TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #1: Absorption and distribution to target tissue(s).

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
IPCS 1994	**Y**	Not Discussed	Not Discussed	Not Discussed
Butterworth et al. 1995a Butterworth et al. 1995b Conolly 1995 Conolly & Butteworth 1995 Wolf & Butterworth 1997 Butterworth & Bogdanffy 1999	Not Discussed			
Golden et al. 1997	**Y**	Evaluated use of oil vehicles as confounding influence due to known effects on peak blood levels and toxicity of other hydrocarbons.	Implied in discussion of differential effects with different vehicles.	Not Discussed
Greim et al. 1997	Not Discussed			
Hard 1998 Hard et al. 2000	Not Discussed			

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #1: Absorption and distribution to target tissue(s).

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Fawell 2000	Not Discussed			
Andersen et al. 2000	Not Discussed			
Meek et al. 2002 Meek et al. 2003	**Y**	Not Discussed	Not Discussed	Detailed discussion of studies on distribution of chloroform to target tissues.
Komulainen 2004	Not Discussed			
Holsapple et al. 2006	Not Discussed			
Boobis 2010 Boobis et al. 2009	**Y**	Not Discussed directly; however, provided detailed discussion of non-linear kinetics of absorption with dose and saturation phenomena.	Not Discussed	Detailed discussion of non-linear kinetics of absorption with dose and saturation phenomena.

TABLE 3: Evaluation of Support for Chloroform Mode of Action

Key Event #2: Oxidative metabolism of chloroform by the P450 enzyme CYP2E1 to highly reactive phosgene.

Citation	**Supports Key Event (Y/N)**	**Data Quality**	**Counterfactual Evidence**	**Alternative Hypotheses**
IPCS 1994	**Y**	Not Discussed	Not Discussed	Evaluated reductive metabolism; concluded to occur only at high
Butterworth et al. 1995a Butterworth et al. 1995b Conolly 1995 Conolly & Butterworh 1995***** Wolf & Butterworth 1997 Butterworth & Bogdanffy 1999	**Y***	Not Discussed	Not Discussed	*****Discussed published data indicating threshold for hepatocyte lethality above the concentration at which the maximal rate of metabolism occurs, suggesting that chloroform plays a direct role in the hepatic response.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #2: Oxidative metabolism of chloroform by the P450 enzyme CYP2E1 to highly reactive phosgene.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Golden et al. 1997	**Y**	Not Discussed	Cytochrome P450-specific inhibitors block cytotoxicity and DNA double-strand breaks characteristic of cytolethality.	Cytochrome P450-mediated oxidative or reductive metabolism may lead to cytotoxicity (isozyme not yet identified). Reductive pathway not excluded but considered minor based upon data.
Greim et al. 1997	**Y**	Not Discussed	CYP2E1-Knockout mice; renal cortex of male but not female mice.	Not Discussed
Hard 1998 Hard et al. 2000	**Y**	Not Discussed	Considered species and sex-specific metabolism patterns.	Not Discussed
Fawell 2000	**Y**	Not Discussed	Not Discussed	Reductive metabolism shown to be implausible.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #2: Oxidative metabolism of chloroform by the P450 enzyme CYP2E1 to highly reactive phosgene.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Andersen et al. 2000	**Y**	Not Discussed directly but implied by citation of framework criteria.	Not Discussed	Weight of evidence strongly supports oxidative rather than reductive pathway at low exposure levels.
Meek et al. 2002 Meek et al. 2003	**Y**	Mentioned strength of blocking studies in demonstrating metabolic route.	Discussed CYP2E1 knockout experiments and P450 inhibition.	Evaluated role of parent chloroform in toxicity.
Komulainen 2004	**Y**	Not Discussed	Not Discussed	Not Discussed
Holsapple et al. 2006	**Y**	Evaluated framework criteria, concluding data to be sufficient for establishing MoA and extrapolation to humans.	Not Discussed	Not Discussed

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #2: Oxidative metabolism of chloroform by the P450 enzyme CYP2E1 to highly reactive phosgene.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Boobis 2010 Boobis et al. 2009	**Y**	Not Discussed directly but implied by citation of framework criteria.	CYP2E1-Knockout mice	Not Discussed explicitly, however implied via thorough and detailed discussion of kinetics, tissue specificity and inter-individual variability of metabolism by CYP2E1 to phosgene.

TABLE 3: Evaluation of Support for Chloroform Mode of Action

Key Event #3: Sustained cytotoxicity to target cells, hepatocytes and / or renal proximal tubular epithelial cells.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
IPCS 1994	**Y**	Not Discussed	Not Discussed directly, but attributed differential results with different routes of oral exposure to cytotoxicity-detoxification threshold.	Considered and rejected genotoxic MoA; attributed carcinogenicity to a non-genotoxic/cytoxicity MoA, noting that more studies found inhibition of tumorigenesis than induction.
Butterworth et al. 1995a Butterworth et al. 1995b Conolly 1995 Conolly & Butterworth 1995 Wolf & Butterworth 1997 Butterworth & Bogdanffy 1999	**Y**	Not Discussed	Emphasized gavage versus oral dosing experiments, which present strong elements of counterfactual design whereby the tumorigenic dose is exceeded but administered in a way that avoids sustained cytotoxicity.	Ruled out genotoxic MoA based on consistency of results showing non-DNA reactivity and lack of consistency with tumor response patterns characteristic of mutagenic carcinogens.

TABLE 3: Evaluation of Support for Chloroform Mode of Action

Key Event #3: Sustained cytotoxicity to target cells, hepatocytes and / or renal proximal tubular epithelial cells.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Golden et al. 1997	**Y**	Evaluated quality of positive and negative genotoxicity data, noting that some positive results were compromised by cytotoxic dose levels.	Emphasized differences between two oral routes of exposure, which present strong elements of counterfatual design whereby tumorigenic response is absent when blood levels remain below cytotoxic level despite administration of a cytotoxic daily dose.	Concluded that genotoxicity / mutagenicity is not plausible and inconsistent with tumorigenicity data. Concluded that DNA damaging MoA is plausible, but only at very high doses that produce DNA damage secondary to damage of other macromolecules.
Greim et al. 1997	**Y**	Not Discussed	Not Discussed	Considered clastogenic mode of action; concluded if operable, occurs only at high doses secondary to cytotoxicity.

TABLE 3: Evaluation of Support for Chloroform Mode of Action

Key Event #3: Sustained cytotoxicity to target cells, hepatocytes and / or renal proximal tubular epithelial cells.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Hard 1998 Hard et al. 2000	**Y**	Not Discussed	Not Discussed	Considered and rejected a direct-acting mutagenic MoA based on a quantitative WoE evaluation of genotoxicity data.
Fawell 2000	**Y**	Not Discussed	Not Discussed	Described data showing genotoxicity in vitro and compared to negative in vivo data, concluding non-genotoxic MoA.
Anderson et al. 2000	**Y**	Not Discussed	Not Discussed	Ruled out genotoxic MoA based on ICPEMC quantitative weighting score indicating lack of support for genotoxicity among 40 studies.

TABLE 3: Evaluation of Support for Chloroform Mode of Action

Key Event #3: Sustained cytotoxicity to target cells, hepatocytes and / or renal proximal tubular epithelial cells.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Meek et al. 2002 Meek et al. 2003	**Y**	Not Discussed	Discussed results with gavage versus oral dosing experiments, which present strong elements of counterfactual design whereby the tumorigenic dose is exceeded but administered in a way that avoids sustained cytotoxicity.	Discussed studies showing lack of evidence for tumor induction at non-cytotoxic doses.
Komulainen 2004	**Y**	Mentions inconsistency of positive genotoxicity data for chloroform.	Not Discussed	Rejected tumorigenic response due to DNA-reactivity based on inconsistency and incoherence of data.

TABLE 3: Evaluation of Support for Chloroform Mode of Action

Key Event #3: Sustained cytotoxicity to target cells, hepatocytes and / or renal proximal tubular epithelial cells.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Holsapple et al. 2006	**Y**	Evaluated framework criteria, concluding data to be sufficient for establishing MoA and extrapolation to humans.	Not Discussed	Considered and rejected tumorigenic response due to DNA-reactivity based on consistency of data.
Boobis 2010 Boobis et al. 2009	**Y**	Not Discussed directly but implied by citation of framework criteria; noted that positive genotoxicity data are from non-standard studies, implying lower reliability.	Emphasized gavage versus oral dosing experiments, which present strong elements of counterfactual design whereby the tumorigenic dose is exceeded but administered in a way that avoids sustained cytotoxicity.	Genotoxicity ruled out on the basis of generally negative data in various assays for DNA reactivity and genotoxicity and mutations in vivo.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #4: Regenerative cell proliferation in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
IPCS 1994	**Y**	Not Discussed	Not Discussed	Not Discussed
Butterworth et al. 1995a Butterworth et al. 1995b Conolly 1995 Conolly & Butterworth 1995 Wolf & Butterworth 1997 Butterworth & Bogdanffy 1999	**Y**	Evaluated methods used to measure cell proliferation versus other measures of cytotoxicity. Concluded high confidence in the quality of labeling data based on study design and measurement factors.	Discussed gavage versus oral dosing experiments, which present strong elements of counterfactual design, whereby the tumorigenic dose is exceeded but administered in a way that avoids sustained proliferative regeneration.	Implied ruling out of mitogenic MoA by comparison with features of known mitogenic carcinogens.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #4: Regenerative cell proliferation in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Golden et al. 1997	**Y**	Not Discussed	Not Discussed	Considered potential for proliferation alone to produce tumors; concluded that a combination of cytotoxicity and proliferation is necessary but may not be sufficient depending upon genetic susceptibility of test species.
Greim et al. 1997	**Y**	Not Discussed	Not Discussed	Not Discussed
Hard 1998 Hard et al. 2000	**Y**	Not Discussed	Not Discussed	Reevaluated the 2-year drinking water bioassay in Osborne-Mendal rats, concluding that cytotoxicity and regenerative hyperplasia occur only at high doses.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #4: Regenerative cell proliferation in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Fawell 2000	**Y**	Not Discussed	Not Discussed	Not Discussed
Andersen et al. 2000	**Y**	Not Discussed	Not Discussed	Not Discussed
Meek et al. 2002 Meek et al. 2003	**Y**	Mentioned strength of labeling experiments to measure proliferation.	Discussed gavage versus drinking water dosing and lack of evidence for proliferation even when daily tumorigenic dose is exceeded but administered in a way that avoids sustained proliferative regeneration.	Considered lack of nasal tumor induction at doses that produce proliferation of nasal epithelium.
Komulainen 2004	**Y**	Not Discussed	Not Discussed	Not Discussed

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #4: Regenerative cell proliferation in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Holsapple et al. 2006	**Y**	Evaluated framework criteria, concluding data to be sufficient for establishing MoA and extrapolation to humans.	Not Discussed	Not Discussed
Boobis 2010 Boobis et al. 2009	**Y**	Not Discussed	Not Discussed	Ruled out growth stimulation based on lack of any evidence for direct stimulation of hyperplasia, inhibition of apoptosis or receptor activation. Also ruled out estrogenicity or other hormonal activity based on lack of evidence.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #5: Threshold development of tumors in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
IPCS 1994	**Y***	Not Discussed	Not Discussed	* Acknowledged cytotoxic MoA, but considered deviation from the default linear model premature at that time due to lack of data on regenerative proliferation in Osborne-Mendel rats, where kidney tumors were observed.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #5: Threshold development of tumors in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Butterworth et al. 1995a Butterworth et al. 1995b Conolly 1995 Conolly & Butterworth 1995 Wolf & Butterworth 1997 Butterworth & Bogdanffy 1999	**Y**	Not Discussed	Emphasized differences in tumor incidence with gavage versus oral dosing, which presents strong elements of counterfactual design, whereby adminstration of higher doses in drinking water fails to produce tumors seen with lower doses by gavage.	Discussed lack of strong evidence for linear, genotoxic MoA and concluded that a non-genotoxic MoA is consistent with thresholds for cytotoxicity.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #5: Threshold development of tumors in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Golden et al. 1997	**Y**	Noted that some cancer bioassays were compromised by exceeding the MTD for chloroform; those showed tumors, albeit at cytotoxic doses.	Emphasized differences in tumor incidence with gavage versus oral dosing, which present strong elements of counterfactual design, whereby administration of higher doses in drinking water fails to produce tumors seen with lower doses by gavage.	Ruled out other MoAs based on strength, consistency and plausibility of evidence for cytotoxicity.

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #5: Threshold development of tumors in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Greim et al. 1997	**Y**	Not Discussed	Not Discussed	Tumours develop only at cytotoxic doses; the main effect is induction of regenerative hyperplasia. An MAK (maximum exposure limit) for humans can be established on the basis of animal studies.
Hard 1998 Hard et al. 2000	**Y**	Not Discussed	Not Discussed	Kidney tumors develop only at high doses associated with cytotoxicity and regenerative hyperplasia.
Fawell 2000	**Y**	Not Discussed	Not Discussed	Not Discussed

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #5: Threshold development of tumors in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Andersen et al. 2000	**Y**	Not Discussed	Not Discussed	Expert panel concluded lack of carcinogenic risk below doses that cause cytotoxicity and inconsistency of data with genotoxic MoA.
Meek et al. 2002 Meek et al. 2003	**Y**	Not Discussed	Emphasized lack of tumor induction at tumorigenic doses that fail to produce cytotoxic tissue levels.	Ruled out other MoAs based on strength, consistency and plausibility of evidence for cytotoxicity.
Komulainen 2004	**Y**	Not Discussed	Not Discussed	Not Discussed

TABLE 3: Evaluation of Support for Chloroform Mode of Action
Key Event #5: Threshold development of tumors in liver and / or kidney.

Citation	Supports Key Event (Y/N)	Data Quality	Counterfactual Evidence	Alternative Hypotheses
Holsapple et al. 2006	**Y**	Evaluated framework criteria, concluding data to be sufficient for establishing MoA for rodent liver tumors and extrapolation to humans.	Not Discussed	Considered possibility of non-relevance to humans, concluding that liver tumor MoA is established in rodents, plausible in humans, with expectation of non-linear dose response and threshold.
Boobis 2010 Boobis et al. 2009	**Y**	Not Discussed	Not Discussed	Considered possibility of linear tumor formation in humans due to individual variability, but concluded that the existence of biological thresholds indicates that population thresholds can also be identified.