Summary of the overall weight of evidence for assessment of the AOP based on questions provided in the OECD's Users' Handbook.

1. Support for Biological Plausibility of KERS	**Defining Question**	**High (Strong)**	**Moderate**	**Low (Weak)**
	a) Is there a mechanistic relationship between KE_{up} and KE_{down} consistent with established biological knowledge?	Extensive understanding of the KER based on extensive previous documentation and broad acceptance.	KER is plausible based on analogy to accepted biological relationships, but scientific understanding is incomplete	Empirical support for association between KEs, but the structural or functional relationship between them is not understood.
MIE => KE1: Chemical binding to tubulin leading to disruption of microtubule dynamics.	**HIGH.** The binding of colchicine to tubulin is one of the best characterized chemical interactions with biological molecules. The depolymerisation of microtubules subsequent to chemical binding is also well characterized.			
KE1 => KE2: Disruption of microtubule dynamics in oocytes leading to meiotic spindle disorganization	**MODERATE.** There is good biological understanding of the relationship between the two KE in vitro, with multiple studies supporting this association. In vivo, and in oocytes in particular, the relationship is inferred by studies that have reported spindle disorganization following exposure to chemicals.			
KE2 => KE3: Spindle disorganization leading to altered chromosome alignment and segregation	**MODERATE** There is extensive evidence that well organized spindle is essential for proper chromosome segregation; however, it is not well characterized what degree of disorganization is necessary to alter chromosome alignment and segregation.			
KE3 => KE4: Altered chromosome alignment and segregation leading to the generation of aneuploidy oocyte	**LOW** It is broadly accepted that an aneuploid oocyte is the consequence of altered chromosome alignment and segregation. However, chromosome congression at metaphase (in both mitosis and meiosis) is a highly dynamic process. Therefore, demonstration of altered chromosome dynamics does not necessarilyy predict the generation of an aneuploid oocyte. Furthermore, the role played by the SAC and the mislocalization of SAC proteins following administration of spindle poisons is still not well understood.			
KE4 => AO: Aneuploidy in oocytes leading to aneuploidy syndromes in offspring	**HIGH.** This is dogma.			
MIE=> K4: Chemical binding to tubulin leading to aneuploid oocytes.	**HIGH.** There is broad understanding and extensive evidence that microtubule depolymerizing agents lead to aneuploidy in mammalian oocytes.			

2. Support for Essentiality of KEs	Defining Question	High (Strong)	Moderate	Low (Weak)
	Are downstream KEs and/or the AO prevented if an upstream KE is blocked?	Direct evidence from specifically designed experimental studies illustrating essentiality for at	Indirect evidence that sufficient modification of an expected modulating	No or contradictory experimental evidence of the essentiality of any of the KEs.

	least one of the important KEs	factor attenuates or augments a KE	
KE4: Aneuploidy in oocytes	**LOW** Essentiality for KEs has not been specifically tested. In general, it is not clear how experiments may be designed to 'block' or 'reverse' the KEs. However, genetic manipulation has provided some support for the essentiality of spindle assembly and integrity checkpoints in preventing aneuploidy.		

3. Empirical Support for KERs	Defining Questions	High (Strong)	Moderate	Low (Weak)
	Does empirical evidence support that a change in KE_{up} leads to an appropriate change in KE_{down}? Does KE_{up} occur at lower doses and earlier time points than KE_{down} and is the incidence of KE_{up} > than that for KE_{down}? Inconsistencies?	Multiple studies showing dependent change in both events following exposure to a wide range of specific stressors. No or few critical data gaps or conflicting data	Demonstrated dependent change in both events following exposure to a small number of stressors. Some inconsistencies with expected pattern that can be explained by various factors.	Limited or no studies reporting dependent change in both events following exposure to a specific stressor; and/or significant inconsistencies in empirical support across taxa and species that don't align with hypothesized AOP
MIE => KE1: Chemical binding to tubulin leading to disruption of microtubule dynamics	**STRONG** Extensive evidence in somatic cells to support this KER (in particular for temporal concordance), but not in a format to support evaluating concordance of dose response. A limited number of studies provide data in support of this in female germ cells.			
KE1 => KE2: Disruption of microtubule dynamics in oocytes leading to meiotic spindle disorganization	**MODERATE** There is strong in vitro data showing that microtubule depolymerisation precedes spindle disorganization. However, there is much more limited data showing that this is occurring in oocytes.			
KE2 => KE3: Spindle disorganization leading to altered chromosome alignment and segregation	**MODERATE** Studies in both somatic cells and oocytes have shown that when spindle abnormalities are detected, they are associated with altered chromosomal alignment at metaphases. Studies have also reported mislocalization of SAC proteins in morphologically abnormal meiotic spindles in mammalian oocytes.			
KE3 => KE4: Altered chromosome alignment and segregation leading to the generation of aneuploidy oocyte	**LOW** As chromosomal congression at metaphase is a very dynamic process, it is generally accepted that the presence of a mislocalized chromosome will not always result in an aneuploid oocyte.			
KE4 => AO: Aneuploidy in oocytes leading to aneuploid offspring	**MODERATE** Only a few studies have compared the incidence of aneuploidy in oocytes and zygotes following exposure to spindle disrupting agents. These studies observed similar frequencies of aneuploidy in oocytes and zygotes indicating that there is no strong selection against aneuploid oocytes at fertilization.			
MIE=> K4: chemical binding to tubulin leading to aneuploidy oocytes	**STRONG** There are many studies showing that chemicals that bind to tubulin induce aneuploidy in oocytes. The dose-related effects reveal that increasing incidence of tubulin binding is concordant with increased incidence of aneuplody. The timing of administration of the			

	chemical in relation to oocyte maturation and ovulation in order to elicit the strongest aneuploid effect is also quite well established with a peak sensitivity around the resumption of meiosis.

4. Inconsistencies and Uncertainties for KERs	
MIE => KE1: Chemical binding to tubulin leading to disruption of microtubule dynamics	There are no inconsistencies. The data are based primarily on effects from acellular biochemical experiments; however, data are also available in oocytes.
KE1 => KE2: Disruption of microtubule dynamics in oocytes leading to meiotic spindle disorganization	No inconsistencies based on the somatic cell literature. However, limited data available in oocytes.
KE2 => KE3: Spindle disorganization leading to altered chromosome alignment and segregation	The presence of a mislocalized chromosome does not necessarily mean the generation of an aneuploidy oocyte
KE3 => KE4: Altered chromosome alignment and segregation leading to the generation of aneuploid oocyte	Limited data available in oocytes.
KE4 => AO: Aneuploidy in oocytes leading to aneuploidy syndromes in offspring	Not all aneuploid oocytes produce viable embryos. Thus, some of the aneuploidies that occur in the eggs will not be seen in the offspring. However, aneuploidies that do not affect the eggs and are viable will be inherited by the offspring.