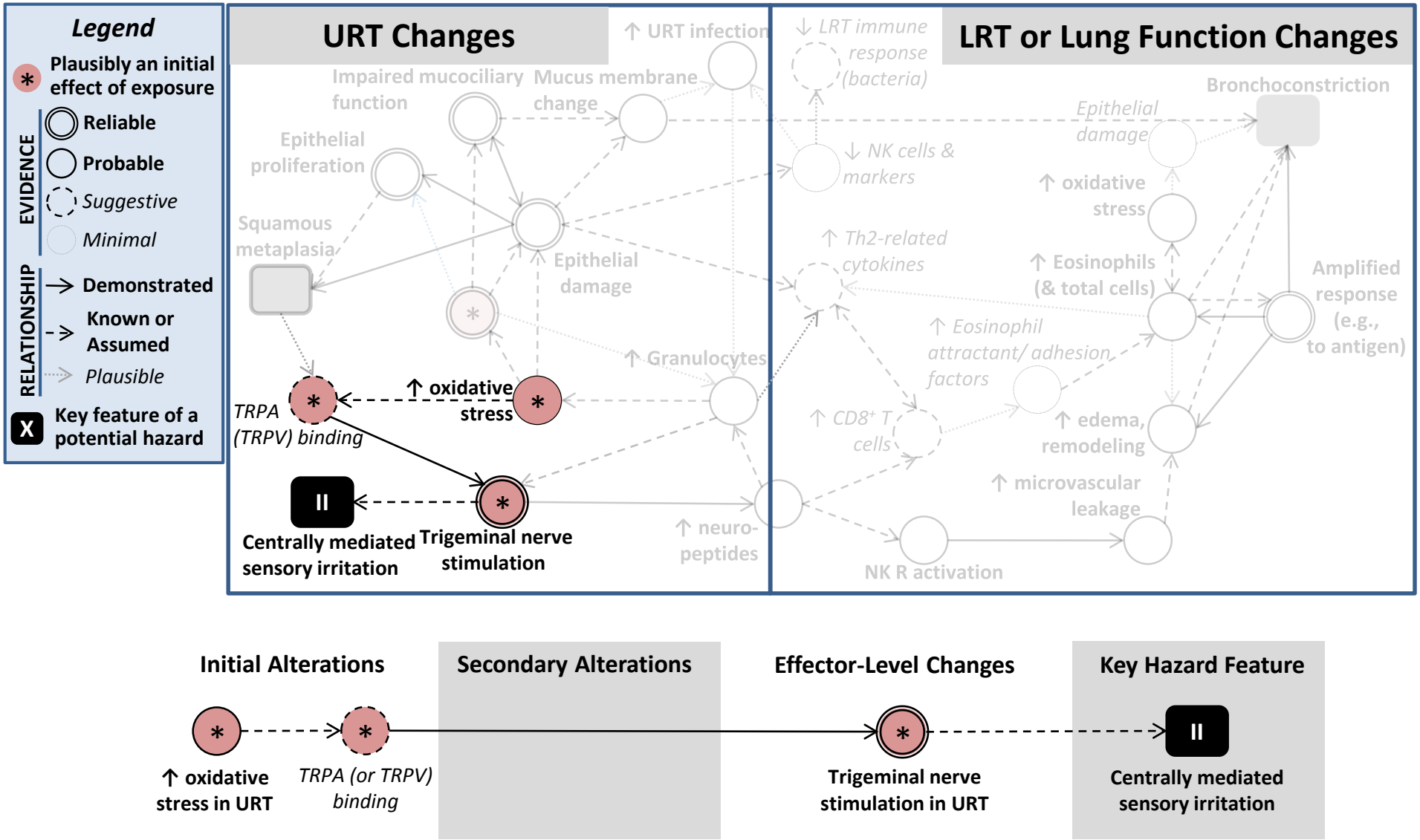
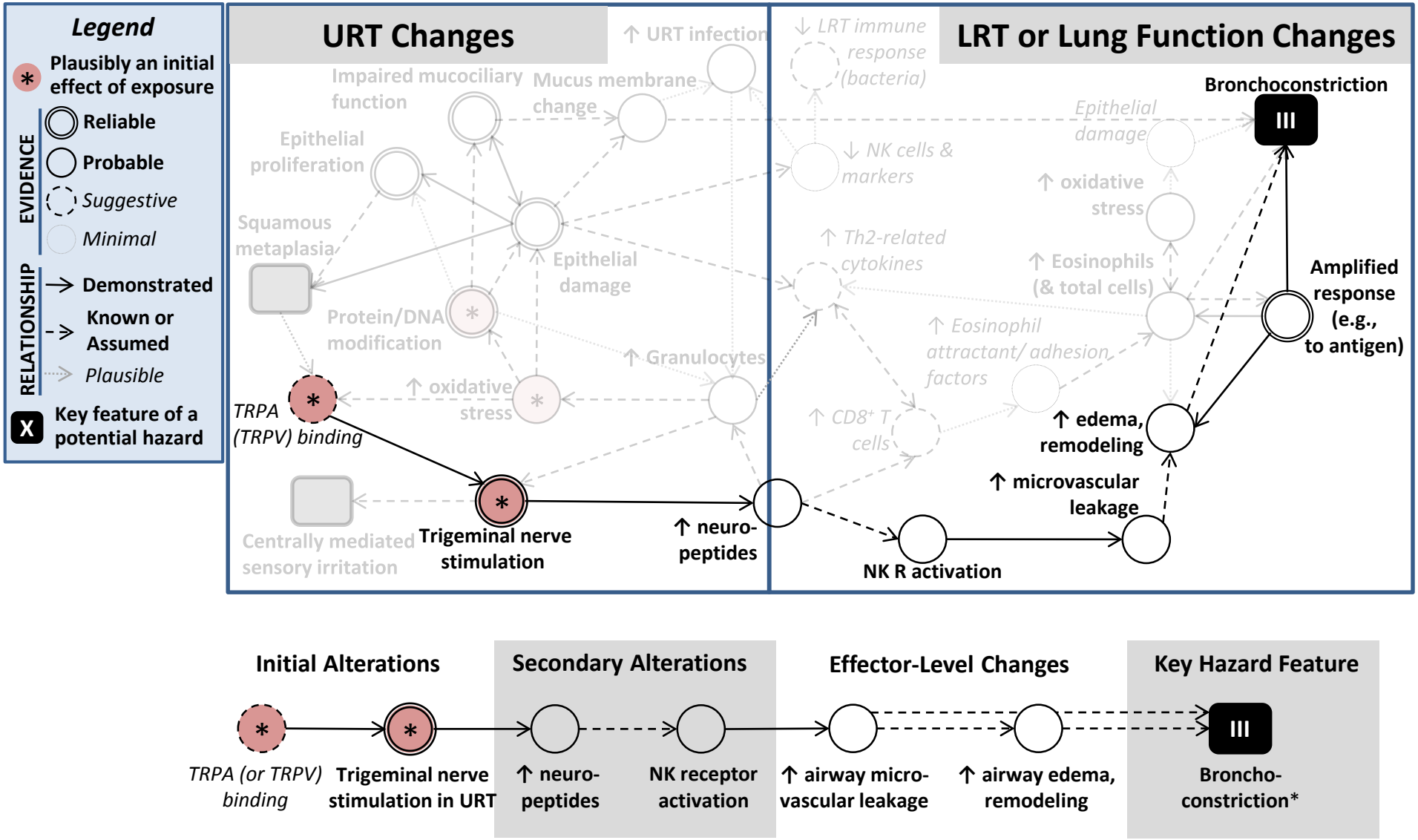


Illustration of extracting the "most likely" mechanism(s) for effects

[Bronchoconstriction]



*evidence of an amplified response to antigen after formaldehyde exposure

Summarizing Conclusions

Decision Point "11": How to present interpretations/ confidence in the identified MOAs?

- MOA/ AOP conclusions can take many forms, depending on the author and purpose

Decision: A qualitative summary reiterates conclusions regarding the strength of each line of mechanistic evidence, including judgments about whether key information is likely missing.

[Alternatives: A more/ less structured approach or framework for deriving overall conclusions]

__Decision point "12"__ (not addressed): How are MOA conclusions used in the assessment?

Illustration of qualitative conclusions regarding potential mechanism(s) for effects

- ***Squamous metaplasia through epithelial cell damage***

Interpretation: Likely a mechanism by which formaldehyde inhalation could cause this effect.

Rationale: All events based on reliable or probable evidence with known or demonstrated interactions between events, indicating that this mechanism is involved. However, modification of epithelial cell health and function in the URT can occur via multiple direct and indirect mechanisms, not all of which are incorporated in the MOA and which are interpreted to vary based on exposure duration and intensity. Thus, the understanding is considered incomplete and other contributing mechanisms are expected.

- ***Sensory irritation through trigeminal nerve stimulation***

Interpretation: Likely the dominant mechanism by which formaldehyde inhalation could cause this effect.

Rationale: A biological understanding exists to identify the physiological sensation of sensory irritation as being due to stimulated sensory fibers of the trigeminal nerve. Based on reliable formaldehyde- specific data supporting activation of trigeminal nerve fibers, alongside a general lack of alternative explanations for chemical-induced sensory irritation, this is interpreted to be the primary mechanism for this health effect.

Looking Forward: To what extent does a more systematic evaluation process influence the overall MOA conclusions and their use in assessments?